# CHARACTERIZATION OF A CAPRINE ADENOVIRUS

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# ABSTRACT

## CHARACTERIZATION OF A CAPRINE ADENOVIRUS

By

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An adenovirus was isolated from the conjunctival drainage of an adult goat with acute conjunctivitis. The virus produced cyotpathic changes including intranuclear inclusions in goat kidney cell culture and in cell cultures derived from several other animal species. It was resistant to ether, chloroform, a pH of 3, and was moderately resistant to heat. Negative contrast electron microscopy showed a particle diameter of about 75-78 nm. The virus did not agglutinate erythrocytes of rat, guinea pig, human (type 0), chicken, sheep, horse, cow or pig. Inoculation of the agent into rats, mice and guinea pigs produced no disease, nor did it cause tumors in hamsters or goats. When inoculated by various methods into goats the agent produced no disease. However, the goats did respond with specific antibody production. Forty-five of 50 serum samples collected from randomly selected healthy goats showed neutralizing antibody to the virus.

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By

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Dedicated to my parents

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# REVIEW OF THE LITERATURE

## History of the Adeno Group

In 1953, Rowe et al. isolated a new cytopathic agent from human adenoidal tissue undergoing spontaneous degeneration in tissue culture (42). They named it the Adenoidal Degeneration Agent (AD agent) (42). A year later, Hillman and Werner isolated a similar virus by inoculating sputum into human epithelial cell cultures (3, 18). In this same year, Huebner et al. defined these agents as the Adenoidal Pharyngeal Conjunctival (APC) viruses. At this time, there were six serotypes in this group, all derived from human sources (2, 3, 21).

By 1955, Ginsberg and his coworkers had serologically connected APC viruses to respiratory disease (2, 3, 15). The term adenovirus was introduced in 1956 by Enders, "for a group of viral agents isolated from the respiratory and intestinal tracts of man and animals" (10). Adenovirus quickly replaced the earlier names.

New isolations occurred rapidly; a total of 18 serotypes being found by 1958. All isolated possessed a group specific antigen when tested by complement fixation and all were individually typed by specific serum neutralization (3, 4). Rosen in 1958 found that several adenoviruses

would agglutinate erythrocytes from a variety of animal species (39). He also showed that this activity could be inhibited with type specific antisera (39).

By 1959, the adenovirus group was large enough to require formal defining. Pereira made the first attempts to formulate a set of criteria for members of the group. They were as follows: (1) resistance to ether treatment, (2) production of a characteristic cytopathic effect in human cell culture, (3) the presence of a soluble group specific complement fixing antigen, and (4) lack of apparent pathogenicity for ordinary laboratory animals (32). Although the cytopathogenic effect must be broadened to include nonhuman cell types, the rest of the characteristics basically hold true today.

Also in 1959, the adenovirus structure, an icosahedron, was described by Horne (20). Its various substructures and antigens were enumerated throughout the 1960s. The name adenovirus became the official internationally accepted group designation in 1965 (50).

Adenoviruses have been isolated from species other than human. Simian adenoviruses were first found in 1958, canine in 1959, bovine in 1960, and a number of others throughout the 1960s and 1970s. The group is still expanding as new serotypes are being sporadically isolated and characterized (4). The group has been extensively studied

not only because of its disease potential, but also for its use as a model of viral oncogenicity.

## Characteristics of the Adenovirus Group

### Virion Size

Average size of the adenovirus virion is about 70-80 nm (20, 49). There is some variation in the literature, possibly due to different earlier methods of measurement. The current method used is direct measurement by electron microscopy, which seems to give fairly uniform results. The figure above is the most currently accepted one (19, 20, 32, 49).

Viral density, measured in cesium or rubidium chloride, is approximately 1.34g/cm$^3$ (8).

### Capsid Components

The adenovirus particle is icosahedral in shape with 252 surface units (capsomers) (45, 49). Each triangular facet has a side of six subunits and maximum diameter of a subunit as estimated from the center to center distance is 70 Å; each subunit may represent a single protein molecule (20). The overall symmetry of the icosahedron is 5-3-2 (34).

There are 12 capsomers at the vertices of the icosahedron, each with five neighbors. These 12 are called pentons and are structurally complex, consisting of a round head (called the penton base) embedded in the capsid with

a long rod 2 nm in diameter (called a fiber) projecting from it (13).

The remaining 240 capsomers each have six neighbors and occupy the faces and edges of each triangular facet. These capsomers are called hexons (7).

## Hexon Structure

A hexon is a polygonal hollow prism of approximately 70-85 Å in diameter. It has a central hole of about 25 Å across. Each hexon consists of three asymmetric units. Each unit contains a single polypeptide chain of about 93,000 daltons (13).

## Penton and Fiber Structure

The penton consists of a polygonal base of about 70-85 Å in diameter. Each penton has one attached fiber, a stringlike structure with a terminal knob. It is of variable length, which appears to depend on the adenovirus serotype. The fiber is composed of three polypeptide chains, of about 61,000 daltons each. The penton base has not been as extensively studied (13). For a structure summary see Table 1 (22).

## Chemical Composition

Chemically the virion is simple, containing DNA (11-14% by weight) and protein (13, 24). The DNA is a linear double stranded molecule which varies in base composition according to serotype (45). It is approximately

Table 1.--Comparative Data on Adenovirus Type 2 Morphologic and Antigenic Subunits and Protein Components.

| Morphologic Subunits | | | | Antigenic Subunits | | Protein Components |
|---|---|---|---|---|---|---|
| Appearance | Name | Number Per Virion | Molecular Weight | Antigen | Specificity | |
| Virion | DNA | | 23,000,000 | | | |
| | Protein | | 150,000,000 | | | |
| | Hexon | 240 | 210,000<br>400,000<br>320,000<br>360,000 | A | Group | II |
| | Hexons | 20 | 3,600,000 | | | II, VIII, IX |
| | Penton | 12 | 280,000<br>1,100,000 | | | III, IV |
| | Penton Base | 12 | 210,000 | B | Subgroup | III |

Table 1.--Continued.

| Morphologic Subunits | | | | Antigenic Subunits | | Protein Components |
|---|---|---|---|---|---|---|
| Appearance | Name | Number Per Virion | Molecular Weight | Antigen | Specificity | |
| | Fiber | 12 | 70,000 | C | Type | IV |
| Core | DNA | 1 | 23,000,000 | P | | V, VI, VII |
| | Protein | | 29,000,000 | P | | V, VI, VII |
| | Protein | | 13,000 | | | VIII, IX |
| | Protein | | 7,500 | | | X |

20-25 x $10^6$ daltons in molecular weight (45). The DNA, along with at least two internal proteins, makes up the viral core (22).

Capsid proteins account for about 58% of the total particle weight of the virion (45). Thus, noncapsid proteins comprise approximately 30% of the total viral weight. When the noncapsid proteins are subjected to acrylamide gel electrophoresis, nine distinct polypeptides are seen. Protein I is complex, II is penton base associated, III is fiber subunit associated, and IV is hexon associated. Proteins VIII, IX, and X are capsid associated and V, VI, and VII are DNA core associated. Specific functions are unknown (45). For a summary of this information and accompanying molecular weights, see Table 1 (22).

## Hemagglutination

Hemagglutination can be accomplished by one of two distinct hemagglutination factors, separable from each other by elution with DEAE cellulose (33, 45). Hemagglutination factor B acts without antiserum and hemagglutination factor C requires heterotypic antisera to hemagglutinate. Possibly the difference lies in the valences of the two factors, but the mechanisms are unknown (33, 45).

Hemagglutination is inhibited by homologous antisera. Specific antibodies probably attach to fibers, because only the fibers adsorb to erythrocytes (4, 34). Aggregates of two or more isolated pentons will agglutinate

erythrocytes by virtue of the hemagglutinin at the tip of the fibers. A single fiber cannot hemagglutinate, as it is univalent (7).

Species of erythrocyte used and temperature of hemagglutination can be varied and used to group certain adenovirus serotypes. This is discussed in the section on classification (33).

## Effect of Temperature and pH on Adenoviruses

Adenoviruses tend to be rather stable to temperature variations. Most types can withstand room temperature for as long as three weeks with no apparent decrease in virus titer (43). They are also stable at 4°C for long periods. At 37°C they are usually stable for at least a week, but at 56°C they are inactivated within 10-15 minutes (8, 37).

Adenoviruses are also stable over a fairly wide pH range with maximal stability occurring at around pH 6. The virus is inactivated slowly at an alkaline pH; at pH 7.5 and greater, inactivation occurs by seven days (8). The virus is stable in an acid pH as low as 3 for as long as 30 minutes (34, 37).

The adenovirus group is also stable to many freeze-thaw cycles (37). For a summary of the latter and temperature stabilities for certain representative adenoviruses, see Tables 2 and 3 (37).

