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Chapter 3

Adenoviruses of Vertebrate Animals

J. B. MCFERRAN

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I. INTRODUCTION

This chapter has been written to be complementary to the review of Taylor (1977). Information is given only where it has been derived from study with animal adenoviruses, particularly with respect to the basic properties of adenoviruses.

The first adenovirus isolated was the virus of infectious canine hepatitis (ICH) (Rubarth, 1947), although Cowdrey and Scott (1930) predicted that the intranuclear inclusions they saw in the hepatic and epithelial cells of two dogs with ICH would eventually be found to be due to a filterable agent. Green and Stillinger (1934) noted similar inclusions when they experimentally infected dogs with material from fox encephalitis. The first avian adenovirus was isolated when material from bovine lumpy skin disease was inoculated into latently infected embryonated eggs and a virus was isolated (Van den Ende *et al.*, 1949).

Human adenoviruses were isolated during investigations into respiratory disease (Rowe *et al.* 1953); (Hilleman and Werner, 1954). Huebner *et al.* (1954) recognized that these agents constituted a new virus group, and Rowe *et al.* (1955) suggested the name "adenoidal-pharyngeal-conjunctival (APC) viruses." However, the name "adenoviruses" was subsequently accepted as the generic name (Enders *et al.*, 1956).

The general properties required for classifying an isolate as an adenovirus were established by an international committee (Pereira *et al.*, 1963). The International Committee on Taxonomy of Viruses recognized the genus *Adenovirus* (Wildy, 1971), and a second report suggested the establishment of the family Adenoviridae with two genera, *Mastadenovirus* and *Aviadenovirus*, with human adenovirus type 2 and Celo virus as the type species (Norrby *et al.*, 1976).

II. DESCRIPTION OF THE VIRION

A. Morphology

The adenovirus virion is considered to be an icosahedral structure 70–90 nm in diameter, composed of 252 capsomers. There are 240 nonvertex capsomers (hexons) of diameter 8–9.5 nm and 12 vertex capsomers (penton base) of diameter 7–9 nm. The vertex capsomers carry projections called "fibers" (Norrby *et al.*, 1976). The virion has no envelope but does possess a core of 60–65 nm (Horne *et al.*, 1959).

All the accepted animal adenovirus species have been shown to possess at least most of these properties. Most isolates made at Stormont have fallen into the size range 74–80 nm (McFerran, unpublished observations). One exception has been the hemagglutinating virus (127) isolated from a depressed egg production syn-

drome. Although it has most of the properties of adenoviruses including size, the typical adenovirus structure of 20 triangular faces and 12 vertices is not resolved (Fig. 1).

There are few records of fiber lengths for animal adenoviruses. The fibers of the canine adenovirus, which causes infectious canine hepatitis (CI), are 25–27 nm long, while a second canine adenovirus associated with respiratory disease (A26/61) has fibers of 35–37 nm (Marusyk *et al.*, 1970). The mouse 1 (AL)

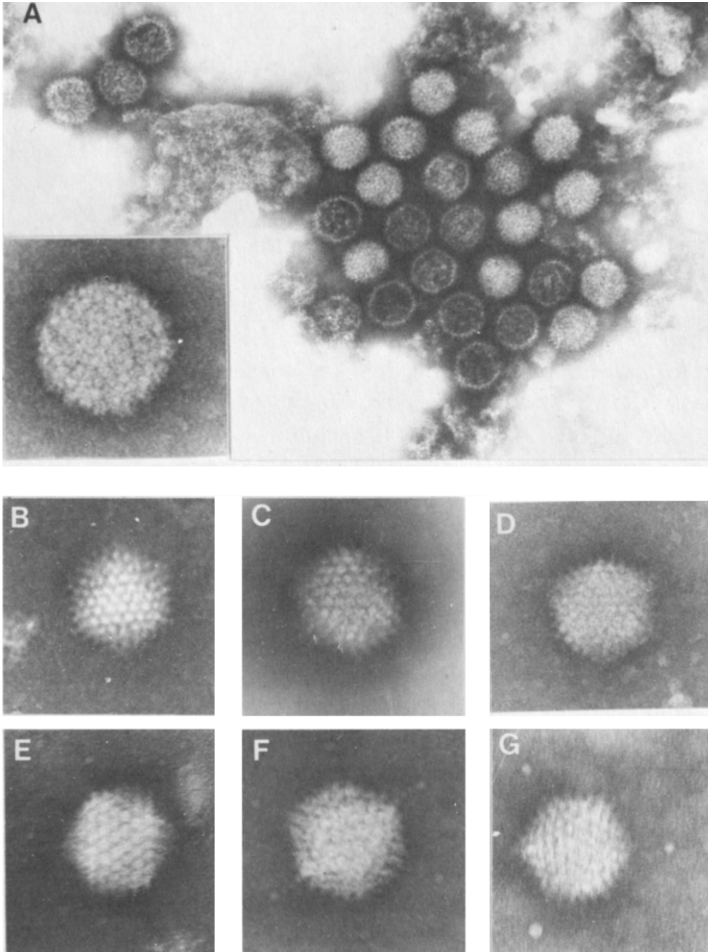


Fig. 1. Morphology of adenoviruses by negative staining with 4% phosphotungstic acid pH 7.2. (a) 127 virus. Note absence of triangular facets, even when capsomers are well resolved, as in inset, (b) ovine, (c) porcine, (d) canine, (e, f) fowl, and (g) turkey adenoviruses.

strain has a fiber length of 29 nm (Wigand *et al.*, 1977). Laver *et al.* (1971) reported two fibers on F1 (Celo), one of which was 42.5 nm and the other 8.5 nm. However, Norrby (1971) found that the fiber length of F1 was 14 nm, and El Mishad *et al.* (1975) demonstrated fibers of 17.5 nm on F1 particles. An F8 isolate has fibers approximately 27 nm long, an untyped isolate has fibers about 14 nm long, and the structures associated with a turkey adenovirus are 27–30 nm in length (Fig. 1). A hemagglutinating virus (127) has fibers 25–30 nm in length (Todd and McNulty, 1978).

B. Physicochemical Properties

The estimated molecular weight of the human and avian adenovirus virion is 173×10^6 (Schlesinger, 1969; Laver *et al.*, 1971). Although it is generally accepted that mature mammalian virion has a density of 1.33–1.34 gm/cm^3 and the avian virion that of 1.35 gm/ml in CsCl (Norrby *et al.*, 1976), there is little evidence that there are differences between mammalian and avaian viruses. Values for bovine adenoviruses are 1.25 gm/ml in sucrose density gradients for B5 (Coria *et al.*, 1975) and 1.34 gm/ml in CsCl for complete B3 virus (Niiyama *et al.*, 1975). Density values in CsCl of 1.29 (Konishi *et al.*, 1977), 1.31 (Ardans *et al.*, 1973), and 1.33 (Harden, 1974) to 1.34 (Dutta, 1975) have been recorded for the equine adenovirus. The dog adenovirus (C1) has a density of 1.35 gm/ml in CsCl (Carmichael, 1964), and a murine adenovirus has a density of 1.34 gm/ml (Wigand *et al.*, 1977). Densities between 1.32 and 1.37 gm/ml in CsCl have been estimated for fowl isolates (Laver *et al.*, 1971; Potter *et al.*, 1971; Yasue and Ishibashi, 1977; Todd and McNulty, 1978). A value of 1.32 gm/ml was obtained for a hemagglutinating adenovirus, 127, probably of duck origin (Todd and McNulty, 1978), and values of 1.32–1.34 gm/ml for the turkey hemorrhagic enteritis/marble spleen disease viruses (Carlson *et al.*, 1974; Iltis *et al.*, 1975). Differences in the buoyant densities of human adenoviruses have been demonstrated, and these have been attributed to differences in DNA content and base composition (Green and Pina, 1964).

The virions consist of 11.3 to 13.5% DNA, with the remainder protein (Schlesinger, 1969). However, one report suggests that one avian virus (F1) has 17.3% DNA (Laver *et al.*, 1971).

1. Viral Proteins

Between 10 and 13 polypeptides have been described for human adenoviruses (Maizel *et al.*, 1968; Anderson *et al.*, 1973; Everitt *et al.*, 1973), and at least 10 polypeptides have been resolved for B3 (Niiyama *et al.*, 1975). A study of six human, a simian, a canine, and an equine adenovirus demonstrated that there are major differences between the polypeptides of adenoviruses isolated from pri-

mates and nonprimates. However, there are also significant differences between the human strains at the molecular level (Marusyk and Cummings, 1978).

While one study of a fowl adenovirus (F1) demonstrated 5–6 polypeptides (Laver *et al.*, 1971), other workers resolved 11–14 polypeptides in F1 (Yasue and Ishibashi, 1977). Another avian adenovirus, 127, has 13 polypeptides, and 7 of these have counterparts in the 13 resolved polypeptides of F1 (Todd and McNulty, 1978).

2. *Viral DNA*

The DNA of F1 has been calculated at 30×10^6 daltons, compared to $23 - 10^6$ daltons for a human adenovirus (Green *et al.*, 1967; Laver *et al.*, 1971; Robinson *et al.*, 1973). The DNA of the B3 virion is a linear duplex, with a buoyant density in CsCl of 1.72 gm/ml (Niiyama *et al.*, 1975). Panigraphy *et al.* (1977) recorded buoyant densities of B1 to B3 as ranging from 1.71 to 1.72. There appears to be an identical structure for the DNA of F1 and mammalian adenoviruses. Thus the DNA was isolated as a circular DNA protein complex and visualized as a linear molecule with the ends joined by a protein (Robinson *et al.*, 1973). There was about 25% homology between the DNAs of B3 and H5 by filter hybridization (Niiyama *et al.*, 1975).

Burnett *et al.* (1972) divided simian adenoviruses into three groups on the basis of DNA homology and into two groups on the basis of base composition. The DNA of a mouse adenovirus has been described as a linear duplex of molecular weight 20×10^6. There was less than 10% nucleotide sequence homology between the mouse and human adenoviruses (Larsen and Nathans, 1977).

It has been possible to establish a correlation between the base composition, the guanine × cytosine (G–C), of the DNA, and the ability of human adenovirus serotypes to produce tumors in newborn hamsters (Pina and Green, 1965; Green, 1970). Thus highly oncogenic human adenoviruses (H12, H18, H31) have a G–C content of 48–49%, weakly oncogenic strains (e.g., H3, H7) 50–52%, and nononcogenic strains (e.g., H8, H9, H10) 57–61%. However, this pattern does not apply to the simian adenoviruses, in which oncogenic viruses have a G–C content of 55.1–61.6% (Table V). Thus the highly oncogenic strains SA7 and SV20 (Hull *et al.*, 1965) have a G–C content of 58–62% (Pina and Green, 1968; Burnett *et al.*, 1972). The highly oncogenic B3 (Darbyshire, 1966) from cattle has been described as having a G–C content of 58% (Niiyama *et al.*, 1975) and would therefore resemble the oncogenic simian viruses. However, Panigrahy *et al.* (1977) established the G–C content of B3 at 48% and that of the nononcogenic B1 and B2 at 61–62%, which would fit into the classification of human adenoviruses.

A value of 44% for the G–C content has been recorded for a mouse adenovirus, but oncogenicity has not been attributed to this isolate (Larsen and Nathans, 1977).

C. Antigenic Characteristics

1. Hemagglutination

a. Subgroup Classification. The human adenoviruses have been divided into subgroups on the basis of their ability to hemagglutinate rat and rhesus monkey red cells (Rosen, 1960). Hierholzer (1973) extended this work and divided human adenoviruses into 10 subgroups, based on their ability to hemagglutinate rhesus monkey, human, and rat erythrocytes at 37°C. Two patterns of hemagglutination were found, complete and partial. Partial hemagglutination could be made complete or more pronounced by adding heterotypic antiserum.

