must be homozygous for the "*formella*" allele to allow high expression, or whether other factors, such as meiotic problems in the hybrid mothers or differential survival, are important cannot be decided at present. Unfortunately, none of the successful backcrosses thus far have been sufficiently marked with AO-1 electrophoretic variants to allow segregation of the regulatory pattern relative to structural alleles to be tested. (The electrophoretic difference noted in Fig. 1c is not fixed.)

Analysis of the mechanism of action of diffusible factors that influence tissue-specific enzyme expression will require biochemical methods. Of interest initially will be experiments to determine whether the posttranslational steps potentially included in the broad sense of gene regulation are important in these cases or whether the patterns reflect different rates of enzyme synthesis. The dramatic example of *trans*-acting regulation reported here should be amenable to biochemical analysis. Expression of AO-1 in salivary glands is a particularly attractive model for analysis of such regulation, since the presence of excellent giant chromosomes would permit joint use of cytological and biochemical methods.

The expression of AO-1 in *grimshawi* × *formella* hybrids provides evidence for a system involving both *cis*- and *trans*-acting factors in controlling various aspects of the program of a single enzyme. The results with ODH-2 in *grimshawi* × *orthofascia* hybrids are suggestive of the same thing. Similar conclusions have been reached with systems involving intraspecific variants in mice and *D. melanogaster* (*1*, *2*). Results that are understandable in terms of *trans*-acting regulators have also been obtained in studies of enzyme expression in interspecific hybrids in another group of *Drosophila* and in teleosts (*11*). Studies of enzyme expression in species hybrids may be useful in seeking models of developmental gene regulation and should yield important information about the evolution of new regulatory patterns.

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8. Cytological evidence (*4*) suggests that all members of the picture-winged group carry the same basic set of genes. All of the 26 species I examined have three nonallelic structural genes coding for proteins with aldehyde oxidase activity. The enzyme AO-1 is consistently distinguishable from the others by its electrophoretic mobility (it is always the slowest), substrate range (the other two appear to correspond to xanthine dehydrogenase and pyridoxal oxidase of *D. melanogaster*), and regulatory patterns present in all species (it always predominates in the adult head and is the only one in the ovary). Electrophoretic variants have been found in various species including *grimshawi* and *formella*. Mobility of AO-1 is shifted independently of the other two isozymes, whereas the AO-1 band in all tissues in which it is found is always affected coordinately.
9. All species have an ODH-1 that is homologous by criteria virtually identical to those applied to AO-1 here and to alcohol dehydrogenase (*3*). However, only 17 of 26 species examined have a distinguishable, faster-migrating band designated ODH-2. These two bands continue to be distinct when run in gels containing nicotinamide adenine dinucleotide, unlike the multiple forms of alcohol dehydrogenase also found in these flies (*3*). Electrophoretic variants of ODH-1 have been found in several species, and in no case is the mobility of ODH-2 coordinately affected. In one species (*D. affinidisjuncta*), one of two allelic forms of ODH-1 comigrates with ODH-2, obscuring the latter. This may be the situation in the species in which ODH-2 has not been found (and in all of which only a single form of ODH-1 has been seen).
10. A possible exception is suggested by cases in *D. melanogaster* in which the ability of homologs to synapse in tissues containing giant chromosomes influences the activity of specific genes [G. Korge, *Chromosoma* **62**, 155 (1977)]. Since homologs in the *grimshawi* × *formella* hybrids do not synapse well (H. L. Carson, unpublished data), this effect may be relevant. However, the best-documented case works in the direction opposite to that observed in the present experiments (that is, a normally inactive gene is activated when tightly paired with a normally active homolog), and it is difficult to imagine a negative influence of one unsynapsed chromosome on its homolog that does not involve a diffusible product.
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31 October 1979

Chronic Arthritis in Goats Caused by a Retrovirus

Abstract. *A virus was isolated from an adult goat with chronic arthritis and shown to belong to the retrovirus group by electron microscopy and biochemical methods. Inoculation of the virus into cesarean-derived specific-pathogen-free goats' kids produced arthritic lesions similar to those in the spontaneous disease. Virus was reisolated from the experimentally induced lesions.*

The concept that viruses can initiate chronic degenerative disease has been supported by studies of a variety of spontaneous retroviral diseases in animals (*1*-*4*). A report of RNA-dependent DNA polymerase in a particulate fraction of brains from patients with amyotrophic lateral sclerosis (*5*) raises further interest in this class of viruses as a cause of progressive degenerative disease in man. The present report describes a chronic disease in goats (caprine arthritis-encephalitis syndrome) characterized by progressive arthritis as well as demyelinating encephalomyelitis. The syndrome is caused by a retrovirus that we have designated caprine arthritis-encephalitis virus (CAEV).