Table 2.--Thermal Stability of Adenovirus Types 12, 14, and 18.

| Adenovirus Type | Infectivity Titer ($logs_{10}TCID_{50}$/0.1 ml) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 4°C months | | | 37°C days | | | 56°C minutes | | |
| | 0 | 3 | 6 | 0 | 10 | 20 | 0 | 4 | 8 |
| 12 | 3.0 | 3.5 | - | 3.1 | 1.7 | .6 | 3.4 | .6 | neg |
| 14 | 4.1 | 4.1 | 3.7 | 3.8 | 1.5 | .5 | 4.3 | 2.5 | .6 |
| 18 | 3.5 | 3.7 | - | - | - | - | 4.3 | - | neg |

Titrations performed in Hela cell cultures. Serial 10 fold dilutions were used, eight tubes per dilution, 0.1 ml inoculum per tube (37).

Table 3.--Effect of Repeated Freeze-Thawing on Adenovirus Types 12, 14, and 18.

| Adenovirus Type | Infectivity Titer ($logs_{10}TCID_{50}$/0.1 ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Freeze-Thaw Cycles | | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 | 30 |
| 12 | 3.4 | - | 3.4 | - | 3.0 | 2.5 | 3.2 |
| 14 | 4.5 | 4.5 | 4.5 | 4.5 | - | - | - |
| 18 | 3.0 | - | 3.5 | - | 3.2 | 3.5 | 3.5 |

Titrations performed in Hela cell cultures. Serial 10 fold dilutions were used, eight tubes per dilution, .1 ml inoculum per tube (37).

# Adenovirus Antigens

## Group Specific and Type Specific Antigens

All adenoviruses except the avian strains share a group specific soluble antigen identifiable by complement fixation (3, 4, 37). The group specific antigen is separable from the virus particle by ultracentrifugation, electrophoresis, and chromatography (4).

Individual serotypes can be differentiated by serum neutralization with homotypic immune antiserum of their type specific antigen. Some cross reaction between closely related types occurs, but it is not particularly common (34, 43).

## A, B, and C Antigens

Cells infected with adenovirus produce, in addition to virus particles, three noninfectious antigenic components designated A, B and C (4, 6). They are virus specific, smaller than the infective particle and separable from each other (4). Separation is performed by fraction elution from DEAE cellulose and agar gel double diffusion (45).

Protein A is a nucleoprotein, group specific and associated with the hexon capsomer (7, 13). Protein B is not a nucleoprotein; it is associated with the toxic or cytopathic component. Protein B is susceptible to trypsin and is mainly type specific. It may also be slightly group reactive, associated with the penton base (13, 33).

Protein C is a fiber associated nucleoprotein and is strictly type specific (13, 45).

### P Antigen

Another antigen, the P antigen, is similar to the T antigen (to be discussed in oncogenic infections) associated with tumors. It is complex: one of its components is found within the viral capsid. Arginine is necessary for the synthesis of the P antigen. The P antigen may have some function in viral maturation. However, the mechanism of its action is unknown (44).

## Replication Cycle

### Steps in Making a Complete Virion: 1 Cycle

There are ten steps in adenovirus replication (22). The first is adsorption or attachment. This involves the viral capsid attaching to and indenting the host cell membrane (45). The amount or rate of attachment is dependent in part on cell concentration (32). Researchers disagree widely on the time necessary for attachment, estimating anywhere from 30 minutes to five or six hours (14, 45).

Following adsorption, penetration occurs. By pinocytosis (or viropexis), the virion indented into the cell membrane enters the cell enclosed in a vesicle (45). This vesicle travels to and is disrupted at the nucleus.

How the virion actually enters the nucleus is unknown. It may enter by pinocytosis or through membrane pores (36).

The third step is uncoating. In this step, the viral DNA is released from its protein coat. The capsid is shed at least partly in the cytoplasm. This step is thought to be a loosening of the penton bond resulting in partial uncoating. Completion of the uncoating step occurs at or inside the nucleus and the DNA is dissociated from the core proteins (36). Very little is known about uncoating. It is considered a slow step (45).

At about three hours after the start of infection, early transcription of viral DNA to mRNA occurs (13). The mRNA travels to the cytoplasm and attaches to polyribosomes, there to begin the next step, early translation (13).

Early translation of mRNA to protein results in the formation of early viral proteins. These are thymidine kinase, deoxycytidylate deaminase, DNA polymerase, aspartate transcarbamylase, and tumor antigens (13). At least some of these are required for viral DNA synthesis (22).

At about six hours after the cycle starts, DNA synthesis begins (13). This occurs rapidly for 10-14 hours (13). Large quantities of viral DNA are producted, as much as double the host cells own DNA. Only 10% of this viral DNA becomes incorporated into mature virus (13, 45). Ongoing synthesis of viral DNA does not occur in association with the nuclear envelope (46); however, the initiation site may be

on the nuclear membrane (46). DNA synthesis occurs de novo from the nucleotide pool of the host cell (13). Host DNA is inactivated, but remains essentially intact (13). This step of DNA replication precedes viral maturation by about 6-10 hours (24, 45).

The seventh step is late transcription. Late mRNA is made from progeny viral DNA (13). Late translation of late mRNA to protein occurs in the cytoplasm about 2-3 hours before viral maturation begins. The proteins produced, i.e., the late proteins, include hexons, pentons and fibers (45).

Following synthesis of viral capsid proteins, condensation occurs. This involves the transfer of viral proteins from the cytoplasm to the nucleus (13). An assembly of all parts into complete virions occurs in the nucleus. It is here that the final step, viral maturation occurs (45). This is one complete viral replication cycle.

## Capsid Synthesis

The synthesis of the capsid proteins requires viral DNA synthesis (13). Hexons and fibers are made simultaneously, about two hours before mature progeny virus appears. The penton base is probably the last part made, appearing almost as the complete virion is made. Only about 5-10% of the synthesized viral structural proteins ever become part of a mature virion (45).

## Latent Period

The latent period in replication of adenovirus varies from 12-18 hours, according to serotype. The eclipse period does not seem to be shortened by high infective doses. The whole process seems less efficient at high doses. This cannot yet be explained, probably because the early replications stages are not well understood (14, 36).

## Rate of Viral Production and Viral Release

Maximum infection occurs at 28-40 hours and liberation of infective virions is slow and incomplete. The liberation mechanism is unknown (45). As the virus is released slowly, it spreads from cell to cell at an uneven rate. Virus production in the culture becomes asynchronous and a continuous production of new virus is observed (14).

The rate of production is relatively slow and it is not much influenced by virus:cell ratios, temperature, or electrolytes. The entire cycle is about 23-26 hours long. However, only 2-6% of the total mature virus formed is spontaneously released from the cells (14).

Figure 1 is an example of the cycle of type 2 adenovirus. It is fairly typical for the group (45).

In cell culture, the virus production reaches a plateau after maximum viral titer is reached. The virus at low titer is still present at 26 days (31).