With the animal adenoviruses, hemagglutination has proved useful only in classifying simian adenoviruses. However, it is clear from the literature that a study comparable to Rosen's has not been undertaken for other species. It is important that the reaction be undertaken at least at 4°C and 37°C and erythrocytes tested for sensitivity, especially from rats and monkeys (Rosen, 1960; Rapoza, 1967). It may also be necessary to add normal rabbit serum to get good settling of the cells (Pereira and De Figueiredo, 1962). Readings should not be taken until the cells are fully settled. Thus Rapoza (1967) did not read his tests until approximately 5 hours, when the guinea pig or monkey cells had settled and titers were maximal. The purity of virus pools is important. Clearly, the presence of a free living parvovirus or other virus which hemagglutinates cells is undesirable. An adeno-associated parvovirus in a pool can reduce the titer of the adenovirus hemagglutinin (Rapoza, 1967).

b. Bovine Adenoviruses. Klein *et al.* (1959) found that B1 agglutinated rat erythrocytes poorly, but other workers (Inaba *et al.*, 1968) described the agglutination at 4°C of erythrocytes from a wide variety of species, including bovine and rat. Mohanty and Lillie (1965) also demonstrated the agglutination of bovine erythrocytes. Klein *et al.* (1960) found that rat and mouse cells were poorly agglutinated by B2, while Japanese workers described the agglutination of cells at 4°C from a number of species, including cattle and mouse (Inaba *et al.*, 1968). However, a B2 adenovirus isolated from sheep agglutinated rat but not eight other cell types (Belak and Palfi, 1974). Inaba *et al.* (1968) found that B3 did not agglutinate the cells tested, while Darbyshire *et al.* (1965) found very poor agglutination with vervet monkey and rat erythrocytes. B4 agglutinates only rat and rhesus monkey erythrocytes to low titers, while B6 failed to agglutinate any of the cells tested (Tanaka *et al.*, 1968; Cole, 1970). Coria *et al.* (1975) found that B5 agglutinated goat only at 4°C and rat cells at 4°, 25°, and 37°C. B7

agglutinated cells from seven mammalian species, and bovine cells were used in a hemagglutination (HI) test for B1, B2, and B7 (Inaba *et al.*, 1968). There appears to be no information on B8 and B9.

c. Ovine Adenoviruses. Of the seven potential ovine serotypes and one caprine serotype examined, only 01, which agglutinated some batches of rat cells poorly (McFerran *et al.*, 1969), and 07, which agglutinated fowl erythrocytes (Davies and Humphreys, 1977b), were found to have activity.

d. Porcine Adenoviruses. Of the five proposed pig adenovirus species, P5 does not appear to have been tested for hemagglutinin, and P2 and P3 did not agglutinate cells from a number of species (Clarke *et al.*, 1967). Kasza (1966) described the agglutination of rat and fowl erythrocytes by P4, and P1 agglutinated guinea pig, human, rat, monkey, and mouse cells (Haig *et al.*, 1964). However, as P1 was subsequently shown to be contaminated by a hemagglutinating pig parvovirus (Derbyshire *et al.*, 1975), its agglutinating activity has still to be determined. Of four unclassified Danish strains, three did not agglutinate the cells tested, while the remaining one agglutinated fowl and guinea pig erythrocytes (Rasmussan, 1969). A French strain was tested against a very wide range of erythrocytes and agglutinated, to very low titers, only patas and vervet monkey cells (Chappuis and Tektoff, 1975).

e. Equine Adenoviruses. Although only one equine serotype is recognized, considerable differences have been recorded in the activity of strains of this virus. Thus some workers found that horse erythrocytes agglutinated (England *et al.*, 1973; Wilks and Studdard, 1973; Harden, 1974; Konishi *et al.*, 1977), while others did not (Petzoldt and Schmidt, 1971; Dutta, 1975). England *et al.* (1973) observed hemagglutination of mouse cells, whereas others did not (Dutta, 1975; Konishi *et al.*, 1977). Rat erythrocytes are agglutinated to varying degrees (Todd, 1969; England *et al.*, 973; Harden, 1974; Konishi *et al.*, 1977; Moorthy and Spradbrow, 1978). Human erythrocytes also appear to have different sensitivities in different laboratories (Todd, 1969; England *et al.*, 1973; Dutta, 1975; Konishi *et al.*, 1977).

f. Canine Adenoviruses. ICH was reported by Fastier (1957) to agglutinate fowl but not human or rat erythrocytes, whereas Italian workers reported positive results with erythrocytes from a large number of species (Mantovani and Gramenzi, 1956). Epsmark and Salenstedt (1961), on the other hand, obtained agglutination only with human and rat cells and not with fowl. These findings are now generally accepted. Infectious canine laryngotracheitis (ICL) virus hemagglutinates human and rat cells to similar titers (Marusyk and Yamamoto, 1971).

The canine adenoviruses differ from the majority of human serotypes in that 80% of the hemagglutinating activity is associated with the intact or "empty" virus particles and the remainder with the soluble penton dimer structure (Marusyk and Yamamoto, 1971). These workers showed that fluorocarbon and ultrasonication quickly inactivated the hemagglutinating activity. This contrasts to many of

the human adenoviruses, with which fluorocarbon can be used for virus purification (Green and Pina, 1963), although H19 and H26 are inactivated by fluorocarbon (Wigand *et al.*, 1966). The ICH hemagglutinin–erythrocyte receptor complex appears to be more stable than human strains, since it is not dissociated either by lowering of the temperature or by the use of receptor-destroying enzyme (Simon, 1962; Norrby *et al.*, 1967; Marusyk and Yamamoto, 1971).

g. Simian Adenoviruses. It is possible to group monkey adenoviruses in a similar fashion to human adenoviruses on the basis of their power to agglutinate erythrocytes from three species (Table V). The main differences are that most strains of monkey adenoviruses agglutinate guinea pig and rhesus erythrocytes at 4°C but not at 37°C, (Rapoza, 1967). This can be contrasted to the human strains, of which only H8, H9, H10, and H19 hemagglutinate guinea pig cells, and with which rat and rhesus monkey cells are agglutinated at 37′C (Hierholzer, 1973). S4 will also agglutinate human, bovine, and ovine erythrocytes (Tyrrell *et al.*, 1960).

Hillis and Goodman (1969) divided the adenoviruses isolated from chimpanzees into three subgroups. Subgroup 1 agglutinated rhesus or vervet monkey erythrocytes; subgroup 2 hemagglutinated rat cells in the presence of heterotypic immune serum; and subgroup 3 did not agglutinate monkey, rat, guinea pig, or human cells. The subgroup 1 viruses include the C1 isolate. These isolates may have some relationship to human 16, but full cross HIs were not undertaken. The C1 isolate has been shown to be related by serum neutralization to H14 (Rowe *et al.*, 1956). The subgroup 2 viruses were divided into four strains: Y25, Y141, those viruses apparently identical to H5, and those, including C2, related to H2. Subgroup 3 had two unrelated strains, Y34 and Y37 (Hillis and Goodman, 1969). Four serologically distinct viruses, PAN5, 6, 7, and 9, were isolated from lymph nodes of chimpanzees.PAN6 hemagglutinated rhesus monkey cells, and they were not related to human or other simian viruses (Basnight *et al.*, 1971).

h. Murine Adenoviruses. No complete or partial hemagglutination has been recorded (Hartley and Rowe, 1960; Missal, 1969; Wigand *et al.*, 1977).

i. Avian Adenoviruses. F1 has been shown to hemagglutinate rat cells (Clemmer, 1964; Burke *et al.*, 1968). Anderson *et al.* (1971) demonstrated that peaks of hemagglutinating activity were recorded at densities of 1.32 and 1.34 gm/ml in association with bands of complete and incomplete particles. El Mishad *et al.* (1975) demonstrated that while rat erythrocytes were agglutinated at 4°C and 37°C, maximum titers were achieved between pH 6 and pH 9, with temperatures between 20°C and 45°C. The hemagglutinin was stable to trypsin, RNase, DNase, and neuraminidase. It was, however, inactivated after 15 minutes at 56°C, and 0.2% formaldehyde reduced its titer by eightfold. By agglutinating rat cells, the virus resembled the viruses of Rosen's human adenovirus group 2, but the fiber length of 17 nm would classify it in the human subgroup 3.

While strains of F1, including Phelps, EV89, and GAL3, did not agglutinate sheep cells (Clemmer, 1964; Burke *et al.*, 1968), F1 (Indiana C) did (Fadly and Winterfield, 1975). F1 did not hemagglutinate a wide range of cells, including rhesus monkey erythrocytes (Kraus, 1965; El Mishad *et al.*, 1975).

There has been little work reported for other avian serotypes, except the failure of eight Japanese serotypes to agglutinate fowl erythrocytes (Kawamura *et al.*, 1964). The turkey isolate (TA-1) failed to agglutinate a variety of cells (Scott and McFerran, 1972).

An adenovirus, 127, probably of duck origin, agglutinates fowl, duck, and turkey but not mammalian erythrocytes to high titers (McFerran *et al.*, 1978a; Adair *et al.*, 1979c). The soluble hemagglutinin banded at a density of 1.24 gm/ml in CsCl gradients and had one major and one minor polypeptide. These corresponded to virus polypeptides P2 and P3, respectively, and the molecular weights of these polypeptides (67,000 and 65,000) are in the range given for the fiber and penton base of human adenovirus (Wadell and Norrby, 1969). The purified soluble hemagglutinin was not inactivated by 30 minutes at 70°C or by 24 hours at 56°C but was totally destroyed by 30 minutes at 80°C. It was inactivated by trypsin, urea, and pyridine (Todd and McNulty, 1978).

2. *Group Antigen*

The mammalian adenoviruses have a soluble group antigen (Enders *et al.*, 1956). Thus hexon preparations of six human, three simian, and two canine adenoviruses showed cross reactivity in the complement fixation (CF) test (Norrby *et al.*, 1971). Exceptions to this have been the subgroup 2 bovine adenoviruses Table II). While the subgroup 1 viruses fully share a common antigen with human adenoviruses, the subgroup 2 viruses have only a weak relationship. The reaction is stronger when human antigen is reacted against bovine subtype 2 sera than with the reverse reaction (Bartha, 1969). Support for these findings has come from other workers (Tanaka *et al.*, 1968; Mohanty, 1971; Rondhuis, 1973; Mattson *et al.*, *1977). However, Inaba et al.* (1968) showed that there was as much sharing of antigens between human strains and B4 and B7 as there was between B4 and B7. B6 shared a common CF antigen with B1–B4 and P4 adenoviruses (Mayr *et al.*, 1970). B4 had a common line of identity with H2 in the immunodiffusion test (Cole, 1970), and a B5 isolate had the adenovirus group antigen (Coria *et al.*, 1975). Baczynski *et al.* (1974) used purified hexon antigens of B5 and B8 in the CF and fluorescent antibody CF tests against antisera to B4, 3, and 4–8 and demonstrated that both subgroups shared an antigen, but titers were two- to fourfold higher with the homologous system.

Although the ovine (McFerran *et al.*, 1971b; Sharp *et al.*, 1974; Bauer *et al.*, 1975), caprine (Gibbs *et al.*, 1977), porcine (Clarke *et al.*, 1967), and equine (Todd, 1969) strains have been reported to share the common group antigen, this is usually based on a nonquantitative immunodiffusion test. There were indica-

tions that not all ovine adenoviruses shared the group antigen to the same degree (McFerran *et al.*, 1971b). The canine adenoviruses have a one-way cross with human adenoviruses. Thus C1 antigen will detect CF antibody in human serum as efficiently as human adenovirus antigen, but human adenovirus antigens do not detect antibody in dog sera (Kapsenberg, 1959; Heller and Salenstedt, 1960; Carmichael and Barnes, 1961). The horse and canine adenoviruses have a cross relationship (Konishi *et al.*, 1977). The murine strains share a group antigen with human adenoviruses, but this is basically a one-way cross, with only antiserum to human adenovirus reacting with the mouse adenovirus (Hasimoto *et al.*, 1966; Wigand *et al.*, 1977).

The avian adenoviruses have a common group antigen, but this antigen is distinct from the mammalian group antigen (Kawamura *et al.*, 1964; McFerran *et al.*, 1975). The viruses of turkey hemorrhagic enteritis and pheasant marble spleen disease share a common antigen (Domermuth and Gross, 1975; Domermuth *et al.*, 1975; Iltis *et al.*, 1975). Opinions are divided as to whether they share an antigen with other avian viruses (Iltis *et al.*, 1977) or do not (Jakowski and Wyand, 1972; Silim *et al.*, 1978). The hemagglutinating adenovirus (127) associated with depressed egg production partially shares an antigen with other fowl adenoviruses (McFerran *et al.*, 1978b).

III. BIOLOGICAL FEATURES

A. Effect of Physical and Chemical Agents

It is generally recognized that the adenoviruses are resistant to lipid solvents and are rapidly inactivated by 56°C (Norrby *et al.*, 1976). They are acid stable (Andrewes *et al.*, 1978), and their heat stability is reduced by divalent ions (Wallis *et al.*, 1962). While the animal adenoviruses have all been shown to be resistant to lipjd solvents, there are differences in response to the other agents.

The equine adenoviruses vary in their stability to pH 3. Some workers have found them stable (Ardans *et al.*, 1973; Dutta, 1975), while others have found that pH 3 caused either complete inactivation or a marked fall in titer (Harden, 1974; Moorthy and Spradbrow, 1978). Harden (1974) found that pH 5 caused a 99% reduction in titer. The bovine and ovine strains tested were resistant to pH 3. Variation on pH sensitivity has been found in the same laboratory between two porcine strains (Chappuis and Tektoff, 1975).