We previously described the pathology of leukoencephalomyelitis in a herd of goats and its transmission with 220-nm filtrates of tissue suspensions (*6*, *7*). Clinical disease of the central nervous system in this herd occurred predominantly

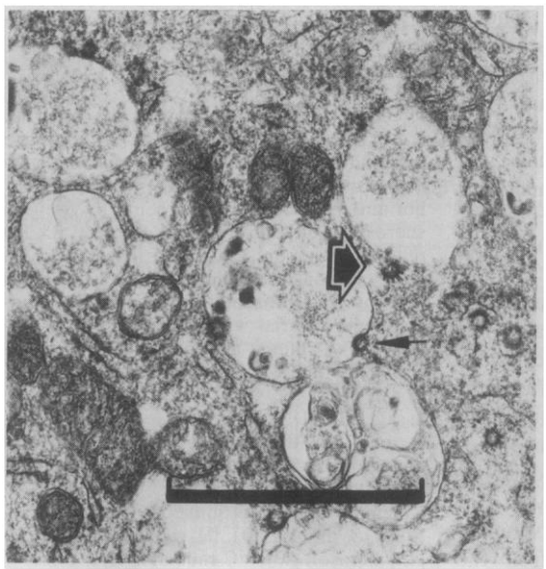

Fig. 1. Electron microscopy of CAEV-infected synovial membrane cells. Fetal caprine synovial membrane cells in plastic flasks at the eighth passage were infected with the 75-63 isolate of CAEV. At 8 days after infection the cells were removed by brief treatment with trypsin, fixed in glutaraldehyde, and centrifuged into a dilute (0.2 percent) agar suspension. The block was postfixed in osmium tetroxide, stained with 1 percent uranyl acetate, and sectioned on a Porter-Blum ultramicrotome. Sections were examined in a Phillips EM-200 electron microscope. The photograph shows particles in the cell cytoplasm and budding into intracellular spaces (small arrow). Membrane projections are visible on some of the particles (large arrow). Scale bar, 1 μm.

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Table 1. Induction of joint lesions and reisolation of virus from cesarean-derived goat kids injected with CAEV. Surgically derived kids were kept in strict isolation and inoculated with virus at 7 to 9 days of age. The inoculum was medium containing CAEV at a 50 percent tissue culture infective dose of $10^{6.2}$ per milliliter. The left carpal joint was injected with 0.25 ml, and 1.0 ml was injected intravenously. Three kids were injected in the same way with medium from control cultures. One kid was not inoculated. All kids were killed between 13 and 45 days after inoculation and examined for histologic lesions in both inoculated and uninoculated joints. Synovial membrane explants for reisolation of virus were established from all kids. Cultures were subjected to three passages before being considered negative. Evidence used for presence of virus was production of characteristic cytopathic effect.

Inoculum	Number of animals	Lesions in		Virus reisolated
		Inoculated joints	Uninoculated joints	
Infected medium	8	8/8	4/8	8/8
Control medium	3	0/3	0/3	0/3
None*	1	0/1	0/1	0/1

*The goats were not inoculated.

in kids 2 to 4 months old and frequently resulted in death or the need for euthanasia. Subsequent studies revealed a significant incidence of chronic arthritis among adult goats. The disease course was not influenced by broad-spectrum antibiotic therapy, and attempts to culture bacteria and mycoplasma from synovial fluid, periarticular tissue, and joint capsule were uniformly negative, as were attempts to isolate *Chlamydia* by serial passage in chicken eggs (*6*). Although survivors of encephalomyelitis had proliferative synovitis and periarthritis up to 3 years later (*7*), the relation between the encephalitic and arthritic components was not clear. Studies on adult goats were begun to clarify this relation.

Gross and microscopic examinations were conducted on 11 adult goats with arthritis; their ages ranged from 5 to 9 years. Tissues were collected and processed as described (*6*). Six goats had central nervous system lesions similar to those in young kids, though less severe. Connective tissue changes were consistent and widespread, affecting several organs, most regularly joints, tendon sheaths, and synovial bursae. Pathology consisted of synovial cell hyperplasia, marked enlargement of synovial villi, perivascular accumulation of lymphocytes and plasma cells, capsular and periarticular collagen necrosis, and mineralization surrounded by zones of intense lymphocytic infiltration (*8*).