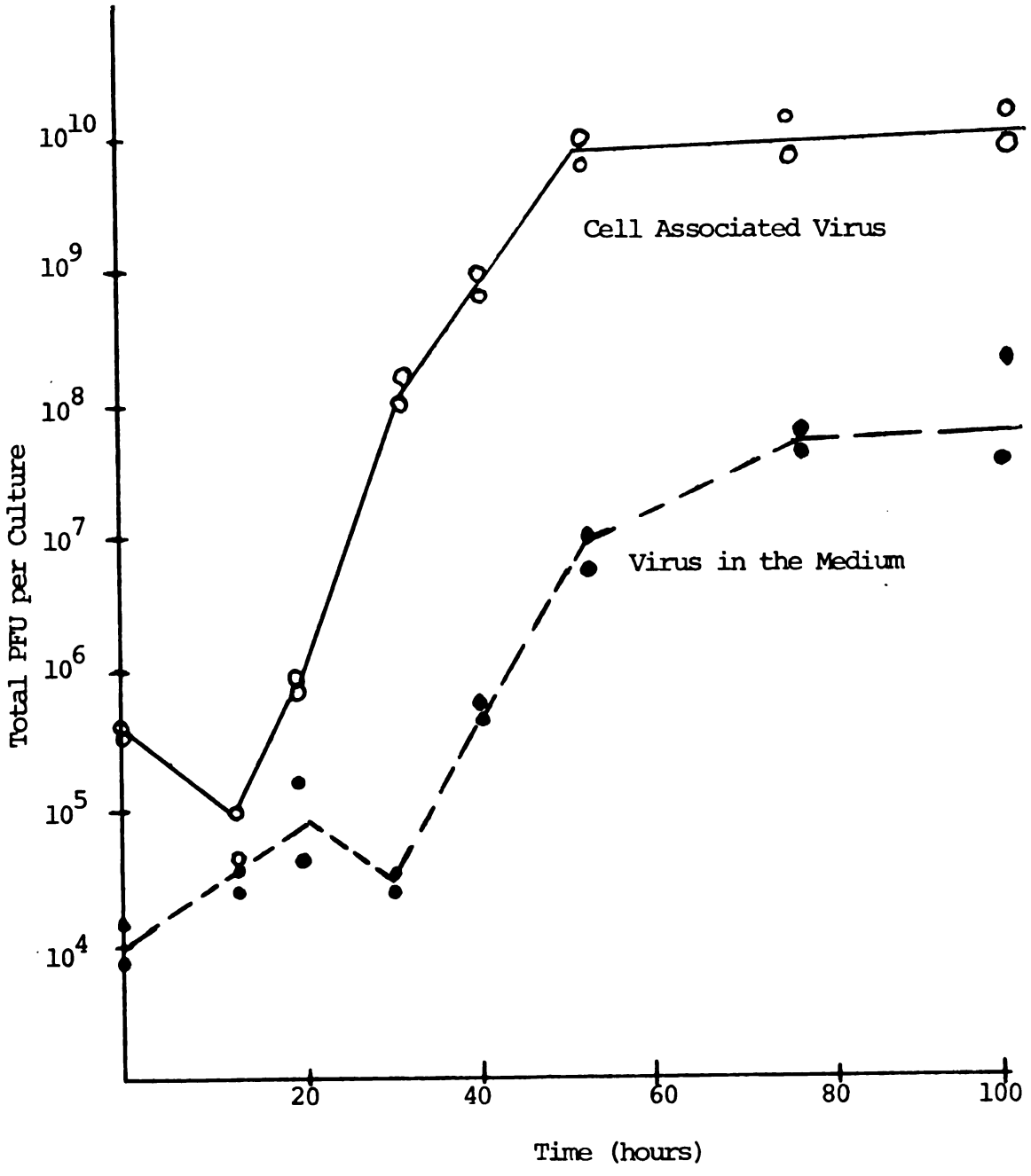


**Fig. 1.--Growth Curve of Type 2 Adenovirus in Monolayer Culutres of Human Tonsil Fibroblasts.**

### Arginine Requirement

Arginine is required for the production of complete infectious adenovirus particles. Omission of arginine completely inhibits the production of new infectious virus. If arginine is restored to the medium of arginine starved infected cells, viral maturation occurs immediately (40, 44). In the absence of arginine, limited quantities of all capsid subunits and early antigens are produced (44, 45). This is the only amino acid for which there is an absolute requirement (40).

The arginine requiring step has to do with the conversion of a P antigen from its early to its late form. When no arginine is present, the balls, rosettes and rings characteristic of late P antigen are not seen (45). The exact mechanisms and purposes of P are not known (45).

Cell cultures contaminated with mycoplasma are often resistant to adenoviruses. Mycoplasma deplete the arginine in culture and the adenovirus is not produced or is produced in very small quantities (40).

## Host Response to Adenovirus

### Characteristics of the Cell Culture Responses

Multiplication of adenoviruses is accompanied by progressive reduction and eventual stopping of cellular reproduction as shown by mitotic and total cell counts. Infected cultures show, in addition, marked metabolic changes

manifested by increased oxygen consumption, organic acid accumulation, and consequent lowering of pH (32).

The infected culture shows rounding and clumping which starts in focal areas and spreads. Within the aggregates, cell membranes remain intact and there is no evidence of syncytia formation (4).

## Cellular Level Response: Nuclear and Metabolic Processes

Productive infection causes profound changes in the host cell. Production of host cell DNA stops abruptly at 6-10 hours after infection. Host RNA and protein synthesis ceases 6-10 hours later and cell division stops. Marked cytologic changes in the nuclei of infected cells accompany the biochemical changes (13). Cellular level metabolic changes are summarized in Table 4 (13).

Intranuclear inclusions are found which differ from type to type. These inclusion bodies contain viral DNA and viral proteins (13).

Types 1, 2, 5, and 6 produce nuclear changes with eosinophilic inclusions and clusters of feulgen negative bodies. These clusters change, becoming clusters of feulgen positive granules. This is followed by nuclear enlargement, intense intranuclear vesiculation, and formation of irregular inclusions (4, 32).

Adenovirus types 3, 4, 7, and 14 cause a different type of effect. First, granular eosinophilic masses form

Table 4.--Metabolic Changes in Cell Cultures Infected With Adenovirus.

| Metabolic Change | Time After Infection |
|---|---|
| Cell Division Ceases | Occurs Immediately |
| Overall DNA and Protein Synthesis rates stay constant | For about 48 hours |
| RNA synthesis (overall) decreases | At about 24 hours |
| Mass/Cell doubles | By 24 hours |
| Total Macromolecular synthesis/ cell is 1.5-2 fold that of uninfected cells | At 24 hours |

and chromatin rearranges itself into a lattice pattern. Then a rarified zone develops beneath the nuclear membrane; the nucleus enlarges and becomes distorted; the clear zone widens, and the central areas become basophilic. Many small crystalline bodies appear in the infected nuclei varying from eosinophilic and feulgen negative to basophilic and feulgen positive. The crystalline inclusions increase in size. There appear to be mature virions in the array (4, 32). Differences between the types are not always clear cut.

Cellular Level Response:
Cytopathic Effect

There are two separate cytopathic effects attributable to adenovirus. The first effect, caused by a protein separable from the virion, leads to early detachment of the affected cells from glass surfaces (32, 45). The event is

a toxic reaction and the protein factor is called the cytopathic factor, cell detachment factor, or toxin (35).

At 3-4 hours after inoculation of the protein into cells, clumping and rounding occurs. Some of the cells leave the glass. If the factor is alone in the cell culture with no infectious adenovirus present, the cells will usually recover completely (4, 32).

The cytopathic factor is heat and ultraviolet stable, and is separable from the virus by centrifugation. Trypsin inactivates it and the effect is neutralizable by homologous antisera. The factor does not cause cell death and it is resistant to both DNAse and RNAse (4, 32).

The cytopathic factor is thought to be associated in some way with the base capsomer of the penton component. The precise mechanism of early cytopathic effect is unknown (45).

Late cytopathic effect is considered to be a manifestation of virus infectivity and consists mainly of nuclear alterations. The factor is resistant to trypsin digestion, sensitive to heat and ultraviolet, and is progressive and irreversible. The effect is probably due to the entire virion and the replication process, but the mechanisms have not been extensively studied (32).

Cellular Level Response:
Acid Production

Fluids from adenovirus infected cell cultures are more acidic than those from companion uninfected cultures.

This production accompanies the extensive adenovirus cytopathic effect. The increased organic acids are lactic, pyruvic, acetic and alpha ketobutyric acids. There is also increased use of glucose by the cells (11).

Increased glycolysis and accumulation of carboxylic acids may or may not be an inherent part of the viral synthesis. It may simply reflect cell injury resulting from the infection. Following this one step further, it may as easily be due to cell damage from nonviral noxious agents. The mechanism of increased glycolysis and accumulation of organic acids is unknown (11).

Cellular Level Response:
Response to Viral Subunits

Exposure of the cell to the fiber component of the adenovirus reduces the capacity of the cell to replicate related adenoviruses or other unrelated viruses. Exposure to fiber also inhibits DNA, RNA and protein synthesis in infected and uninfected cells. This effect is detectable at about 20 hours after inoculation. Similar effects are not seen with the hexon subunit (45).

Hexon protein can bind to cellular and viral DNA. Fiber and hexon each can inhibit DNA dependent RNA polymerase, and DNA polymerase in vitro in infected or uninfected cells (45).

# Classification

## To Classify as Adenoviridae

The most important characters are the size and shape of the virus as determined by electron microscopy. Approximately 70-75 nm, nonenveloped icosahedral virion with 252 capsomers is almost enough to place a virus in the adenovirus family (1, 17, 41).

Certain other criteria besides size and shape were recommended by a special study group of the Virus Subcommittee of the International Nomenclature Committee in 1965. These included the possession of DNA and the presence of at least one common adenovirus antigen (with the exception of avian adenoviruses) (50).

Some auxiliary characters add support to grouping a virus as an adenovirus. These include ether, chloroform, and trypsin resistance; nuclear inclusion body formation; inapparent infection in laboratory animals, and typical adenovirus cytopathic effect in cell culture (12, 17, 29). These characteristics are at times open to exception and equivocation. As a result, they should only be considered as supporting but not defining characteristics (17, 45).

## Adenovirus Subgroups

The adenovirus family is at present divided initially according to natural hosts; human adenoviruses, simian adenoviruses, bovine adenoviruses, canine adenoviruses, murine adenoviruses, porcine adenoviruses, ovine adenoviruses,

equine adenoviruses, and avian adenoviruses. Even as the family expands, this type of classification will probably prevail (44, 45).

Within a group isolated from a given species, identification can be made by specific viral neutralization tests. As they are isolated and identified, adenoviruses are given a serotype number, which then identifies that type specifically. Some of the adenovirus groups can also be separated at the serotype level by their hemagglutination ability. This is useful as it may give some idea of the relationships between certain serotypes (41).