The activity of equine adenoviruses was not removed by 30 minutes at 56°C, although the titer was reduced (England *et al.*, 1973; Harden, 1974). When divalent cations at 50°C were examined, one strain was unaffected (Dutta, 1975) and another destabilized (Harden, 1974). The bovine adenoviruses show consid-

erable variation in their response to heat, and this property is discussed under their classification. The ovine adenoviruses resist 56°C, and divalent ions increase their inactivation (McFerran *et al.*, 1971b; Bauer *et al.*, 1975). The ICL (A26/61) canine adenovirus is inactivated by 40 minutes at 56°C (Yamamoto, 1966). The fowl adenoviruses are apparently the most variable. At 56°C, F1 (U. Conn) was inactivated after 90 minutes (Petek *et al.*, 1963), while F1 (Phelps) was still viable (Yates and Fry, 1957). F1 (EV-89) was still infective after 22 hours (Burke *et al.*, 1959). The infectivity of F1 (93) was reduced by 30 minutes at 70°C but not removed until 80°C was reached (Clemmer, 1964), and F2 (65) was not completely inactivated by 40 minutes at 60°C (Cho, 1971). Strains tested in the same laboratory have shown variations in thermostability, suggesting that these variations cannot be ascribed just to differences in technique (Rosenberger *et al.*, 1974; Mustaffa-Babjee and Spradbrow, 1975). Fowl adenoviruses have been found to be destabilized by divalent cations (Petek *et al.*, 1963; Burke *et al.*, 1965; McFerran *et al.*, 1972), although other workers could not confirm this (Kawamura *et al.*, 1964). Some other variations could be due to technique, however. Thus 3–30 minutes at 56°C inactivated both mouse adenovirus types, and divalent ions had no effect (Hartley and Rowe, 1960; Hasimoto *et al.*, 1966; Missal, 1969). But Wigand *et al.* (1977) found that if cell culture supernatant was used, 30 minutes at 56°C inactivated the virus, whereas if the supernatant was diluted 1:20 in distilled water, the virus was thermostable. Yamamoto (1967) drew attention to the importance of pH when testing for the effects of cations on thermal stability.

B. Growth in Cell Culture

The human adenoviruses have been divided into two subgroups on the basis of their growth in cell culture (Boyer *et al.*, 1957, 1959). This not only aids in classification but also appears to extend to other properties. The members of subgroup A of human adenoviruses tend to cause sporadic outbreaks of disease and exist in occult form in the tonsils, while the members of subgroup B tend to cause epidemics and do not normally persist in the tonsils (Parker *et al.*, 1961). In addition, there appears to be a correlation between cytopathology subgroup and virus multiplication cycle (Ginsberg, 1958), neutralization reaction (Denny and Ginsberg, 1964), G–C content (Philipson and Pettersson, 1973), and hemagglutinating characteristics (Rosen, 1960). It has been possible to classify the animal adenoviruses studied into these human adenovirus subgroups (see Table I).

Electron microscopic studies on the development of human adenoviruses in cell cultures have been described (Martinez-Palomo *et al.*, 1967; Weber and Stich, 1969). Viral multiplication occurred in the nucleus of infected cells. Virus particles, some with electron-dense and some with electron-lucent cores, accu-

TABLE I
Classification of Adenoviruses Based on Their Cytophathology[a]

Stage of development	Subgroup A	Subgroup B
Early	Refractile pearl-like inclusions, each with a halo Fluorescent inclusions of different sizes, mainly in periphery	Several irregularly shaped granular eosinophilic areas Granular flourescent inclusions. Nuclear membrane often stained
Middle	Increasing granularity of background nucleoplasm, sometimes condensing into basophilic bodies. Basophilic inclusions develop and reticular networks seen in some nuclei. Nuclei enlarged. Fluorescent material throughout the nucleus, becoming concentrated into definite inclusions	Eosinophilic inclusions fill the nucleus. One or two large inclusions or several smaller inclusions, each with a halo and with granular basophilic material in spaces between. Nucleus enlarged. Flourescent material concentrated into several large bodies
Late	Further condensation, giving rise to central basophilic inclusions with surrounding halo. Nucleus contracted. Many nuclei with fluorescence only in peripheral area	One or more central basophilic inclusions in the nucleus. Halo present Nucleus filled with several large fluorescent bodies
Human[b]	H1, H2, H5, H6	H3, H4, H7
Bovine[c]	B4, B5, B6, B8	B1, B2, B3, B7, B9
Ovine[d]	06, 07	01, 02, 03, 04, 05
Porcine[e]	—	P1, P2, P3, P4
Canine[f]	C1	A26/61
Fowl[g]	F1, F2, F4, F5, F8	F3, F5, F6, F7, F9
Turkey[e]	—	T1, T2
Duck[h]	127	—

[a] Based on the classification suggested for human adenoviruses of Boyer *et al.* (1957, 1959).
[b] Boyer *et al.* (1957, 1959).
[c] Adair and Curran (1978–1979)
[d] Adair *et al.* (1979b).
[e] Adair (1976).
[f] Adair (1979).
[g] Adair (1978).
[h] Adair *et al.* (1979c).

mulated in the nucleus, sometimes forming crystalline lattices. Four types of inclusion, differing in density and morphology, were shown to be composed of viral proteins, some in association with viral DNA. Large protein paracrystals with a well-defined structure were described.

The morphogenesis, structure of virus particles, and virus-associated inclusions are similar in all the animal adenoviruses (Figs. 2 and 3) studied to those described for human adenoviruses. For further information, see for bovine Adair and Curran (1978–1979), for ovine Adair (1979b), for porcine Chandler (1965), for equine Shahrabadi *et al.* (1977), for canine Givan and Jezequel (1969) and Yamamoto (1969), for simian Fong *et al.* (1965, 1968), for murine Blailock *et al.* (1968), and for avian adenoviruses Maeda *et al.* (1967) and Adair *et al.* (1979a).

The cell growth of each species is considered in Section IV. In general, they grow best in cells from the homologous species, and some have a very limited range of cells in which they will grow. The turkey hemorrhagic enteritis marble spleen disease viruses are unique in that they have not so far been propagated in cell cultures, although a wide range has been tried (Carlson *et al.*, 1974; Iltis *et al.*, 1975; Domermuth and Gross, 1978).

C. Oncogenicity

1. Bovine Adenoviruses

Both B3 (Darbyshire, 1966; Gilden *et al.*, 1967; Nishibe *et al.*, 1970) and B8 (Rondhuis, 1973) produce tumors when inoculated into newborn Syrian hamsters *(Mesocricetus auratius)*. No oncogenicity has been shown for B1, B2, or B6 (Rondhuis, 1973; Panigraphy *et al.*, 1977). Mohanty (1971) could find no oncogenicity for serotypes 1–8, omitting B3. The conflicting results in these studies may be due to the use of different strains of B8.

Low doses of B3 produced a solid tumor arising from the mesenchymatous tissue. These tumors had a latent period of at least 1 month and occasionally produced metastases. High doses produced hemorrhagic cystic lesions with a latent period of less than 1 month (Darbyshire *et al.*, 1968).

B3 transformed a variety of hamster cells but not rat or mouse cells *in vitro* (Panigrahy *et al.*, 1976). B3 was nononcogenic in mice, despite the use of antilymphocytic serum (Darbyshire *et al.*, 1968; Panigrahy *et al.*, 1976).

Rondhuis (1973) found that B8 had a low oncogenic potential for hamsters. Thus although some tumors arose within a month, most developed 5 to 15 months after inoculation. These were of the fibrosarcoma type, and about 10% showed metastases.

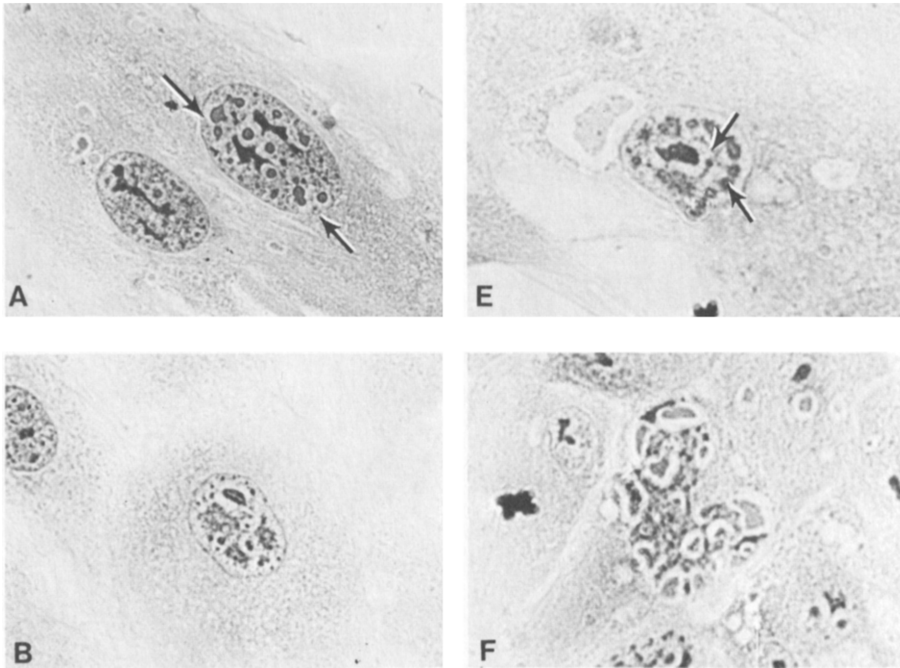


Fig. 2. Adenovirus cytopathology, subgroup A. (a) Early stage. Multiple circular pearl-like inclusions (arrows) are present in nuclei. (b) Middle stage. Background nucleoplasm has condensed to form basophilic inclusions with an irregular profile; nucleolus is placed in a peripheral position. (c) Late stage. Central nuclear basophilic inclusion, surrounded by a halo. H&E. (d) Late stage. Antigen is limited to the peripheral nuclear area. FA staining. Subgroup B. (e) Early stage. Multiple, irregularly shaped nuclear inclusions (arrows). (f) Middle stage. Deformed nucleus containing irregular eosinophilic inclusions, each surrounded by a clear zone. Granular basophilic material fills spaces between them. (g) Late stage. Nucleus containing several basophilic inclusions. H&E. (h) Late stage. Several large nuclear inclusions, stained predominantly at their periphery. FA staining.

2. Ovine, Porcine, Equine, and Murine Adenoviruses

Serotypes of these species do not appear to have been tested for oncogenicity.

3. Caprine Adenoviruses

The isolate 435 did not produce tumors after 15 months (Gibbs *et al.*, 1977).

4. Canine Adenoviruses

The viruses ICH and ICL both produced tumors in hamsters (Sarma *et al.*, 1967; Dulac *et al.*, 1970). Both ICH and ICL have low oncogenicity (Sarma *et al.*, 1967; Kinjo *et al.*, 1968). Virus was not recovered from primary and

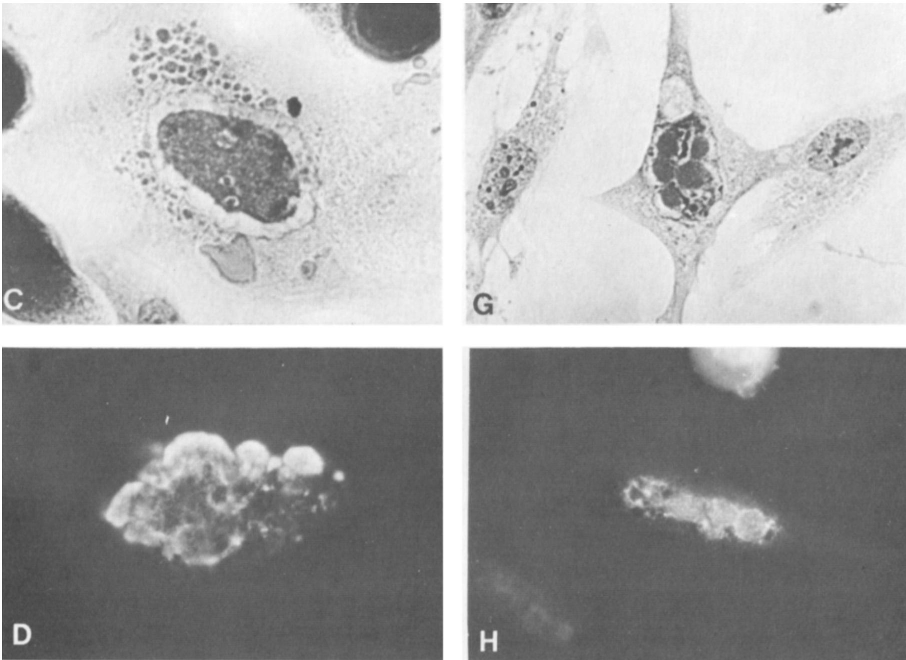


Fig. 2. Continued

transplanted tumors, but antibody to tumor antigen and to the cell culture T antigen of ICH was demonstrated in the sera of tumor-bearing hamsters (Sarma *et al.*, 1967; Kinjo *et al.*, 1968). The ICL virus produced undifferentiated sarcomas following 187 to 273 days of incubation. These tumors were also transplantable, and the hamsters developed antibody to T antigen. Sera from hamsters with tumors due to virus generally had two to four times higher titers to ICL than to ICH T antigens (Dulac *et al.*, 1970).