Virus was initially isolated by explantation (*9*) of synovial membrane from an 8-year-old arthritic male goat (75-5 virus). The explants produced confluent monolayers by 3 weeks after plating. At 4 weeks, cellular vacuolation and scattered syncytial cells were observed. By 6 weeks, multinucleate syncytia involved most of the monolayer. Inoculation of cultures of fetal synovial membrane cells with medium from the primary explants resulted in similar syncytia developing within 3 to 6 days. Cultures of many goat cell types other than synovial membrane were examined for their ability to grow virus produced by explants, but none yielded an observable infection.

For ultrastructural studies by transmission electron microscopy we used cultures of fetal synovial membrane cells infected with a virus isolate from a 5-year-old male Toggenberg with severe chronic arthritis (75-63 virus). Particles exhibiting the size, morphology, and budding maturation process characteristic of retroviruses were seen in the cytoplasm (Fig. 1) and occassionally in the nucleus of infected cells. No viral particles were seen in uninfected cells.

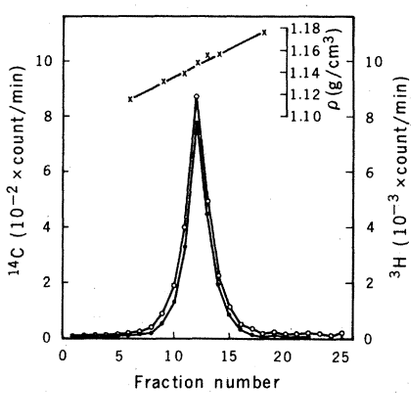

Fig. 2. Association of RNA-dependent DNA polymerase with CAEV. Virus was harvested from the medium of cultures of 75-63 CAEV-infected synovial membrane cells after 12 hours of labeling with 1 μCi of [2-^{14}C]uridine per milliliter. The ^{14}C-labeled virus was purified as described (*10*) and centrifuged to equilibrium in a 20 to 45 percent (by weight) sucrose gradient (Spinco SW41 rotor, 35,000 rev/min, 16 hours, 2°C). Portions (25 μl) of each fraction were diluted in 2 ml of cold 5 percent trichloroacetic acid, and the acid-insoluble material was collected onto Whatman GF-C filters and analyzed for radioactivity with Beckman EP scintillation solution. Portions (300 μl) of each fraction were assayed for DNA polymerase activity as described (*15*), the reaction mixtures of 810 μl containing $3.35 \times 10^{-3}M$ $MgCl_2$ at *p*H 7.8. Density (ρ, see inset) was determined by weighing 100-μl portions of indicated fractions. Symbols: (●) ^{3}H radioactivity (DNA polymerase); (○), ^{14}C radioactivity.

The prototype 75-63 virus was purified from the medium of infected synovial membrane cultures as described (*10*) and examined with respect to nucleic acid type, buoyant density, and endogenous RNA-dependent DNA polymerase activity. Figure 2 shows that the peak for CAEV labeled with [^{14}C]uridine coincides with that for endogenous DNA polymerase activity at a density of 1.15 g/cm^3 after isopycnic centrifugation in sucrose. The virus does not incorporate labeled thymidine.

Experiments designed to induce arthritis with the 75-63 virus and to recover virus from the animals with the experimental disease were conducted by inoculating cesarean-derived, specific-pathogen-free goat kids. Table 1 shows that experimental infection with medium from 75-63 virus-infected cell cultures consistently produced acute synovitis [synovial cell hyperplasia, mononuclear cell infiltration, and villous hypertrophy (*11*)] in inoculated joints and with a lower prevalence in uninoculated joints of the infected animals. Mild perivascular mononuclear cell infiltrates were found in brain and spinal cord of three of these goats. No lesions were discernible in kids injected with medium from uninfected cells or in the uninoculated control. In addition, virus was consistently isolated by explantation of synovial membrane from the joints of infected kids, but no isolates were obtained from the controls.

Brain lesions have been induced in neonatal goats by two different isolates of CAEV. These experiments, together with studies of animals with spontaneous disease (*7*), indicate that the virus causes both the encephalitic and arthritic components of caprine arthritis-encephalitis syndrome. Disease expression is highly variable, and many infected goats exhibit little or no clinically apparent disease. Reports of paralytic disease in goats from other states (*12*) and countries (*13*) as well as our immunologic screening studies indicate a wide distribution of the virus. However, evidence of infection has not been observed in numerous cell strains derived from ten different goat fetuses, in spite of careful scrutiny. Thus, CAEV appears not to be endogenous, but horizontally transmitted; in fact, the virus is probably acquired from the mother early in life (*6*). One possibility that could influence the type and severity of disease is that the level of maternal antibody passively transferred by colos-

trum varies widely in ungulates, depending in part on the efficiency of transfer and the vigor and specificity of the doe's immune response (*14*). Other variables to be considered are the dose of infecting virus, variations in the efficiency of the immune response, nonimmunologic controls over intracellular virus expression, and perhaps virus strain differences that may affect tropism.