Human adenoviruses can be grouped into the following fairly stable hemagglutination subgroups.

| Sub-Group | Serotype Number | Cell Species Hemagglutinated | |
|---|---|---|---|
| | | Rhesus Erythrocytes | Rat Erythrocytes |
| 1 | 3,7,11,14,16,21 20,25,28 | hemagglutination | no hemagglutination |
| 2 | 8,9,10,13,15,19, 22,23,24,26,27, 29,30 | no hemagglutination occasional positive | hemagglutination |
| 3 | 1,2,3,4,5,6,12, 18,31 | no hemagglutination | partial hemagglutination |

Simian adenoviruses are also classified into subgroups by hemagglutination, and in this way they can also be separated from human adenoviruses. Simian adenoviruses usually hemagglutinate guinea pig erythrocytes while human adenoviruses usually do not (38).

Simian adenoviruses can also be classified into three subgroups (38). Chimpanzees have a separate classification, but this is not well defined (38).

Adenoviruses from other animal species have not been studied in enough detail to be classified by HA (25, 26).

| Sub-Group | | Cell Species Hemagglutinated | | | | | |
|---|---|---|---|---|---|---|---|
| | | Rhesus | | Rat | | Guinea Pig | |
| | | 4°C | 37°C | 4°C | 37°C | 4°C | 37°C |
| 1 | M11(sv36) | + | + | | | | |
| 2 | M2(sv23),M3(sv32,27,39) M4(sv15),M6(sv17) M9(sv27,31) | + | - | + | + | + | - |
| 3 | M1(sv1),M5(sv11), M7(sv20),M8(25), M10(sv30,34,38) | - or partial | | | | | |

(+) = hemagglutination (-) = no hemagglutination

## Host Range

### In the Host Animal

Adenoviruses have been isolated from human and animal species, including chimpanzees, monkeys, dogs, cattle, mice, chickens, sheep and horses (12). Other animal species can be expected to yield adenoviruses, on the basis of serological evidence (4).

Any individual adenovirus tends to cause disease in or to infect only the species from which it was initially isolated. In other species, it may cause latent or abortive infections or rarely disease (41). For example, human adenoviruses injected into rabbits produce a latent infection with an antibody response but with no disease signs (32). Human denoviruses injected into dogs may cause an asymptomatic viremia (4).

In a few cases, human adenoviruses may cause disease in other species. For example, some human adenovirus serotypes can cause bronchopneumonia in piglets (23, 32) or tumors in hamsters (41).

The usual species specificity of human adenoviruses is also true for those serotypes isolated from animals. As with the human serotypes, there are a few exceptions. For example, canine heptatitis virus may infect and cause disease in foxes. Usually, if an adenovirus can proliferate in another species, it will produce a short symptomless infection (41).

## Habitats Within the Host Animal

Adenoviruses are found in many places in the animal body and its secretions. Isolates correlating species of animal with site of isolation are contained in Table 5 (34, 41). In any species, isolates can often be obtained from lymphatic tissues and the kidneys (34).

Table 5.--Adenovirus Isolates.

| Species | Site of Adenovirus Isolations | | | | |
|---|---|---|---|---|---|
| | Feces | Urine | Nose or Eyes | Liver | Central Nervous System |
| Bovine | + | | + | | |
| Canine | | + | + | + | + |
| Mouse | | + | | | |
| Simian | + | | + | | + |
| Chicken | + | | | + | |
| Human | + | | + | | |
| Equine | + | | + | | |
| Porcine | + | | | | |
| Ovine | + | | + | | |

## In Tissue Culture

Adenoviruses tend to multiply best in cells closely related taxonomically to their natural host. For example, human adenoviruses multiply to their highest titer in human cells, but when inoculated in high doses can produce

cytopathic effects and cause the formation of small amounts of infectious virus in a wide variety of cells. Human adenoviruses can grow reasonably well in monkey, rabbit, procine and some bovine cells (41).

As with human adenoviruses, simian adenoviruses prefer simian cell lines; bovine in bovine, and so on (41). All adenoviruses show epitheliotropism in tissue culture. Ciliated respiratory epithelial cell cultures, however, resist infection by adenovirus. It is thought that adenoviruses may "hide" or persist in some cell cultures asymptomatically for long periods of time (32).

## Species That Contain Adenoviruses

### Human

There were 33 human adenovirus serotypes isolated by 1969 and more can be expected to be found from time to time. The usual way to distinguish between serotypes is by serological procedures including hemagglutination. Human adenoviruses tend to grow best in primary human kidney tissue culture. Most of the research in the Adenoviridae has been done on members of the human adenovirus group (1).

### Simian

At least 18 serotypes of adenovirus have been isolated from monkeys. Here also, more serotypes will probably be added in the future. All simian adenoviruses grow best

in monkey kidney cells and share a common complement fixing antigen with members of the human group (1, 34).

A few of the strains are oncogenic and several have been associated with enteric and respiratory infections (34). Isolations have been made from kidney, tonsils, feces, monkey cage air, nasal and ocular secretions (34). Simian adenoviruses do not cross react with human adenoviruses by neutralization or hemagglutination inhibition (38). Aside from the human adenoviruses, simian adenoviruses are probably studied more frequently than any other group.

Bovine

Ten serotypes are known and all have the mammalian adenovirus antigen, but cross react with neither human nor simian adenoviruses by neutralization (5, 26). They grow well in bovine kidney tissue culture and are usually isolated from bovine respiratory secreations, the gastrointestinal tract, testes and kidney (25, 34). Type 3 is oncogenic in hamsters, and several other serotypes are associated with respiratory disease in calves (5, 25). Types 1, 2, and 3 have been the most studied of the group; the others are relatively unknown (26, 34).

Canine

There are two serotypes of canine adenovirus, each having the adenovirus group antigen. The canine adenoviruses grow well in dog, ferret, raccoon and pig kidneys (19).

Both are isolated from the urine and the respiratory tract (34).

Both canine adenoviruses are associated with diseases. Serotype II is associated with canine laryngotracheitis; while the other (serotype I) causes infectious canine hepatitis. The infectious canine hepatitis virus causes severe respiratory and hepatic disease, conjunctivitis, and fever. Dogs are routinely vaccinated against it (5).

Murine

There are probably two serotypes of murine adenoviruses neither of which has been extensively studied. These grow well in mouse embryo kidney tissue culture. They may cause disease in suckling mice, but are more likely to cause asymptomatic infections. Both have the mammalian adenovirus antigen. They are regularly isolated from various mouse tissues and urine (34).

Ovine

At least eight serotypes have been found in sheep. All of them have the common adenovirus antigen. They grow well in sheep kidney tissue and are usually isolated from the feces (1, 29, 30). Disease producing ability is unclear (32).

Euqine

At least one serotype of adenovirus has been found that infects horses. It has been associated with

respiratory disease in immuno deficient foals. Equine adenoviruses are best grown in equine embryo kidney cells (9).

## Avian

Avian adenoviruses are also known as the Gallus Adeno-like virus (GAL) (1). There may be as many as 40 strains, but little differentiation of isolates has been done as yet. They grow well in most chick embryonic tissues. Some strains are oncogenic, and some may be involved in respiratory disease. Isolates have been recovered from feces and embryo tissues (1, 34). There is no cross reaction with the mammalian adenovirus complement fixing group antigen (1). How the group is related to other adenovirus groups and how its members relate to one another is still unknown.

## Porcine

There are at least three serotypes of porcine adenoviruses, each of which shares the common adenovirus antigen. They grow well in pig or calf kidney cell cultures. Porcine adenoviruses have been isolated from the gastrointestinal tract of pigs and two of the serotypes are associated with respiratory infection in suckling pigs (23, 45).

## Adenoviruses in Other Species

There is serologic evidence of adenoviruses in other species, including goats, oxen, and deer (6). For example, of 50 goats tested for antibodies to the adenovirus common

antigen, 35 had specific antibodies, and four gave doubtful positive reactions (6). This suggests that at least some new adenovirus isolations can be expected to occur.

## Types of Infection

In the host animal, adenoviruses appear to produce four basic types of infections. These are disease-producing infections, latent infections, abortive infections and oncogenic infections.

## Disease Producing Infection

### Human Diseases

The most extensively studied adenoviruses have been those associated with human respiratory diseases. Definite virus-associated illness is now limited to about ten adenovirus serotypes. These types produce five major patterns of illness which are described in Table 6. The infections are self-limiting, usually followed by complete recovery and persistent type specific immunity (8, 16).

There are reports suggesting an etiological relationship between adenovirus infection and outbreaks of diarrhea. Adenovirus types 3 and 7 have been isolated from feces of such cases. However, as adenovirus types can be isolated from the feces of apparently healthy individuals as well, the adenovirus cannot yet be said to be the cause of these enteric disturbances. The isolations may be incidental (22).