5. Simian Adenoviruses

Oncogenicity in hamsters has been established for the following monkey serotypes: S1 (Huebner *et al.*, 1962), S5, S11, S12, S14, S15, S16 (Hull *et al.*, 1965), S2, S6, S7 (Gilden *et al.*, 1968), and S8 (Slifkin *et al.*, 1968). These strains have differing degrees of oncogenicity. Thus S16 produced tumors in all animals inoculated after 41 days, whereas S11 produced only one tumor after 229 days. In a comparative study, Burnett *et al.* (1972) found S5 and S16 the most oncogenic and were unable to confirm the oncogenicity of S6 or S14. Strain S16 will also produce tumors in suckling rats (Hull *et al.*, 1965). Monkey adenovirus-induced tumors rarely metastasize unless virus is inoculated intraperitoneally. The tumors are usually invasive and poorly differentiated. Types S5, S8,

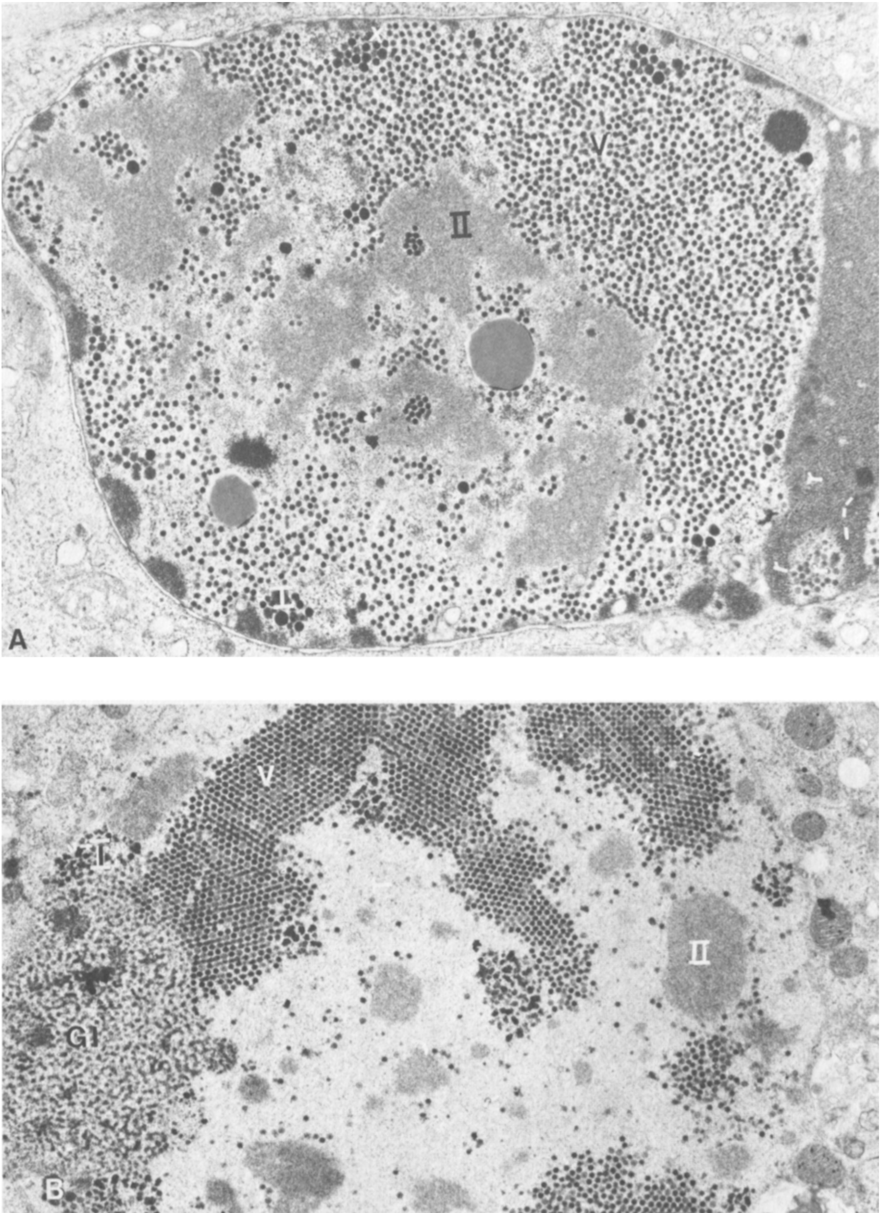


Fig. 3. Thin section of nuclei infected with (a) bovine adenovirus type 1 and (b) fowl adenovirus type 1. Note type 1 (I), type 2 (II), granular inclusion (GI) material, and virus particles (V).

and S16 induced tumors resembling lymphomas, while the other strains produce palisading, pseudoaciner, and spindle cell patterns (Merkow and Slifkin, 1973).

6. *Avian Adenoviruses*

F1 will produce fibrosarcomas (Sarma *et al.*, 1965), hepatomas (Anderson *et al.*, 1971), ependymonas (Mancini *et al.*, 1969), and adenocarcinomas (Stenback *et al.*, 1973). *In vitro* transformations of human and hamster cells have been reported (Anderson *et al.*, 1969a,b).

Attempts to demonstrate oncogenicity with other avian serotypes have been unsuccessful (Sarma *et al.*, 1965; Fadly *et al.*, 1976).

D. Adenovirus-associated Viruses

There are four adenovirus-associated viruses (AAV) which have been associated with primates. Of these two, AAV1 and AAV4, are possibly of simian origin (Rapoza and Atchinson, 1967; Parks *et al.*, 1970).

AAV have been recognized in bovine (Fig. 4a) (Luchsinger *et al.*, 1970), ovine (Clarke *et al.*, 1979), equine (Dutta, 1975), canine (Sugimura and Yanagawa, 1968), fowl (Dutta and Pomeroy, 1967; McFerran *et al.*, 1971a), and turkey (Scott and McFerran, 1972) adenovirus strains. AAV4 agglutinates human erythrocytes at 4°C (Ito and Mayor, 1968). The bovine AAV agglutinates sheep, human, guinea pig, and mouse cells at 4°C and 22°C, and horse cells only at 4°C (Luchsinger *et al.*, 1970). The ovine strain agglutinates guinea pig and human erythrocytes at 4°C and guinea pig cells at 22°C (Clarke *et al.*, 1979).

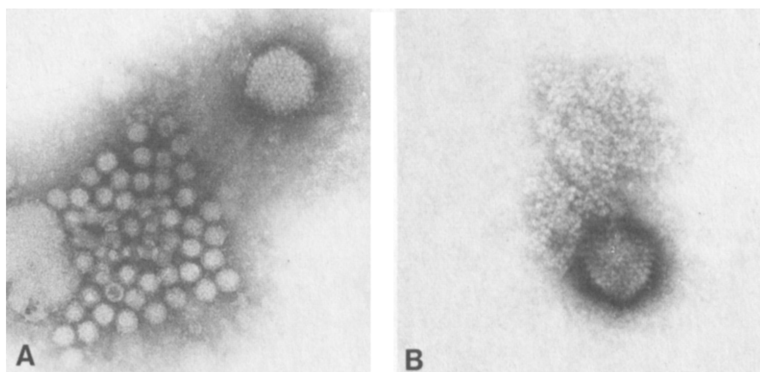


Fig. 4. (a) Bovine adenovirus and adeno-associated (parvo) virus. (b) Fowl adenovirus and an accumulation of disrupted capsid material. The latter can be distinguished from adeno-associated virus by virtue of its smaller size.

Avian adenoviruses are distinct from mammalian AAV types (Yates *et al.*, 1973; El Mishad *et al.*, 1975). Although many stock cultures of fowl adenoviruses have AAV present (El Mishad *et al.*, 1975), this may be due to AAV infection in the parent flock supplying the eggs for cell culture. Immuno-electron microscopy examination of both field isolates and stock pools has shown relatively low levels of infection with AAV (McFerran, unpublished observations), and Yates *et al.* (1976) found that only 2 of 38 adenovirus isolates were infected with AAV.

AAV can reduce both pathogenicity (Pronovost *et al.*, 1978) and oncogenicity (Kirschstein *et al.*, 1968), as well as reducing the replication of adenoviruses in cell cultures (Hoggan *et al.*, 1966).

IV. INFECTION

A. Infection in Cattle

1. Classification

a. Groups. Nine bovine adenovirus serotypes are recognized on the basis of cross neutralization tests (Bartha *et al.*, 1970; Guenov *et al.*, 1971; Mohanty, 1971; Adair and McFerran, 1976) (Table II). A tenth serotype has been proposed but not yet accepted (Kretzschmar, 1973).

b. Subgroups. Bartha (1969) suggested that the bovine adenoviruses could be divided into two groups. Subgroup 1 viruses are inactivated by 56°C for 30 minutes, replicate in bovine testicular and kidney cells, are readily isolated on first passage in cell culture, have single, irregularly shaped inclusion bodies, and share an antigen with human adenoviruses. Subgroup 2 viruses are reduced in titer but not inactivated by 56°C for 30 minutes, replicate only in bovine testes cells, form multiple regular inclusion bodies, and are difficult to adapt to cell culture. There is only a partial sharing of an antigen with human adenoviruses.

Bartha and Kisary (1970) modified this classification, as they found that resistance at 56°C could be modified by passage. Thus the prototype strains of B1 and B2 were still themolabile after 10 passages but thermolabile after 60 passages. One B3 strain was reduced in titer but not inactivated by 56°C (Mattson, 1973), while another B3 isolate was virtually inactivated (Lehmkuhl *et al.*, 1975). B4 and B6 strains were either inactivated or markedly reduce in titer by 30 minutes at 56°C (Tanaka *et al.*, 1968; Cole, 1970; Coria *et al.*, 1975), whereas although B7 had a 99% fall in titer after 30 minutes at 56°C, 2000 $TCID_{50}$ was still present after 120 minutes. Clearly, more work is required to establish if this is a reasonable parameter for classification.

The serological relationship between the proposed subgroups 1 and 2 and human adenoviruses is discussed in Section IV. It is clear that although differences

TABLE II
Bovine Adenoviruses

Serotype	Strain	Erythrocytes agglutinated	Cytopathology subgroup[b]	Bartha subgroup[c]	Original reference
1	B-10	Wide variety	B	1	Klein *et al.* (1959)
2	B-19	Wide variety	B	1	Klein *et al.* (1960)
3	WBR-1	Rat[a] and vervet monkey[a]	B	1	Darbyshire *et al.* (1965)
4	THT/62	Rat[a] and rhesus monkey[a]	A	2	Bartha and Aldassy (1970)
5	B4/65	Goat and rat	A	2	Bartha *et al.* (1970)
6	671130	None	A	2	Rondhuis (1968)
7	Fukuroi	Wide variety	B	2	Inaba *et al.* (1968)
8	Misk/67	N.I.[d]	A	2	Bartha and Aldassy (1970)
9	Sofia-4	N.I.[d]	B	1	Guenov *et al.* (1968)

[a] To low titers only.
[b] See text. Based on Adair and Curran (1978–1979).
[c] Based on Bartha (1969).
[d] N.I., no information available.

do exist between bovine subgroups 1 and 2, they may also exist between species within these subgroups and between adenoviruses of different species. Therefore, more work is required to establish the true situation.

Phillip and Sands (1972) showed that B4 and B7 had inclusions which were numerous single irregular and multiple round types corresponding to both of Bartha's subgroups. However, Adair and Curran (1978–1979) have shown that there is reasonable agreement between the pattern of growth in cell culture and Bartha's subgroups. Thus B1–3 and B9 fell into group B, and B4–6 and B8 fell into group A. B7, however, is in Bartha's subgroup 2 but falls into cytopathology group B. A number of workers have confirmed that subgroup 2 viruses do not replicate in bovine kidney (BK) cells (Bartha and Aldasy, 1966; Tanaka *et al.*, 1968; Cole, 1970; Phillip and Sands, 1972). It appears that these viruses grow in the epithelial cells of bovine testes (BT) cultures but not in the epithelial cells of BK (Aldasy *et al.*, 1965; Mattson *et al.*, 1977; Sibalin *et al.*, 1978). However, other workers have found that B4 (THT), B5 (B4/65), B6 (671130), B6 (KC-2), B7 (Fukuroi), and B8 (Misk/67) all grow as well in (BK) as in (BT) cells. Other strains of species B4 (KC-6), B5 (BIL), and B6 (RG) all required one to two blind passages in bovine embryonic corneal (BEC) cells before producing a cytopathic effect (CPE), and B4 (Negano) could not be adapted to BK cells (Mohanty, 1971). Although in general the subgroup 2 viruses conform to Bartha's classification in that they are difficult to isolate in cell cultures and grow to low titers (Cole, 1970; Mohanty, 1971), others have recorded high titers (Tanaka *et al.*, 1968; Stauber *et al.*, 1976). Furthermore, the subgroup 1 viruses can also be difficult to isolate and can grow to very low titers (Klein, 1962; Darbyshire *et al.*, 1965, 1969; Lehmkuhl *et al.*, 1975).