Encephalomyelitis in caprine arthritis-encephalitis resembles visna of sheep (*7*), and immunodiffusion data indicate antigenic similarity but not identity between CAEV and visna virus. This is consistent with a recent observation of antibody to visna virus in goat serum (*15*). It seems likely that CAEV is part of a family of antigenically related ungulate retroviruses that cause chronic degenerative disease of several organ systems. The connective tissue component of the caprine arthritis-encephalitis syndrome is apparently unique, in that it represents the only reported viral-induced chronic arthritis of mammals.

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21 September 1979

Genetic Differences in Physiological Tolerances of Amargosa Pupfish (*Cyprinodon nevadensis*) Populations

Abstract. *Amargosa pupfish from a constant-temperature spring have a narrower range of temperature and oxygen tolerances than pupfish from the much more variable Amargosa River, indicating that the two populations have diverged genetically since their isolation. The reduced tolerances of the fish inhabiting a constant environment support the predictions of evolution theory.*

Approximately 10,000 years ago, the lowering of water levels in the Death Valley area of the western United States caused the isolation of populations of desert pupfish (genus *Cyprinodon*) into different habitats. A major question for evolutionary biologists concerns the extent to which the several species and populations of pupfish (*1*, *2*) have differentiated genetically (*2*, *3*). Despite the morphological divergence among these fishes (*2*), investigators studying other aspects of pupfish biology (biochemistry, behavior, and physiology) have been unable to demonstrate genetic differentiation among the populations (*3*).

Of particular interest is the extent of differentiation in physiological tolerances. Pupfish habitats differ widely in temperature, salinity, and dissolved oxygen. For example, some populations inhabit springs with constant temperatures, while others inhabit rivers with variable temperatures. This latter observation led Brown and Feldmeth (*4*) to compare temperature tolerances of pupfish inhabiting variable and constant environments. They argued that natural selection should produce fish with reduced thermal tolerances in constant springs, compared to those experiencing variable temperatures. They concluded, however, that "there is no evidence of genetic differences in short-term thermal tolerances between any of the populations tested." The fish they compared were collected directly from the field, rather than raised in the laboratory in similar conditions from egg to adult. In addition, their methods of analysis could not have detected slight, but real, differences (*5*).

In the course of experiments to evaluate genetic differences between two subspecies of *Cyprinodon nevadensis*, *C. n. mionectes* and *C. n. amargosae*, we determined their physiological tolerances to temperature and oxygen. These two populations inhabit very different environments: *C. n. mionectes* is found in Big Spring, a constant temperature (27.3°C) desert spring, while *C. n. amargosae* is found in the Amargosa River, which varies in temperature from near 0° to 40°C. Populations of these two subspecies have been isolated for perhaps 400 to 5000 years (*1*, *6*). We predicated that *C. n. mionectes* would show a narrower range of thermal tolerances than *C. n. amargosae* because of the lack of temperature fluctuations in Big Spring. In addition, we expected that *C. n. mionectes* would not tolerate oxygen levels as low as *C. n. amargosae* could because the latter must frequently face nearly anoxic conditions in the summer when flow in the river is reduced and the fish are isolated in side pools.

All experiments were performed on fish that had spent their entire lives under identical conditions. Adults of both subspecies were collected in the field and acclimated to 27°C for 1 month before eggs were collected. Ten males and 50 females of each subspecies were the source of the fish tested. Offspring were isolated before hatching and maintained under identical conditions (temperature, water, and feeding regimes). Some experiments were performed on the F_2 offspring of these fish, also isolated and reared under identical conditions. Any differences persisting after one or two generations under identical conditions are considered to be genetically based. Hybrids between the F_1 fish were available for some tests. Hybrid intermediacy also may constitute evidence of genetic divergence of stocks (*7*). Several experiments were carried out to determine oxygen and temperature tolerances:

1) For oxygen shock experiments (*8*), fish were transferred from their aquariums to tanks with oxygen levels at 1.00 or 1.45 ppm, and time until loss of equilibrium was recorded for each individual.

2) For determining critical oxygen minima (*9*), ten fish were placed in test aquariums; the oxygen concentration was reduced until the fifth fish lost equilibrium and was recorded at that point.

3) In thermal shock experiments (*10*),

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