Table 6.--Illnesses Associated with Human Adenovirus Infection.

| Disease | Occurrence | Serotypes Order of Association | | Respiratory Tract Involvement | | | | Constitutional Reaction | Other |
|---|---|---|---|---|---|---|---|---|---|
| | | Common | Rarer | Common Cold | Pharyngitis | Bronchitis | Pneumonia | | |
| 1. Acute Respiratory Disease | epidemic winter and summer in recruits | 4,7 | 3,14, 21 | often present | frequent with fever and laryngitis | frequent with fever and laryngitis | infrequent but occurs | headache malaise high fever | none |
| 2. Pharyngo-conjunctival fever | summer epidemics in civilians. Some sporadic. Associated with swimming pools. | 3,7 | 4,14 | often present | frequent; high fever and cervical lymph-adenopathy, hoarseness | uncommon | rare | headache malaise high fever | acute follicular conjunctivitis usually uni-lateral with preauricular lynph adeno-pathy |
| 3. Febrile pharyngitis | sporadic or epidemic; resembles #1. often in children | 3,7 | 1,2,5 | often present | most frequent often with fever | frequent especially in older children | infrequent but can be severe | high fever malaise headache | nausea vomiting diarrhea especially in children |
| 4. Pneumonia | highly fatal illness in infants; sporadic or epidemic | 3,7 | | occurs | very frequent | very frequent | primary with acidophilic necrosis of tracheal and bronchial mucosa resembling tissue culture cytopathic effect | high fever prostration | conjunctivitis skin rash diarrhea intussusception and central nervous system invasion in some cases |
| 5. Kerato-conjunctivitis (EKC) | Epidemic disease in shipyard workers; also spread from infected eye solutions | 8 | 11 | unusual | uncommon | not reported | not reported | usually afebrile | usually unilateral severe, acute conjunctivitis followed by corneal sub-epithelial kenatosis; preauricular lymphadenopathy |

Diagnosis

Diagnosis of human adenovirus infections is usually by serological means. All mammalian adenoviruses have a common soluble complement fixing antigen, which can be identified using hyperimmune serum (12, 13). Each adenovirus also has a type specific neutralizable antigen which specifies the adenovirus serotype. The two common methods of testing for this antigen are by hemagglutination inhibition and neutralization by acute and convalescent serum samples (13). Sera from a patient can be used in any of these tests for adeno antigen.

If viral isolation is desired, ocular secretions, feces, lymph tissue, or respiratory tract samples are inoculated into HeLa, KB or human embryonic kidney. Specific cytopathic effects are observed and the virus is identified serologically (13).

A relatively new method of diagnosis is by immune electron microscopy. This is a rapid sensitive technique enabling the direct observation of virus-antibody complexes. The technique, which will probably become more common as electron microscopy becomes more available, is as follows: (1) add .1 ml virus suspension (from lysed cells) to .1 ml antiserum, (2) incubate the mixture at 37°C for 1 hour, (3) mix 1 drop of the mixture with 1 drop of phosphotungstic acid, (4) place on grid and dry, (5) examine under the electron microscope. The time needed for identification by this method is 4-5 hours as opposed to a

minimum 10-12 hours for isolation. This method may prove useful in rapid diagnosis of epidemics in children or in military populations (27).

Vaccine

Two types of vaccines are available. These are polyvalent formalin treated vaccine and polyvalent live virus vaccine. Both are experimentally successful and have been used in military populations in which adenovirus caused illnesses are most common. The vaccines are not used in civilian populations where the frequency of serious adenovirus disease is low. It is important that the vaccine not be used unnecessarily as there is thought to be some oncogenic potential associated with some serotypes. The vaccine that has been used usually contains adenovirus types 3, 4, and 7 (4, 5, 13, 16).

Aside from its oncogenic potential, the vaccine is not usually used, due to difficulties in producing enough virus. It is also possible that any cells used may have a carrier virus contaminant which could be harmful to humans. As a result of these difficulties, the vaccine is used only in special circumstances. At the present time, work is being done on a viral components vaccine; however, it is not yet ready for use (13).

## Adenovirus Diseases in Nonhuman Mammals

Adenoviruses cause diseases in several animal species. The best known is infectious canine hepatitis. Human adenovirus types 1, 2, 5, and 6 can cause bronchopneumonia in young colostrum deprived pigs (23, 34).

Other species specific adenoviruses seem to cause generalzied respiratory diseases in susceptible members of their species. For example, an equine adenovirus is usually specific for horses. Susceptibility within a species appears to vary with the animal's age, physical condition, immune status and other unknown factors. Essentially, the animal respiratory diseases seem very similar to their human counterparts; however, less research on the diseases has been done in animals than in humans (7, 34). Individual animal adenovirus isolates are discussed in the sections on host range and classification.

## Latent Infections

Latent infections may be the most common type of adenovirus infection. In these persistent inapparent infections, overt disease is not produced, but the virus is not eradicated. This type of infection frequently occurs in the adenoids and tonsils (13). Fifty to 80 percent of the tonsils and adenoids removed surgically yielded an adenovirus when explants were cultured in vitro (13).

Human adenovirus types 1, 2, 5, and 6 are mainly associated with sporadic infections and are the types most frequently found latent in human adenoids and tonsils (4, 8). Types 3, 4, 7, and 14 are rarely latent. When an adenovirus is inoculated into a host animal for which it is not specific, the most likely infection (if any infection occurs at all) is latency (34). The mechanisms of latency are not yet known.

## Abortive Infection

Abortive infection consists of a single incomplete cycle from which no infective virus is produced. It occurs when an adenovirus is inoculated into nonpermissive host cells. The single incomplete cycle usually produces virus specific RNA, tumor antigen, increased thymidine kinase and some viral DNA (45). Viral structural proteins are not produced or are produced in minute amounts (7). Individual cells involved may be killed. The precise replication cycle deficiency is not yet known (45).

Several adenoviruses, at least, show this infection type in nonpermissive cells. For example, a number of human adenoviruses will only multiply is monkey cells that also house an Sv 40 genome (complete or incomplete) (45). In the absence of this helper, abortive infection occurs. The presence of the helper allows the translation of late adenovirus RNA (45). The helper function involves functional complementation and genome linkage, not recombination

or other genetic alterations (7). How common the abortive infection in tissue culture and the host animal may be is unknown. It is speculated that this is a very frequent event (45).

## Oncogenic Infection

The final basic pattern of adenovirus infection is tumor formation or oncogenesis. Certain adenovirus types, when injected subcutaneously into newborn hamsters, cause the formation of sarcomas (16, 38). This is true for several human derived adenovirus types, including types 3, 7, 11, 12, 14, 16, 18, 21, and 31, but probably more are involved (16).

Viral induced tumors can be cultured in vitro and they become continuous cell lines. Productively infecting adenoviruses do not grow well in these lines. For example, adenovirus type 2 can be induced to grow in this type of cell line, but with very low yields (48).

Primary tumor cell cultures are morphologically heterogenous, in cell type. They consist of fibroblast-like cells and polygonal cells. The fibroblasts tend to eventually die out. Remaining cells appear cuboidal or round; the cells seem undifferentiated. There are only small amounts of cytoplasm visible and contact inhibition is lost. The cells are sensitive to calcium and seem to prefer low levels (48). The rate of replication of tumor cells is about the same as for normal cells.

Tumor cell cultures tend to be hardy and viable, maintaining their malignancy for many passages (45). The cellular appearance and character seem to be determined more by the viral genome rather than by the target cell (48).

## T Antigens

Transformed adenovirus infected cells produce specific subgroup reactive T antigens. Specific transplantation antigens have also been demonstrated. The extent to which these antigens are produced is variable and has not been quantitated (48).

Various T antigens show some cross reactivity. This cross reactivity allows subdivision of the human adenoviruses into subgroups of different oncogenicity. These groups in general correspond with the subgroup relationships defined by hemagglutination properties (48).

## Human Adenovirus Groupings by Oncogenicity

The adenoviruses have been divided into three basic groups. These groups are the highly oncogenic, weakly oncogenic, and nononcogenic adenoviruses.

Highly oncogenic adenoviruses have the smallest DNA molecules (about 20 x $10^6$ daltons) of any of the adenoviruses. Their base composition (guanine + cytosine = 48-49%) is closest of all adenoviruses to that of their host DNA (41%). Nononcogenic adenoviruses have the highest

guanine + cytosine content (57-61%). They also have the largest DNA molecules (23-25 x $10^6$ daltons) (13, 45).

The similarity of highly oncogenic adenovirus DNA to its host DNA may be consistent with the findings that at least some part of the DNA of the oncogenic adenovirus is integrated into the host cell genome (13, 45). The significance of the similarity and amount of likeness needed to integrate are unknown.

DNA-DNA hybridization studies demonstrate that adenoviruses of each oncogenic subgroup share 70-100% of their nucleotide sequences. For example, the DNA's of the highly oncogenic adenoviruses hybridize with each other to a great extent, but cross hybridize to only a small degree with weakly oncogenic adenoviruses and even less with nononcogenic adenoviruses. However, all human adenoviruses share common genetic information encoded in 10-25% of their genome (37, 45).