It would appear, therefore, that the two subgroups proposed for bovine adenoviruses are, at best, only a guide to classification.

2. *Distribution*

Bovine adenoviruses are widespread. Antibody studies have shown that between 25 and 87% of sera examined possess antibody to one or more types (Klein *et al.*, 1959; Tanaka *et al.*, 1968; Rondhuis, 1970; Phillip and Sands, 1972; Rossi *et al.*, 1973; Mattson *et al.*, 1977).

3. *Epizootiology and Pathogenesis*

A number of authors have shown that at least some strains are found more often in the oronasal secretions and in conjunctival swabs than in feces. Virus has not been detected in the feces in B3-infected animals (Mattson, 1973; Mattson *et al.*, 1977), while other isolates were made from the feces (Klein *et al.*, 1959, 1960). Following intratracheal inoculation of B3, there were more isolates from the nose (40) and conjunctiva (25) than from the feces (14) (Darbyshire *et al.*, 1966). Using B4, Aldasy *et al.* (1965) made more isolations from the feces than

nasal swabs, but with a high recovery from the conjunctiva. Cole (1971) found that following intratracheal inoculation, B4 was excreted in nasal secretions and in the feces from the fifth to the eleventh day after infection. The urine may well be a source of infection (Belak *et al.*, 1977) and thus may be an important factor in intensive-production units, where food can be contaminated with urine or urine drinking occurs.

It is evident from the large number of adenoviruses isolated from all cultures derived from fetuses that transplacental infection occurs. The existence of such carriers may be very important in the epizootiology. Mattson (1973) suggested that latent infection was reactivated in the dam at calving and showed that calves should be infected in spite of maternally derived antibody.

Cole (1971) studied the distribution of B4 (BIL) and found virus in the respiratory tract and drainage lymph nodes from days 1–12 postinfection (PI), with maximum dissemination in visceral organs from days 5–7 PI, when virus was found in the liver. But as neither histological nor fluorescent antibody (FA) examination showed evidence of virus growth, the virus in the liver may be in macrophages.

4. Association with Disease

a. Normal Animals. Many bovine adenoviruses have been isolated from apparently normal animals. Among these were prototypes 1 and 2, isolated from feces (Klein *et al.*, 1959, 1960), and type 3 from the conjunctiva (Darbyshire *et al.*, 1965). Isolations have also been made under conditions not associated with adenoviruses, such as the isolation of a type 6 virus from the lymph node of a leukotic cow (Mayr *et al.*, 1970). Some isolations have been made from organs taken for cell culture from normal animals. Thus the prototype 9 and a B2 virus were isolated from primary bovine kidney cells (Mohanty and Lillie, 1970; Guenov *et al.*, 1971)) and an untyped isolate also from kidney (Schopov *et al.*, 1968). The B6 prototype strain came from a calf testes cell culture (Rondhuis, 1968), as did B4 strains (Bartha and Csontos, 1969) and B4 and B6 (Phillip and Sands, 1972) strains. Burki *et al.* (1978) made 14 isolations from the testicles of normal slaughterhouse calves.

b. Conjunctivitis. A number of strains of adenovirus have been isolated from the conjunctiva of cattle with infectious keratoconjunctivitis in Australia (Wilcox, 1969). Experimental infection of calves with these isolates produced a mild conjunctivitis after an incubation period of 7–14 days. *Neisseria catarrhalis* and *Moraxella bovis* may be associated as secondary invaders (Wilcox, 1970).

c. Respiratory Disease and Enteritis. Adenoviruses have been associated with both upper and lower respiratory tract disease. In some of these outbreaks, diarrhea has been a minor or major problem. The first associations of bovine adenoviruses with respiratory tract disease came from serological (Darbyshire and Pereira, 1964; Harbourne, 1966) and histological evidence (Omar, 1966).

B1 and B2 have not often been recorded from field outbreaks of disease. Saxegaard and Bratberg (1971) isolated B1 from an outbreak of anorexia, nasal discharge, and diarrhea. Attempts at experimental reproduction of disease using these serotypes have produced only mild illness (Mohanty and Lillie, 1965; Darbyshire *et al.*, 1969), while other workers were unable to reproduce disease (Klein *et al.*, 1960; Ide *et al.*, 1969). Tury *et al.* (1978) claim to have produced microscopic changes in the respiratory tract, kidney, liver, intestine, and lymph nodes with a B2 type virus isolated from sheep. B3 has been isolated from an outbreak of acute respiratory tract disease in feedlot cattle (Lehmkuhl *et al.*, 1975) and has also been associated with a disease syndrome in a beef herd over a 4-year period (Mattson, 1973). This occurred in 1- to 4-week-old calves and was characterized by ocular and nasal discharge, tympanies, colic, and diarrhea. Experimental infections of calves with B3 have ranged from no disease (Ide *et al.*, 1969) to clinical disease. Only mild clinical signs were seen when day-old colostrum-deprived calves were infected with B3 (WBR-1), but areas of collapse, emphysema, and consolidation were seen in the lungs (Darbyshire *et al.*, 1966). Intratracheal inoculation of 3-month-old calves with B3 (FOS-213) produced hyperpnea, dyspnea, and anorexia (Lehmkuhl *et al.*, 1975).

A number of serotypes have been associated with pneumonoenteritis in calves. These include B4 (Aldasy *et al.*, 1965) and B8 (Bartha *et al.*, 1970). This condition occurs mainly in 2-week-old to 4-month-old calves. Initially, upper respiratory tract signs are seen, quickly followed by increased salivation and diarrhea. Following a 1–2-week course, most calves recover, although with a marked loss of condition; however, about 10% develop more severe respiratory signs, and many die. At autopsy the main findings depend on the stage of the disease. There is usually a hemorrhagic tracheitis, and lung changes vary from hyperemic through catarrhal pneumonia to a purulent pneumonia, often associated with pleurisy and endocarditis (Aldasy *et al.*, 1965). Experimental infection of calves with B4 reproduced the syndrome, albeit in a milder form.

Mohanty (1971) carried out experimental infections with B4, B5, and B8. Calves infected with B4 and B5 had clinical respiratory signs, while those given B8 did not. Some of the calves given B4 and B5 had macroscopic and microscopic lung changes. B4 appears to be the most important pathogenic bovine adenovirus in central Europe (Burki, 1973).

In Australia, B4 (BIL) and B6 (RG) have been isolated from the lungs and noses of calves with pneumonia. The two BIL strains were from the lungs of calves with acute exudative pneumonia, while seven RG strains were from the lungs or noses of calves with varying degrees of penumonia or bronchitis and one RG strain was from a nasal swab of a normal calf. Following intratracheal inoculation of B4 (BIL) into calves, a mild interstitial pneumonia was induced (Cole, 1971).

A virus isolated from the blood of a cow with anorexia, pyrexia, diarrhea, and

respiratory signs (Inaba *et al.*, 1968) was designated "prototype 7" (Matumoto *et al.*, 1970). Adenoviruses have also been isolated from cases of enzootic pneumonia in calves. These include the proposed B10 virus (Kretzschmar, 1973) and untyped isolates (Lupini *et al.*, 1970).

d. Weak Calf Syndrome. This syndrome has been described in the United States (Card *et al.*, 1974; Cutlip and McClurkin, 1975). Calves are usually weak and listless at birth, with a locomotor disturbance due to a polyarthritis, and often have diarrhea. At necropsy there is blood-tinged synovial fluid and subcutaneous hemorrhages in the hock, metacarpal, and metatarsal regions. Mortality is 6–15% of calves in the herd.

B5 viruses have been isolated from the synovial fluid (Coria *et al.*, 1975) and a B7 type virus from the buffy coat (Stauber *et al.*, 1976). Experimental inoculations of calves with the B5 (Idaho) isolate resulted in a mild self-limiting illness, with pyrexia and mild diarrhea. Following intraveneous infection, lesions developed which closely resembled the natural disease seen in Idaho and Montana. It has been suggested that this condition may be due to interaction of cold wet weather, bovine viral diarrhea, and adenovirus (Cutlip and McClurkin, 1975; McClurkin and Coria, 1975).

5. Diagnosis

Most isolations of bovine adenoviruses have been made in either calf kidney (CK) or calf testes (CT) cells. However, these cells are not necessarily the most sensitive. Thus Stauber *et al.* (1976) had the highest titers (10^7) for B7 in calf salivary gland cells, and Coria *et al.* (1975) found that B4 and B5 grew well in bovine turbinate cells.

One difficulty is that many bovine isolates (including subgroup A) required 20–50 days of incubation before a good CPE was observed. Part of the difficulty may be that the CPE depends on the multiplicity of infections, as even with recognized isolates, tubes near the end point may take up to 17 days to produce a discernible CPE (Coria *et al.*, 1975; Lehmkuhl *et al.*, 1975).

Elazhary and Derbyshire (1978) found that higher titers of B3 were obtained if they were grown in suspension cultures of MDBK cells and suggested that the virus might have a preference for young, actively dividing cells. This is of interest because a similar observation has been made for sheep adenoviruses (McFerran *et al.*, 1971b). It is therefore suggested that suspected specimens be passaged in freshly dispersed cells.

The observation that B3 grows in bovine tracheal organ culture is of interest (Bouffard and Derbyshire, 1978), not only because the virus was shown to replicate only in cells of epithelial origin but also because of its potential use in diagnosis. It may well be that bovine tracheal organ culture will be most useful in isolating not only adenoviruses but also bovine respiratory syncytial virus (Thomas *et al.*, 1976) and bovine coronavirus (Stott *et al.*, 1976).

B. Infection in Sheep

1. Classification

Five ovine serotypes are recognized (Adair and McFerran, 1976) (Table III). Two isolates (WV419 and WV757) from New Zealand (Davies and Humphreys, 1977b) may be new serotypes, but this has not yet been confirmed. They apparently grow best in lamb testicular cells and may be counterparts of the subgroup 2 bovine adenoviruses.

2. Distribution

Adenoviruses have been isolated from sheep in Australia (Showdon, 1971), Hungary (Belak and Palfi, 1974), New Zealand (Davies and Humphreys, 1977b), Turkey (Bauer *et al.*, 1975), and the United Kingdom (McFerran *et al.*, 1971b; Sharp *et al.*, 1974). Little immunoprecipitin antibody is detectable (Darbyshire and Pereira, 1964; Timoney, 1971), but virus-neutralizing antibody is widespread.

3. Epidemiology and Pathogenesis

These viruses have been isolated from both nasal swabs and feces. Following experimental infection, one strain was isolated from the respiratory tract for 7 days, from the intestine for 10 days, and from the kidney for up to 14 days (Davies and Humphreys, 1977a). It appears that sheep can be infected not only with ovine adenoviruses but also with bovine strains (Belak and Palfi, 1974).

4. Association with Disease

Viruses have been isolated from clinically normal sheep (McFerran *et al.*, 1971b; Bauer *et al.*, 1975; Davies and Humphreys, 1977b), from sheep with diarrhea (McFerran *et al.*, 1971b), and from respiratory tract disease in intensively reared lambs (Belak and Palfi, 1974; Sharp *et al.*, 1974).

TABLE III
Ovine Adenoviruses

Serotype	Strain	Erythrocytes agglutinated	Cytopathology subgroup[a]	Original reference
1	S1	Rat[b]	B	McFerran *et al.* (1969)
2	PX515	None	B	McFerran *et al.* (1969)
3	PX616	None	B	McFerran *et al.* (1969)
4	7769	None	B	Sharp *et al.* (1974)
5	SAV	None	B	Bauer *et al.* (1975)
6[c]	WV419	None	A	Davies and Humphreys (1977b)
7[c]	WV757	Fowl	A	Davies and Humphreys (1977b)

[a] Adair *et al.* (1979b).
[b] To low titers only.
[c] Probable new serotypes.

Experimental infection has produced equivocal results. Inoculation of 04 into lambs did not produce clinical signs, although the virus replicated and stimulated an antibody response (Sharp *et al.*, 1974). A similar failure to reproduce disease was recorded for 05 (Bauer *et al.*, 1975). A New Zealand isolate, WV757, produced a mild illness in 3- to 4-month-old lambs (Davies and Humphreys, 1977a).

An isolate, serologically identical to B2, produced severe respiratory and intestinal signs in colostrum-deprived lambs following an incubation period of 3 days (Belak *et al.*, 1975). Furthermore, in-contact lambs also became ill. The main pathological lesion was a catarrhal pneumonia.

C. Infection in Goats

1. Classification

Two adenoviruses, 1-435 and 2-480, which on the basis of the serum neutralization (SN) test are distinct serotypes, have been isolated from goats (Gibbs *et al.*, 1977).

2. Distribution

Neutralizing antibody to a caprine adenovirus (435) was found to be widespread in goat, sheep, and cattle sera both in Nigeria and in England (Gibbs *et al.*, 1977). It is not known if the antibody in sheep and cattle is due to infection with 435 virus or to the broad antigenicity of 435-detecting antibody to other adenoviruses.

3. Epidemiology and Pathogenesis

No information is available.

4. Association with Diseases

These viruses were isolated in lamb kidney cells from the intestines of goats with peste des petit ruminants in Nigeria. They are not thought to have any part in the etiology of this condition (Gibbs *et al.*, 1977).

It is probable that goat cells are at least as sensitive to infection as lamb cells. Isolate 435 did not produce a CPE in bovine kidney, BHK21, or Vero cells (Gibbs *et al.*, 1977).

D. Infection in Pigs

1. Classification

Four serotypes are recognized (Table IV). The initial isolate, 25R (Haig *et al.*, 1964), was designated "P1," and two new isolates, 6618 and A47, "P2" and "P3," respectively (Clarke *et al.*, 1967); this designation was followed by other

workers (Adair and McFerran, 1976). However, these numbers were transposed (Christofinis *et al.*, 1972), and their notation was followed by the WHO/FAO committee on classification (Anonymous, 1973). Bibrack (1969) suggested that a German and an American isolate (Kasza, 1966) were identical and should be considered P4. The relationship between these strains is clear-cut (Derbyshire *et al.*, 1975; Adair and McFerran, 1976).

Derbyshire *et al.* (1975) suggested that strain 100 should be considered a new serotype. They also described strains of broad antigenicity. A French isolate, not related to the four recognized serotypes, has also been described, but full cross neutralization tests were not undertaken (Chappuis and Tektoff, 1975).

2. *Distribution*

Porcine adenoviruses are widely distributed. Thus 20% of sera examined from Bulgarian and Hungarian pigs had group antibody (Guenov and Bodon, 1976), and 18% of Australian pig sera tested had type antibody to a local isolate (Kwon and Spradbrow, 1971). Antibody to types 1–3 was widespread in England (Clarke *et al.*, 1967), and Bibrack (1970) demonstrated that antibody to type 4 was common in Bavarian pigs.

3. *Pathogenesis*

Serotypes 1–3 and eight field isolates (including potential serotype 5) all appear to have a predilection for the alimentary tract (Derbyshire *et al.*, 1975). Even following intranasal or aerosol exposure, the main site of replication was the alimentary tract, although virus was also recovered from the respiratory tract (Sharpe and Jessett, 1967; Jericho *et al.*, 1971).

P4 appears able to grow in a wider variety of tissues, including the nervous, respiratory, and alimentary tracts. Furthermore, it can be reisolated for up to 48 days following infection. As the virus was more widely distributed following intranasal than oral infection, it has been suggested that aerial transmission is important in the spread of this virus (Shadduck *et al.*, 1968).

TABLE IV
Porcine Adenoviruses

Serotype	Strain	Erythrocytes agglutinated	Cytopathology subgroup[a]	Original reference
1	25R	Wide variety[b]	B	Haig *et al.* (1964)
2	6618 (A47)	None	B	Clarke *et al.* (1967)
3	A47 (6618)	None	B	Clarke *et al.* (1967)
4	F618	Rat, Fowl	B	Kasza (1966)
5	100	—	—	Derbyshire *et al.* (1975)

[a] Based on Adair (1976).
[b] But the strain tested was later found contaminated with a hemagglutinating parvovirus.

One feature of pig adenoviruses has been the number of reported isolations made from cell cultures of apparently healthy pigs (Kohler and Apodaca, 1966; Mahnel and Bibrack, 1966; Rasmussen, 1969; Kwon and Spradbrow, 1971; Kawamura *et al.*, 1972; Chappuis and Tektoff, 1975). Bibrack (1970) isolated adenoviruses from 35% of kidneys obtained from pigs at slaughter, and Guenov *et al.* (1968) found that up to 10% of pig kidneys contained latent adenoviruses. These viruses grew to very low titers.

It is possible that this high incidence of latent infection with adenovirus is responsible for the contamination of hog cholera strains. Thus Bodon (1966) found that seven supposedly cytopathic strains of hog cholera virus were in fact contaminated with adenovirus.

Although transplacental passage of pig adenovirus has not been recorded, intrauterine infection of pig fetuses resulted in widespread dissemination of virus and abortion 9 days after infection (Sharpe, 1967).

4. *Association with Disease*

a. Normal Animals. Many adenoviruses have been isolated from normal swine (Derbyshire *et al.*, 1966; Rasmussen, 1969), in addition to the numerous isolations made from kidney cells grown from healthy pigs.

b. Encephalitis. P4 virus was isolated from the brain of a pig with encephalitis (Kasza, 1966), and encephalitis has been experimentally reproduced following intracerebral inoculation (Shadduck *et al.*, 1967). Edington *et al.* (1972), although producing no clinical signs, did obtain meningoencephalitis and a severe peritubular mononuclear infiltration of the kidneys in pigs following oral or intranasal inoculation with P4. These lesions in the central nervous system (CNS) were not seen until 2 weeks after infection.

c. Respiratory. Following experimental infection with P4, an interstitial pneumonia was produced in addition to lesions in the kidneys, thyroid, and lymph nodes. The cells affected were mostly members of the reticuloendothelial system (Shadduck *et al.*, 1967). Bibrack (1970), in an investigation of natural cases of enzootic pneumonia, did not make many adenovirus isolations. Kasza *et al.* (1969) suggested a synergistic effect between P4 and *Mycoplasma hypopneumoniae* and drew attention to the difficulties of isolation of the virus even from experimental cases.

d. Enteritis. The prototype 1 virus was isolated from a case of diarrhea (Haig *et al.*, 1964), and pigs with diarrhea had intranuclear inclusions in the epithelial cells of the villi of the ileum and jejunum (Fujiwara *et al.*, 1968). Some workers produced a mild diarrhea when isolates were given orally (Derbyshire *et al.*, 1975), while others were unsuccessful (Shadduck *et al.*, 1967; Sharpe and Jessett, 1967).

Following aerosol exposure of P2 (6618), focally distributed intranuclear inclusions and subepithelial lymphoid accumulations were found in epithelial cells of the villi of the jejunum. In the lungs there was an increase of lymphoid tissue

in connective tissue at all levels of the bronchial tree, and inclusion bodies were seen within these proliferations (Jericho *et al.*, 1971).

5. *Diagnosis*

Primary pig kidney (PK) cells are probably the most sensitive, but the P4 strains required up to 42 days in cell cultures before the initial isolation (Shadduck *et al.*, 1968; Kasza *et al.*, 1969). P1, P2, and P3 grew to 10^7 in PK cells (Sharp and Jessett, 1967), whereas some of the isolates from latently infected cells reached 10^3 at maximum (Guenov *et al.*, 1968). There have been reports that some strains grow in MDCK and in calf, human, and patas monkey kidneys (Kasza, 1966; Chappuis and Tektoff, 1975).

Both the SN and double immunodiffusion (DID) techniques have been used for detecting antibody. The SN test can be made more sensitive by adding the virus serum mixture to the cells in suspension and then allowing the monolayer to form (Kwon and Spradbrow, 1971).

E. Infection in Horses

1. *Classification*

Only one serotype is recognized. However, 16 horses were found in Australia with adenovirus CF antibody but without antibody to E1, suggesting that other adenoviruses infect horses (Studdert *et al.*, 1974).

2. *Distribution*

Antibody surveys indicate that between 10 and 25% of horses have precipitating antibody (Darbyshire and Pereira, 1964; Afshar, 1969; Timoney, 1971). However, using the HI test, 73% of horse sera tested had antibody (Studdert *et al.*, 1974). A comparative study revealed that 100% had SN antibody, 68% had CF antibody, and the immunodiffusion test indicated 19% affected (Harden *et al.*, 1974).

3. *Epidemiology and Pathogenesis*

There is no direct evidence of carriers or *in utero* infection, but indirect evidence suggests this possibility. Thus the virus has been recovered from the nasal mucus of a 3-day-old Clydesdale foal (Wilks and Studdert, 1973). Furthermore, on four farms during a 4-year observation period, multiple incidences of disease occurred within groups of foals. One mare gave birth in successive years to three foals that died of pneumonia, indicating not only a genetic deficiency but also virus persistence (McChesney *et al.*, 1973). There is often a very high incidence of antibody on farms (Todd, 1969; Studdert *et al.*, 1974). Foals before suckling had no antibody to adenovirus, which develops after ingestion of

colostrum (Harden *et al.*, 1974). Serological studies indicate that reinfection is frequent, and infection or reinfection can occur in the presence of high levels of circulating antibody. However, subclinical infections appear to occur quite frequently between 90 and 150 days, when maternal antibody levels have fallen (Harden *et al.*, 1974; Studdert *et al.*, 1974).

4. Association with Disease

a. Subclinical Infection in Horses. There have been quite a number of isolations from clinically healthy horses, including the Clydesdale, Thoroughbred, and Arabian breeds (Petzoldt and Schmidt, 1971; Harden *et al.*, 1972; Wilks and Studdert, 1973). In addition, antibody has been found in horses with no history of respiratory tract disease (Darbyshire and Pereira, 1964; Afshar, 1969; Timoney, 1971; Harden *et al.*, 1974).

b. Respiratory Tract Disease. An association is emerging between acute, nonfatal respiratory tract disease in horses and equine adenovirus infection. Virus has been isolated from nasal swabs taken from horses with rhinitis, mucopurulent discharge, and coughing (Todd, 1969; McChesney *et al.*, 1973; Dutta, 1975; Moorthy and Spradbrow, 1978).

c. Fatal Pneumonia. Foals between 10 and 46 days were affected, and the disease lasted 10–56 days. The foals were thin, depressed, and tired rapidly. Persistent nasal and ocular discharge and occasional diarrhea were evident. Dyspnea, polypnea, and a nonproductive cough were features (McChesney *et al.*, 1973; Thompson *et al.*, 1975). Hematological examination revealed an absolute lymphopenia and neutropenia in the foals which died. No HI antobidy could be detected. In-contact foals were infected with equine adenovirus and developed respiratory signs and HI antibody, but did not show the severe lymphopenia (McChesney *et al.*, 1973). In the foals which died, rhinitis, tracheitis, pulmonary atelectasis, bronchopneumonia, and lymphoid atrophy were seen. Microscopically, there was necrosis of and large basophilic intranuclear inclusions in the epithelial cells of the respiratory tract, urinary tract, conjunctiva, pancreas, and focally in the intestine (McChesney *et al.*, 1973; McGuire and Poppie, 1973). These foals lacked bone marrow-derived (B) lymphocytes, as shown by the lymphopenia and hypogammaglobulinemia (IgM and IgA could not be detected) and by the absence of germinal centers in the lymph nodes and spleen. They also lacked thymus-derived (T) lymphocytes, as indicated by thymic hypoplasia and the absence of thymic-dependent lymphoid tissue (periarteriolar lymphocytic sheaths) in the lymph nodes and spleen. These immunological deficiencies suggest that there might be a genetic component of this condition (McChesney *et al.*, 1973; McGuire and Poppie, 1973). Following a study of the percentage of affected foals in Australia, Thompson *et al.* (1975) suggested that the immunodeficiency was due to a simple recessive autosomal gene. These authors consider that the basic reason for the fatal pneumonia is the immunodeficiency and not the adenovirus.

5. *Diagnosis*

The equine adenovirus differs from most of the other isolates in being able to grow in a range of cells. Primary horse kidney (HK) cells appear the most sensitive. An equine dermis cell line has been reported equally sensitive (Konishi *et al.*, 1977) and has been used for virus isolation (Ardans *et al.*, 1973), but other workers have found it less satisfactory (England *et al.*, 1973; Studdert *et al.*, 1974). An equine kidney (EK) cell line has also been used for isolation (Moorthy and Spradbrow, 1978). E1 causes a CPE in a range of cells, fetal PK cells have been shown to be as sensitive as HK cells (Konishi *et al.*, 1977), and bovine cells are virtually as sensitive as HK cells (England *et al.*, 1973).

Serological studies can be undertaken using the HI test, with human erythrocytes as the indicator system (Studdert *et al.*, 1974).