## Evidence of Adenoviruses in Tumors

There is evidence of the presence of adenoviruses both in tumor tissue and in the host animal. Occasionally, animals bearing large virus induced or transplanted adenovirus induced tumors produce type specific antibodies which can react with fiber antigens or can neutralize a specific adenovirus serotype (45).

Large masses of tumor cells used as antigens in immuno-diffusion tests occasionally reveal the presence of hexon or fiber antigens, as well as an additional, still unidentified D component. Density gradient centrifugation of concentrated tumor extracts has been reported to yield a band of incompletely formed capsid like particles; however, no viral capsid antigens have been detected (45).

Tumors or transformed cells induced by adenoviruses produce rapidly labeled RNA species which specifically hybridize with the denatured DNA's of the virus that caused the tumor in question (45). This is evidence for the maintenance and transcription of viral genetic information in the transformed cells. One half of the early viral RNA from normal adenovirus infected cells corresponds to the RNA taken from tumor cells. It probably codes for the transplantation and T antigens, among other things (45). So, while there is evidence of the presence of adenoviruses in tumors in vivo and in vitro, most of the detail about the infection is unknown. This area is probably the most intensely studied at the present time of all adenovirus studies.

## Summary of Adenovirus Tumorgenesis

What is necessary for a tumorigenic relationship between the host and the adenovirus? First, the cell must survive the encounter with the adenovirus. Secondly, the virus must be an oncogenic adenovirus. Thirdly, the

adenovirus must incorporate a part or all of its viral genome stably into the cell. This viral genome must transmit vertically to daughter cells. Lastly, there must be some expression of the viral genome functions which results in changing normal cells to tumor cells (45).

## Adeno Associated Viruses

Many adenoviruses, when isolated and observed under the electron microscope, have satellite viruses accompanying them (9, 38). These viruses, called adeno associated viruses, are morphologically, antigenically and biologically distinct entities, and belong to the Parvoviridae (9, 47). They are 18-20 nm in diameter and icosahedral (28). While adenoviruses have a density of 1.34 $g/cm^3$, adeno associated viruses band in cesium chloride with a density of 1.43 $g/cm^3$ (28). They contain double stranded DNA, protein, and have no envelope (28). Adeno associated viruses are ether, heat and detergent resistant (47).

Adeno associated viruses need adenovirus to replicate and in the absence of adenovirus, no infectious adeno associated virus is produced (38). Adeno associated virus infectivity potential is usually greater than adenovirus infectivity potential. Thus adenoviruses cannot be separated from adeno associated viruses by simple terminal dilution (47). Adeno associated viruses have been found associated with many types of adenoviruses and they appear to suppress adenovirus growth (38). In a dual infection,

adenovirus-adeno associated virus produce cytopathic effects more slowly than adenoviruses alone (47).

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# ARTICLE: CHARACTERIZATION OF A CAPRINE ADENOVIRUS

## SUMMARY

An adenovirus was isolated from an adult goat with acute conjunctivitis. This appears to be the first adenovirus isolate reported in the goat. The virus produced cytopathic changes including intranuclear inclusions in goat kidney, bovine fetal peritoneum, bovine fetal kidney, rabbit kidney and equine kidney cell cultures. It was resistant to ether, chloroform, pH 3 and was moderately resistant to heat. Negative contrast electron microscopy showed a particle diameter of about 75-78 nm. The virus did not agglutinate human type 0, rat, guinea pig, chicken, equine, procine, ovine or bovine erythrocytes. Inoculation of the agent into rats, mice and guinea pigs produced no disease, nor did it cause tumors in hamsters or goats. When inoculated into goats, the agent produced no clinical signs. However, the goats did respond with specific antibody production. Also, 45 of 50 serum samples collected from randomly selected healthy goats showed neutralizing antibody to the virus.

# INTRODUCTION

Adenoviruses cause respiratory infections and conjunctivitis in humans and in other animal species (3). As yet, isolations of adenoviruses from goats have not been reported. However, an early serologic study showed that 35 out of 50 goats tested had antibody to the common group specific complement-fixing adenovirus antigen. Four other goats gave dubious positive reactions (3). Consequently the isolation of an adenovirus from goats might be expected.

The purpose of this report is to describe the isolation and characterization of an adenovirus isolated from an adult goat with severe conjunctivitis.

## MATERIALS AND METHODS

### Viral Isolation

Conjunctival swabs from a goat with severe ocular lesions (Fig. 1) were processed for virus isolation as described elsewhere (8). Bovine fetal peritoneal (BFP) cells, grown in modified Eagles minimum essential medium (MEMEM) (8), were inoculated, incubated at 37 C and examined daily for cytopathic effect. Once isolated, the virus was routinely cultured in cell cultures of BFP, bovine fetal kidney (BFK) and goat kidney (GK). Methods for preparation of cells were essentially the same as described by Roberts and Carter (8).

### Preparation of Stock Virus

Cell cultures with second and third passage levels of virus showing a 2-3+ infection were frozen and thawed three times. The resulting suspension was centrifuged at a low speed to remove cellular debris and the supernatant was distributed to ampules in 1 ml amounts. This virus stock was frozen and stored at -70 C (8).

### Titration of Virus

All viral titrations were done by either microtitration (8) or by test tube systems. Readings for cytopathic

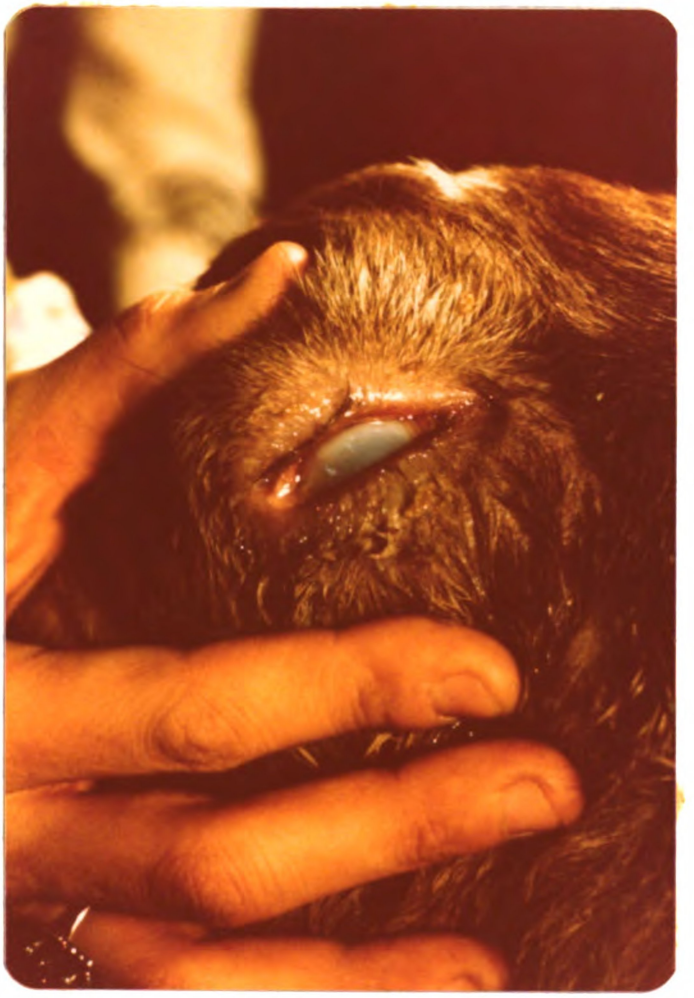

Fig. 1. Ocular lesions in an adult goat, characterized by edema, corneal opacity and swelling of the eyelids.

effect were performed on days 2 through 10. Titers ($TCID_{50}$) were calculated according to the method of Reed and Muench (7). Virus stock contained approximately 1000 $TCID_{50}$/ml.

Sensitivity to Ether

The method of Andrewes and Horstmann (1) was used. A mixture of 1 part anhydrous ether was added to 4 parts of virus stock. This mixture was shaken well at intervals over a 24 hour period at 4 C. The ether was then allowed to evaporate and the virus was titrated, with an untreated control. Comparatively, control viruses were bovine adenovirus type 3 and infectious bovine rhinotracheitis.

Sensitivity to Chloroform

The method of Feldman and Wang (4) was used. One part of chloroform was added to nine parts of virus stock. The tubes were shaken for 10 minutes at room temperature and centrifuged at 33g for 5 minutes. The uppermost clear layer was then titrated. The controls, handled in the same way, were the viruses used in the ether study. Untreated virus was also titrated.

pH Stability

The method of Ketler et al. was used (5). The virus stock was diluted 1/10 in MEMEM which had been acidified with .1 N HCL to pH levels 2, 3, and 4. These dilutions were held at room temperature for 0.5 and 1 hour. The virus

was then titrated. Virus diluted with nonacidified MEMEM and held at room temperature was used as a control.