F. Infection in Dogs

1. *Classification*

ICH virus (C1) and the virus associated with infectious laryngotracheitis (A26/61) (Ditchfield *et al.*, 1962) can be distinguished using the HI test. But while antiserum to C1 neutralized only C1, antiserum to A26/61 neutralized both viruses to the same titer (Ditchfield *et al.*, 1962). However, it should be noted that the antiserum to A26/61 had apparently been prepared in dogs previously given C1-attenuated vaccine. Using both the HI and SN tests, it was possible to divide seven isolates into C1 or A26/61 types (Swango *et al.*, 1969). But using purified reagents, it was shown that antiserum to C1 hexons would neutralize both C1 and A26/61 viruses, whereas antiserum to A26/61 hexons would neutralize only A26/61 virus. Using antisera prepared to the fibers, the HI test indicated a similar one-way cross, but in the SN test these fiber antisera were type specific (Marusyk, 1972). When antiserum to the virion was tested against complete soluble hemagglutinin as antigen, the two types of virus were readily differentiated (Marusyk, 1972; Wright *et al.*, 1972).

There are other reasons why A26/61 should be considered a separate serotype. The viruses have different fiber lengths and the viral capsid components carry different ionic charges, as demonstrated by anion exchange chromatography (Marusyk *et al.*, 1970). The C1 and A26/61 viruses have different hemagglutinin receptors on erythrocytes (Marusyk and Yamamoto, 1971), and DNA–DNA hybridization studies indicate a genetic relationship of 70% between these viruses (Marusyk and Hammarskjold, 1972). Also, the pattern of replication differs, with C1 behaving like cytopathology subgroup A and A26/61 resembling cytopathology subgroup B (Adair, 1979).

One reason for considering C1 and A26/61 as the same serotype is that A26/61 protects dogs against infection with C1 (Fairchild *et al.*, 1969).

2. *Distribution*

These viruses are widely distributed.

3. *Epidemiology and Pathogenesis*

Following oral infection, C1 replicated in the tonsils and then traveled via the drainage lymph nodes and lymph to the blood. The viremia occurred 4–6 days PI and lasted 1–3 days. Virus was in the kidney from day 5–7 to at least day 23, and there was irregular excretion in the urine for up to 161 days PI. Virus was isolated from the tonsillar swabs from days 1–7, with peak titers on days 2 and 3. Minimal quantities of virus were excreted in the feces (Poppensiek and Baker, 1951; Salenstedt, 1963; Hamilton *et al.*, 1966). Leukopenia accompanied the viremia and pyrexia and was followed by an intense leukocytosis (Hamilton *et al.*, 1966). The minimal dose required for oral infection was between 40 and 400 $TCID_{50}$ (Salenstedt, 1963).

Damage to the vacular endothelium and to liver cells was widespread (Rubarth, 1947). Lesions occurred in the liver from day 7 to 11 and were maximal in the CNS at days 8 and 9, and nephritis was seen from day 11 (Hamilton *et al.*, 1966). Nephritis resulted from virus in the glomerular epithelial and mesengial cells in the larger renal vessels, causing marked cellular degenerative changes in both glomeruli and tubular epithelium. Swelling of the affected cells resulted in partial or complete occlusion of both capillaries and urinary spaces, leading to proteinuria and viruria. At this stage, there was no deposit of immune complexes (Poppensiek and Baker, 1951; Wright *et al.*, 1973). These interstitial lesions appeared to be a type IV (cell-mediated) hypersensitivity reaction in which epithelial cells containing viral antigen were surrounded by macrophages and lymphocytes (Morrison *et al.*, 1976).

At the height of the disease, when antibody was developing but had not yet reached high levels, circulating immune complexes were formed (Morrison and Wright, 1976). It has been suggested that the glomerulonephritis associated with ICH is due to the deposition of these complexes (Kurtz *et al.*, 1972), and this has been confirmed by the identification of IgG and C1 antigen in the glomeruli of naturally occurring cases (Morrison *et al.*, 1975). These proliferative glomerulonephritic lesions are transient and do not lead to permanent damage.

During the viremic phase, the virus enters the anterior chamber of the eye and infects the endothelial cells, producing a primary iridiocyclitis. This may resolve without sequellae or the virus may persist. This results in a type III hypersensitivity reaction, with the formation of immune complexes in the aqueous fluid. These complexes are phagocytosed and the corneal epithelium is attacked, leading to corneal edema and the characteristic "blue eye" (Carmichael, 1964).

The pathogenesis and pathological changes induced by C1 depend on the route of entry. An isolate from a dog with acute hepatitis (Wright *et al.*, 1970) pro-

duced severe respiratory tract disease in dogs when given intratracheally or by aerosol (Wright *et al.*, 1970, 1971). The route of infection may well affect the pattern of excretion. Thus although classical ICH virus was not airborne (Baker *et al.*, 1950) when dogs were infected by the respiratory route, airborne infection occurred (Wright *et al.*, 1971).

Following combined intranasal and intratracheal inoculation of an A26/61 type virus, a viremia occurred, and unlike C1, virus was recovered from throat swabs for 8 days and rectal swabs for 10 days. Only sporadic isolations were made from urine (Swango *et al.*, 1970).

4. Association with Disease

a. Canine Adenovirus 1 (ICH) Virus

1. Subclinical. C1 virus has been isolated from the respiratory tract of normal dogs (Campbell *et al., 1968) and also from kidney cells grown from healthy dogs.*

2. Generalized Infection. This condition was initially described by Rubarth (1947). It varied in degree from fatal disease to mild illness. Following an incubation period of 2–14 days, the signs seen were a biphasic pyrexia, anorexia, ocular and nasal discharge, conjunctivitis, hyperemia of the oral mucus membranes, and vomiting. Hepatitis was rare, and there was subcutaneous edema of the head, neck, and body (Rubarth, 1947; Pay, 1950).

The basic lesions were hepatitis, lymphadenitis, and vascular damage. Histological examination revealed a severe hepatitis with focal parenchymal necrosis, with intranuclear inclusions (Fig. 5c). When examined using immunofluorescence, antigen was present in large amounts in the liver and also in the lymph nodes, spleen, and tonsils (Rubarth, 1947; Wright *et al.*, 1972).

It has also been suggested that C1 infection can give a chronic hepatitis (Gocke *et al.*, 1970), but this has not been confirmed.

3. Respiratory Infection. C1 has been isolated from the turbinates of dogs suffering from severe rhinitis (Wright *et al.*, 1972).

C1 has also been isolated from dogs suffering from mild to severe respiratory tract disease, where there were lesions in both the upper and lower respiratory tracts (Binn *et al.*, 1967; Studdert and Studdert, 1972; Wright *et al.*, 1972). It has been possible to reproduce respiratory tract disease in dogs using a C1 virus from a fatal case of ICH (Wright *et al.*, 1971) and using a C1 virus isolated from a natural outbreak of respiratory disease (Wright *et al.*, 1972). Following aerosol exposure, the dogs were dull and pyrexic and had respiratory signs. At autopsy there was dull red consolidation of the lungs, with focal areas of pulmonary edema and a severe necrotizing bronchiolitis. There was also focal necrosis in the turbinates, nasal epithelium, and tonsils. Viral inclusions and the antigen were detected in necrotizing bronchiolar epithelial cells and in turbinate and tonsillar cells (Wright *et al.*, 1972).

4. Ocular Disease. Unilateral or bilateral corneal edema occurred in about 20% of animals recovering from C1 infection about 2–3 weeks PI. Usually transient, it became protracted, with permanent impairment of vision (Carmichael, 1964; Curtis and Barnett, 1973; Carmichael *et al.*, 1975).

5. Other Conditions. C1 infection has also been associated with encephalopathy (Salyi and Knapp, 1958; Wright, 1967) and with focal interstitial nephritis (Poppensiek and Baker, 1951; Hamilton *et al.*, 1966). It has been suggested that C1 infection could be an important cause of chronic renal filure (Bush and Evans, 1972), but other workers could not incriminate the virus (Wright *et al.*, 1976).

b. A26/61 Toronto Virus. This virus was isolated from dogs with pharyngotracheitis (Ditchfield *et al.*, 1962). Other workers have also associated this virus with respiratory tract disease (Binn *et al.*, 1967), and experimental work has confirmed this. Thus, following a combined nasal–tracheal route of infection, signs and lesions were confined to the respiratory tract. There was a proliferating necrotizing bronchitis and bronchiolitis, and large intranuclear inclusions were seen in the bronchial epithelial and alveolar septal cells (Swango *et al.*, 1976).

5. Diagnosis

The canine adenoviruses are easily isolated in canine kidney cell cultures and in the MDCK cell line (Cabasso *et al.*, 1954; Yamamoto, 1966; Danskin, 1973). C1 virus will also replicate in pig, raccoon, and ferret kidney cells (Fieldsteel and Yoshihara, 1957; Bolin *et al.*, 1958; Emery and York, 1958). The virus, in common with other adenoviruses, affects the epithelial cells (Cabasso *et al.*, 1954). Hemagglutinin is produced in the cell fluid, and the isolate can be typed against appropriate serum. Mouse ascitic fluid is often used as a source of antibody (Wright and Burns, 1966). Organ culture of dog trachea can also be used (Thompson *et al.*, 1972). Immunofluorescent staining of liver sections from cases of ICH has proved both quick and sensitive (Wright and Burns, 1966).

Serological investigations can be undertaken using the SN (Carmichael *et al.*, 1963) or HI test. Following thermal inactivation and kaolin treatment, the sera are tested for HI antibody using human cells (Espmark and Salenstedt, 1961; Marusyk and Yamamoto, 1971).

G. Infection in Monkeys and Apes

1. Classification

Viruses derived from rhesus *(Macaca mulatta)*, patas *(Erythrocebus patas)*, and vervet *(Cercopithecus aethiops)* monkeys fall into 16 species (Hull *et al.*, 1956, 1958; Tyrrell *et al.*, 1960; Malherbe and Harwin, 1963; Pereira *et al.*,

1963; (Table V). SV23 and SV39 could be distinguished using the HI test but are similar using the SN test and are classified as one species, S6. S10 and S14, although similar using the HI test, could be distinguished using the SN test and are therefore classified separately (Rapoza, 1967; Hull, 1968). Viruses isolated from vervet monkeys were identified as either S3 or an unclassified type, V340 (Kim *et al.*, 1967), and isolates from baboons *(Papio* species) were either S16 or V340 (Eugster *et al.*, 1969).

There was no crossing between S1–15 and 22 human adenoviruses, using both HI and SN tests, except that S2 and S7 neutralized H25 and H12, respectively, at very low dilutions (Hull, 1968).

Viruses have been isolated from chimpanzees *(Pan troglodytes troglodytes)* by a number of workers (Rowe *et al.*, 1956; Hillis and Goodman, 1969; Basnight *et al.*, 1971). Unfortunately, most of these isolates do not appear to have been compared. The relationship of some of the chimpanzee and human adenoviruses has been discussed in Section II.

2. *Distribution*

Neutralizing antibody to SV15 and V340 is widespread in primates, including man (Kim *et al.*, 1967).

3. *Epidemiology and Pathogenesis*

Some strains (S1, S2, S3, S4, S6, and S10) are more often isolated from the respiratory tract, while other strains are frequently isolated from feces (Hull, 1968).

4. *Association with Disease*

a. None. Adenoviruses have been isolated from normal animals or from latently infected cells (Malherbe and Harwin, 1963; Bullock, 1965; Hillis and Goodman, 1969; Basmight *et al.*, 1971).

b. Pneumoenteritis. Eugster *et al.* (1969) described outbreaks in young baboons 1–2 weeks postpartum from which V340 and three SA7 viruses were isolated. Kim *et al.* (1967) isolated V340 and S3 viruses from vervet monkeys with chronic interstitial type pneumonia and enteritis. S10 strains were isolated from a number of monkeys with pneumonia and diarrhea (Bullock, 1965). An S4 virus was isolated from patas monkeys suffering from an epidemic of conjunctivitis and rhinorrhea (Tyrrell *et al.*, 1960).

c. Enteritis. A number of workers have reported the isolation of adenoviruses from monkeys with diarrhea (Hoffert *et al.*, 1958; Rapoza *et al.*, 1961; Hull *et al.*, 1968).

d. Other Conditions. Chandler *et al.* (1974) have reported the association of adenovirus infection with necrotizing pancreatitis in a rhesus monkey.

TABLE V
Monkey Adenoviruses

Serotype[a]	Hemagglutination subgroup[b]	Erythrocytes agglutinated[b]	Oncogenic serotype[c]	Buoyant[d] dencity (DNA)	%[d] G + C	DNA homology group[e]
S13 (SV36)	I	Rhesus at 4°C and 37°C. No agglutination of guinea pig or rat	—	1.71	49.9	
S3 (SV15), S4 (SV17), S6 (SV23), S9 (SV31), S10 (SV32), S14 (SV37)	II	Guinea pig at 4°C. Rhesus at 4°C. Rat at 4°C and 37°C; complete agglutination	(S6, S14)	1.71–1.76	53.8–56.0	II
S1 (SV1), S2 (SV11), S5 (SV20), S7 (SV25), S8 (SV30), S11 (SV33), S12 (SV34), S15 (SV38)	III	Rat 4°C and incomplete at 37°C[f] Rhesus and guinea pig at 4°C[g]	S1, S2, S5, S7, S8, S11 S12, S15	1.71–1.72	55.1–61.6	I
S16 (SA7)	IV	No agglutination of rat, rhesus, or guinea pig	S16	1.716	57.2	III

[a] Based on classification given in Andrewes *et al.* (1978). Note that these numbers differ from those proposed by Pereira *et al.* (1963).