Heat Stability

Virus stock was held at 4 C and at room temperature for 7 days and at 56 C for 3, 5, 7, 9, 11, 12, 13, 14, and 15 minutes. Each sample was then titrated. A freshly thawed sample of virus from stock was titrated as a control.

Electron Microscopy

The method used was essentially the same as described by Spradbrow (12). Viral size was determined by examining distilled water lysates of virus infected cells with the electron microscope. Infected cells showing obvious cytopathic effects were scraped from the glass surface. The cells were sedimented by low speed centrifugation and the supernatant was discarded. The cells were suspended in 5 volumes of sterile distilled water. One drop of this suspension was placed on a collodion coated grid and allowed to stand for 30 seconds. Excess fluid was drained by touching the grid corner to filter paper. One drop of 1% phosphotungstic acid was added to the grid and allowed to stand for 30 seconds. Excess fluid was removed with filter paper and the grid was examined with the electron microscope.

The method used for thin section electron microscopy was described in Roberts et al. (9). Thin sections of pelleted cell cultures were fixed in glutaraldehyde, embedded

in resin, and stained with a lead citrate-uranyl acetate double stain. They were then examined with the electron microscope.

Hemagglutination

The procedure used for hemagglutination was that described by Salk (11). Viral stock dilutions of 1/2, 1/4, 1/8, and 1/10 in phosphate buffered saline (pH 7.2) were used in the test. Human type 0 rat, guinea pig, chicken, porcine, ovine, equine, and bovine erythrocytes were tested. The red cells were washed in normal saline and diluted to 0.5%. One half ml of each viral dilution was added to 0.25 ml of each type of erythrocyte. The test volumes were then incubated at 4 C, 25 C, and 37 C. Controls consisted of 0.25 ml of each erythrocyte type mixed with either 0.5 ml of MEMEM or 0.5 ml of phosphate buffered saline. Hemagglutination tests were read by the pattern method (10).

Inclusion Bodies

The method used was described by Rovozzo and Burks (10). Cells were grown on coverslips and infected with virus. When a 2-3+ cytopathic effect was evident, coverslips were removed and stained with hematoxylin and eosin. The coverslips were then examined with the light microscope for cellular changes. Uninfected cells were used as a control.

Acridine Orange Stain

The cells were grown and handled as previously mentioned for hematoxylin and eosin staining. The procedure used was that described elsewhere (6, 10). Uninfected cells and cells infected with bovine adenovirus type 3 and Parainfluenza 3 virus were used as controls.

Laboratory Animal Inoculations

Six weanling hamsters were inoculated subcutaneously with 0.4 ml of undiluted stock virus. They were examined weekly for 2 months after which they were killed and autopsied.

Nine weanling mice, 6 rats, and 6 guinea pigs were also inoculated; separate groups of 3 mice, 2 rats and 2 guinea pigs were inoculated intraperitoneally, subcutaneously or intranasally and intraocularly with 0.1 ml undiluted virus. They were examined at least twice weekly post inoculation for 2 months, then killed and autopsied.

Goat Inoculations

Each of 3 adult goats was inoculated via one of the following routes: intraveneously, (IV), intraocularly (IO), and intranasally (IN). The inoculum was undiluted virus; the amounts were 2 ml IV, 1 ml IO, and 3 ml IN. All 3 were bled and checked for antibody by serum neutralization with the virus prior to inoculation. Post infection samples were also obtained and tested for seroconversion by serum neutralization.

Host Range Studies

Cell cultures of equine kidney, rabbit kidney, feline kidney, canine kidney and human origin (HeLa and Hep-2) were used. The medium used was MEMEM. Cells were infected with undiluted virus and examined daily for cytopathic effects. Three subcultures were performed. Following the third subculture, material was passed back into BFP cells. Fertile eggs were inoculated with 0.1 ml of virus by the allantoic and the chorioallantoic membrane routes. Fluids and membranes were harvested 5 days after infection and repassaged in BFP cells as described for cell culture.

Serologic Survey

Fifty serum samples were obtained from apparently healthy goats. All were tested for antibody to the virus by microtiter serum neutralization. The method used was described by Carbrey (2) except that the microtiter transfer plate method[1] was used.

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[1]Cooke Laboratory Products, Division of Dynatech Laboratories, Inc., 900 Slaters Lane, Alexandria, Va 22314.

# RESULTS

## Cytopathogenicity

On initial isolation in BFP cells, approximately 10 days were required before cytopathic effects were evident. The initial changes included small areas of rounded cells and vacuolation. On subsequent passages, the effect was evident at 24-72 hours with infected cells showing rounding, enlargement and vacuolation. The effect started with scattered individual cells and usually within 24 more hours spread to form focal areas (Fig. 2). Within 5-10 days, the effect covered the entire cell sheet, and cells detached from the glass.

## Host Cell Range

The virus grew readily in BFK and BFP cells. Replication in GK cells was slower, requiring 2-3 days longer to reach a 4+ cytopathic effect. The virus established a 4+ cytopathic effect in rabbit kidney cells in 5 days and a 2-3+ cytopathic effect in equine kidney cells in 6 days. No replication was observed in feline kidney, canine kidney, HeLa, or Hep-2 cells. No growth or physical changes were seen in fertile chicken eggs.

## Inclusion Bodies

The nuclei of infected cells showed eosinophilic and basophilic inclusions of various sizes and numbers (Fig. 3). The percentage of cells with inclusions varied depending on the cell type infected. Inclusions in the GK cells were more numerous, but smaller than those found in BFK and BFP cells.

## Ether and Chloroform Sensitivities

Neither ether nor chloroform had any effect on the replication capabilities of the virus. The titers of the treated virus and the untreated controls were eseentially the same (Table 1).

## pH Sensitivity

The virus was inactivated at pH 2. At pH 4, titers were normal, while at pH 3 growth was slightly affected. The titer of the virus was not consistently high enough to permit precise measurements (i.e., a significant 1 log difference).

## Thermal Stability

The virus was able to cause a 3+ cytopathic effect at a $TCID_{50}$ of about 10, even after 9 minutes at 56 C. After 12 minutes at 56 C, replication did occur, but at a slower rate; titers dropped about 1 log unit. After 13 minutes, inactivation was complete.

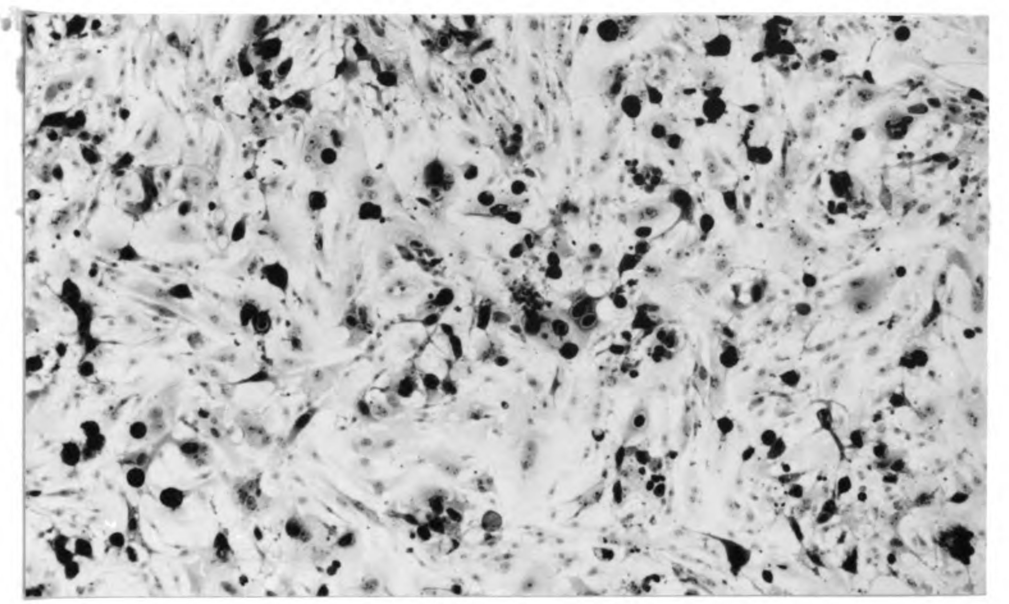

Fig. 2. Cytopathic effect caused by the virus in BFP cells. Hematoxylin and eosin stain; x 300.

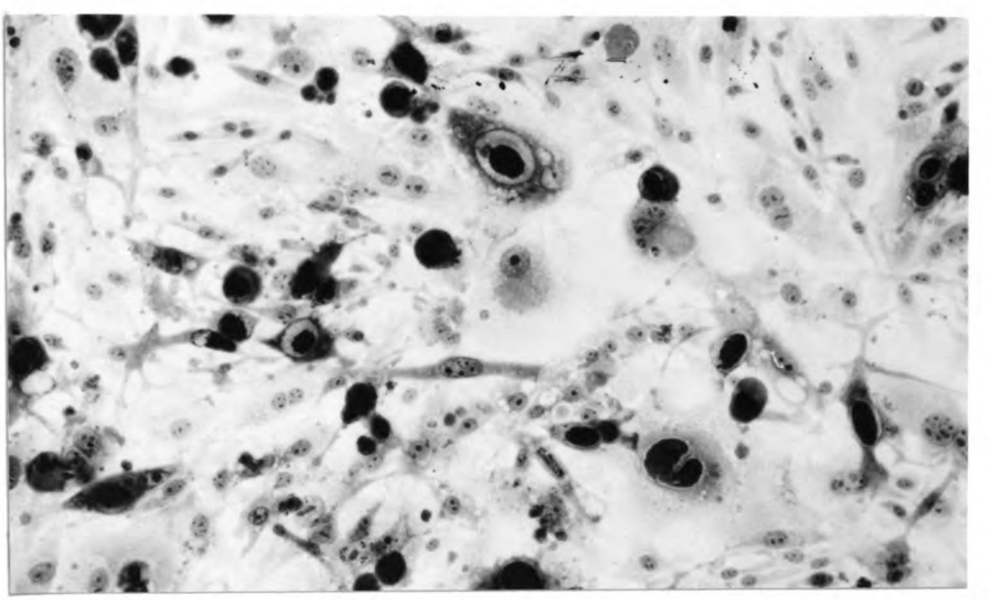

Fig. 3. Cytopathic effect caused by the virus in BFP cells showing enlarged nuclei and basophilic intranuclear inclusions. Hematoxylin and eoxin stain; x 625.

Table 1.--Ether and Chloroform Sensitivities.

| Treatment | Viral Titer($TCID_{50}$) |
|---|---|
| Ether | 5.3 |
| Chloroform | 5.6 |
| Untreated | 5.0 |

The virus could be maintained at room temperature or 4 C for at least a week with no loss of titer. The titer of the virus was not consistently high enough to permit precise measurements.

Electron Microscopy

Particles from cells lysates and thin sections were observed to be nonenveloped virions, icosahedral in shape, and approximately 75-78 nm in diameter (Figs. 4 and 5).

Hemagglutination

Human type 0, rat, guinea pig, chicken, ovine, porcine equine, and bovine erythrocytes were not agglutinated at 4 C, 25 C, or 37 C.

Acridine Orange Stain

Stained infected cells showed increased green nuclear fluorescence. The fluorescence appeared in clumps as opposed to the diffuse nuclear staining of the uninfected controls. The nuclear changes suggest that the viral isolate contains DNA.

Animal Inoculations

The virus did not cause signs of clinical illness or tumors in mice, rats, guinea pigs or hamsters.

The goat inoculated by the IV route exhibited an elevation in temperature at about 72 hours after inoculation. This returned to normal by 120 hours. Serological testing

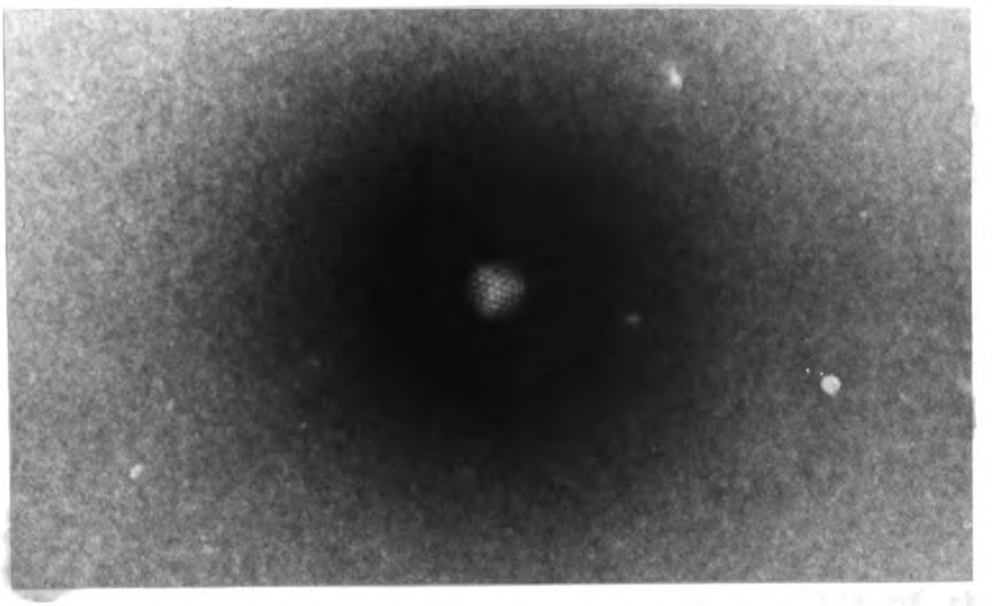

Fig. 4. Electron micrograph showing an adenovirus particle from a distilled water lysate. Notice the size and icosahedral shape of the virion. Phosphotungstic acid stain; x 100,200.

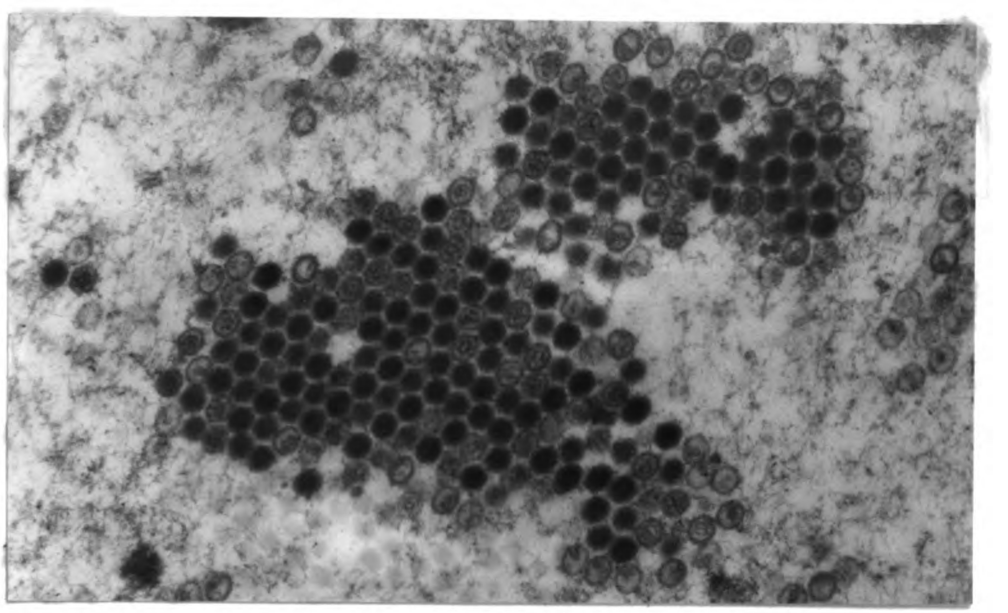

Fig. 5. Electron micrograph showing a thin section of a crystalline mass of adenovirus particles in an infected cell nucleus. Lead citrate uranyl acetate stain; x 60,000.

performed 4 weeks after infection showed specific virus neutralizing antibody with a titer of greater than 1:640.

The goats inoculated by the IO and IN routes showed no disease signs; serological testing at 9 weeks after infection demonstrated specific virus neutralizing antibody with a titer of greater than 1:640.

Serological Survey

The results of the serological survey of healthy goats are presented in Table 2. Approximately 90% of the goats tested showed neutralizing antibody.

Table 2.--Serological Evidence of Adenovirus in Goats.

| Number of Goats | Antibodies for Adenovirus |
|---|---|
| 45 | present (titer greater than 1:640) |
| 5 | absent |

# DISCUSSION

The virus described in this report was determined to be an adenovirus on the basis of physiochemical, cultural, and morphological characteristics. Whether it was responsible for the ocular lesions in the goat from which it was isolated is not clear. Circumstantial evidence suggests that the virus was the causative agent as bacterial pathogens and mycoplasma were not isolated from conjunctival swabs, and chlamydia were not demonstrated with special stains. However, we were unable to reproduce the disease. We were severely limited in this respect because only 3 of 16 goats available for purchase were free of antibody to the virus and only one of these was inoculated intraocularly.

Small sample size is only one possible explanation for our failure to reproduce the disease. The adenovirus may have been a latent organism activated by some other factor. As a result, it could be either a partial cause of the disease, or simply a nonpathogenic incidental isolate.

Antigenic comparisons with adenoviruses of other animal species will have to be done before this isolate can be considered to represent a distinct adenovirus of goats, and further pathogenesis studies are needed to determine its role in disease. However, the fact that it was isolated

from the eye in the absence of other pathogens, and that a high percentage of goats had serological evidence of exposure suggest that it should be considered as a possible cause of commonly occurring infectious conjunctivitis ("pink ege") in goats.

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