[b] Based on Rapoza (1967).

[c] Based on Gilden *et al.* (1968), Huebner *et al.* (1962), Hull *et al.* (1965), and Slifkin *et al.* (1968). Burnett *et al.* (1972) found that S6 and S14 are not oncogenic.

[d] Based on Goodheart (1971). Note that Burnett *et al.* (1972) obtained similar results, except that they found S12 had a density of 1.72 and S12 had a density of 1.71.

[e] Based on Burnett *et al.* (1972). Note that S5 and S8 have also been included in this group. They are not as closely related as members of other groups are to one another.

[f] All gave 4–32 fold higher titers at 4°C than at 37°C, except S7 (SV25) and S8 and (SV30).

[g] Serotypes S2 (SV11), S7 (SV25), S8 (SV30), and S16 (SA7) did not agglutinate these erythrocytes.

5. *Diagnosis*

The monkey adenoviruses apparently grow only in cells of simian origin and probably best in primary homologous kidney cells. All grow in the monkey cell line LLC-MK_2 except S13 (Hull, 1958). The isolates from chimpanzees also grow in human cells (Basnight *et al.*, 1971).

The HI test, using sensitive rat erythrocytes at 4°C, is suitable for all strains, except for S13 when rhesus cells must be used and S16 when the SN test is required.

H. Infection in Mice

1. *Classification*

There are two serotypes of mouse adenovirus (van der Veen and Mes, 1974), represented by the FL strain (Hartley and Rowe, 1960) and the K87 strain (Hashimoto *et al.*, 1966). Wigand *et al.* (1977) disagreed and demonstrated a one-way cross, with antiserum to K87 neutralizing both antigens to the same titer but antiserum to FL only neutralizing itself.

The isolate of Missal (1969) appears to be unclassified.

2. *Distribution*

Two out of 11 mice colonies tested had CF antibody, and infection in these colonies ranged from 45 to 96% (Hartley and Rowe, 1960). Another survey found that 11% of mouse colonies tested were infected (Parker *et al.*, 1966).

3. *Epidemiology and Pathogenesis*

The FL strain of virus was found in the urine and nasal tissue but not in the saliva or feces. Infection is transmitted to in-contacts in the same cage but not in the same room. Maternal antibody is protective (Hartley and Rowe, 1960). The FL strain given intraperitoneally to adult mice produced a persistent infection of the kidneys, shown by an extensive mononuclear cell infiltration of the cortex and medulla and tubular necrosis. These changes predisposed to pyelonephritis (Ginder, 1965). Similar adenovirus effects have also been described in dogs (Ginder, 1974). In adult mice with an inapparent infection, a viruria lasting for months or years was found (van der Veen and Mes, 1973).

The K87 strain produced an intestinal infection, with peak fecal titers for 1–2 weeks after infection and virus continuing to be excreted for up to 4 weeks. No excretion was detected in oronasal excretions or urine (Sugiyama *et al.*, 1967).

4. *Association with Disease*

The FL strain was isolated from an apparently latently infected culture (Hartley and Rowe, 1960) and the K87 strain from the feces of apparently healthy mice (Hashimoto *et al.*, 1966).

The FL strain causes a generalized infection in newborn mice and an inapparent infection in adults (Hartley and Rowe, 1960; Heck *et al.*, 1972). In newborn mice, lesions and intranuclear inclusions are seen in a number of organs, including myocardium and brown fat. The FL strain inoculated into mice with an Ehrlich's ascitic form of carcinoma produced a fatal illness. The main areas of adenovirus replication were the lung and ascitic cells (Schmidt-Ruppin, 1968). No disease was produced by other strains in both suckling and weaned mice (Sugiyama *et al.*, 1967; Missal, 1969).

5. *Diagnosis*

Mouse kidney cells, and preferably young primary cultures, are the most sensitive. Some mouse cell lines are sensitive, while other mouse cell lines and cells from other species, including rat, are not (Hartley and Rowe, 1960; Larsen and Nathans, 1977; Wigand *et al.*, 1977). There is one report of cytopathology in PK cells (Missal, 1969).

Isolation of some strains is difficult, requiring up to 56 days of incubation before a good CPE is produced (Hashimoto *et al.*, 1966).

I. Infection in Fowl

1. *Classification*

The fowl adenoviruses have been classified by a number of workers (Clemmer, 1964; Kawamura *et al.*, 1964; Khanna, 1966; Burke *et al.*, 1968; McFerran *et al.*, 1972; Calnek and Cowen, 1975; McFerran and Connor, 1977; Grimes and King, 1977a). A proposed classification is shown in Table VI. Although the strains were initially found to be relatively clearly defined, prime strains were found (McFerran *et al.*, 1972). Strains of broad antigenicity were recognized when an isolate of Khanna (1966) was found to neutralize both 58 and 764 (McFerran *et al.*, 1975). The presence of prime strains and strains of broad antigenicity has been confirmed by others (Cowen *et al.*, 1977; Grimes and King, 1977a).

2. *Distribution*

The fowl adenoviruses have a worldwide distribution. Serological investigations have shown that birds on a farm can be infected with many different serotypes. Thus Grimes *et al.* (1977a) found, when testing 16 flocks for adenovirus antibody, that titers to the eight serotypes used could be found in 8 flocks and to seven serotypes in 6 flocks. Antibody to F2, F5, F6, and F8 was found in all flocks and F2 and F8 also had the highest titers, while only F1 was found on 11 farms. Although some of this antibody could be due to a heterotypic antibody response, virus isolation studies support the concept that adenovirus infection is widespread (Khanna, 1966; Yates *et al.*, 1976; Cowen *et al.*, 1978b).

TABLE VI
Classification of Fowl Adenoviruses

Proposed Serotype	Proposed type strain	Japanese[a]	Northern Ireland[b]	USA[c]	Erythrocytes agglutinated	Cytopathology[d] subgroup	Original reference (type strain)
1	CELO	OTE	112	E1	Fowl	A	Yates and Fry (1957)
2	GAL-1	SR48	685	Z-7 DP1 2-1	None	A	Burmester *et al.* (1960)
3	SR-49	SR49	75	—	None	B	Kawamura *et al.* (1964)
4	KR-5	KR-5	506	J-2	None	A	Kawamura *et al.* (1964)
5	340	TR-22	340	M-2	None	A/B[e]	McFerran *et al.* (1972)
6	CR-119	CR-119	—	—	None	B	Kawamura *et al.* (1964)
7	YR-36	YR-36	—	X-11	None	B	Kawamura *et al.* (1964)
8	TR-59	TR-59	58	T-8	None	A	Kawamura *et al.* (1964)
9	764	—	764	B-3	None	B	McFerran *et al.* (1972)
10	A2	—	—	A2	None		Calnek and Cowen (1975)
11	C-2B	—	—	C-2B	None		Calnek and Cowen (1975)
12	380	—	380	—	None		McFerran and Connor (1977)

[a] Based on the classification of Kawamura *et al.* (1964)
[b] Based on the classifications of McFerran *et al.* (1972, 1975) and McFerran and Connor (1977).
[c] Based on the classification of Calnek and Cowen (1975).
[d] Based on Adair (1978).
[e] See text.

3. Epidemiology and Pathogenesis

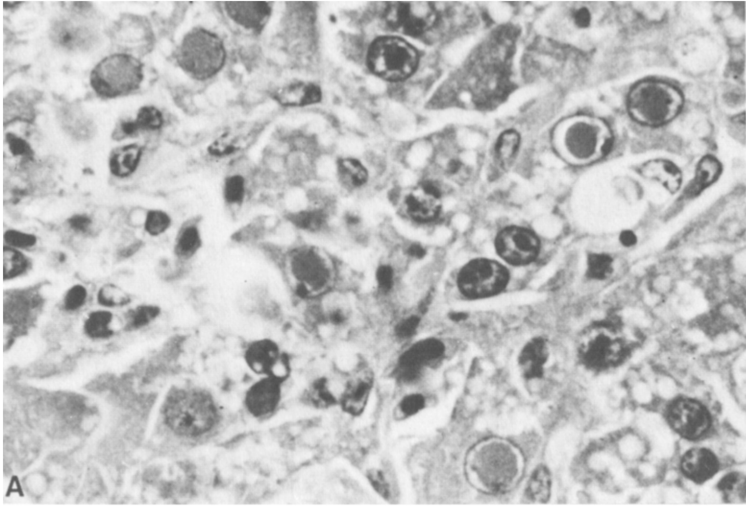
Virus was excreted in the feces for up to 20 days after infection, with peak titers 4 to 7 days after infection (Kohn, 1962; Kawamura *et al.*, 1963; Clemmer and Ichinose, 1968). Clemmer (1972) was able to distinguish between a juvenile and an adult pattern of virus excretion in the feces, depending on the age of infection. Day-old chicks excreted higher titers of virus for longer periods than did 21-day-old birds. Little information is available on the oronasal secretions as a source of infection, but as virus is often present in the conjunctiva, nasal mucosa, pharynx, and trachea, it seems reasonable to assume that they have a role. Egg transmission is important, and many workers have recorded the presence of adenovirus in uninoculated embryonated eggs or cell culture (Yates and Fry, 1957; Burke *et al.*, 1965; Khanna, 1966; Cook, 1968).

Experimental infection of birds with F1 and F2 viruses by natural routes resulted in widespread dissemination of virus, with maximum virus titers in the trachea and feces (Kohn, 1962; Kawamura *et al.*, 1963).

In a longitudinal survey of broiler flocks (McFerran *et al.*, 1981), birds were excreting adenovirus from 2–3 weeks of age, and at least two serotypes were found in each flock. The virus was confined to the intestine, bursa of the Fabricius, and upper respiratory tract, in contrast to the pattern seen in experimental infection of antibody-free birds with massive doses of virus. In the type of husbandry practiced, with depletion of the flocks and with cleansing and disinfection of the houses between flocks, carryover of virus was not important. The viruses found in these flocks were antigenically similar to those present in the parent flocks of the same organization. Presumably following vertical transmission, the virus becomes unmasked with the loss of maternal antibody at 2–4 weeks, and spreads slowly through the flock. Experimental work has shown that maternal antibody is lost within the first 4 weeks of life and that its presence does not prevent infection with some serotypes (Kohn, 1962), but presumably it does prevent latent virus from becoming activated. Because of the rather frequent isolation of more than one serotype from a bird, it is probable that there is little cross protection on the mucosal surface between species.

4. Association with Disease

a. Inclusion Body Hepatitis (IBH). This disease was first recorded in the United States (Helmboldt and Frazier, 1963). It is normally seen in birds 5–7 weeks of age (Howell *et al.*, 1970; Macpherson et al., 1974) but has been recorded in birds between 2 and 18 weeks (Hoffmann *et al.*, 1975). The main sign was a sudden increase in mortality, with very few sick birds. Mortality reached 10% and usually peaked on the third and fourth days of onset, with deaths stopping after the fifth day but occasionally continuing for 2.5 weeks (Howell *et al.*, 1970; Pettit and Carlson, 1972; Fadley and Winterfield, 1973; McFerran *et al.*, 1976a).



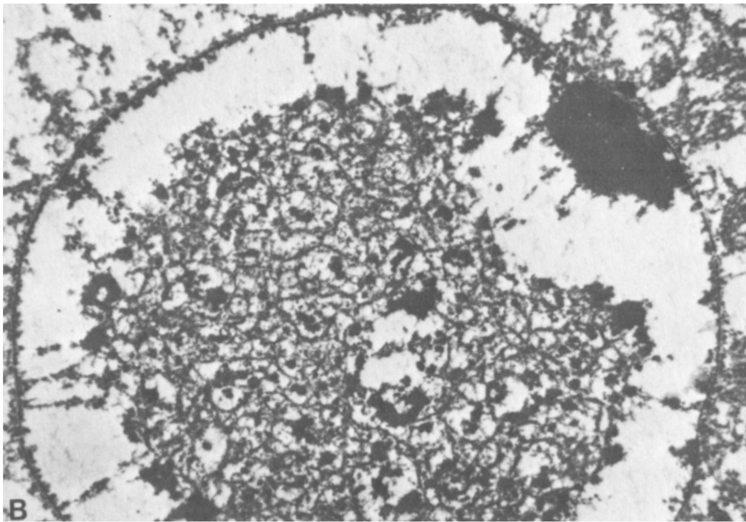


Fig. 5. (a) Liver from natural case of inclusion body hepatitis of fowl. Note large eosinophilic intranuclear inclusion bodies. (b) Thin section of affected nucleus from above. Note absence of virus particles. (c) Liver from dog with infectious canine hepatitis. Note numerous large basophilic intranuclear inclusion bodies.

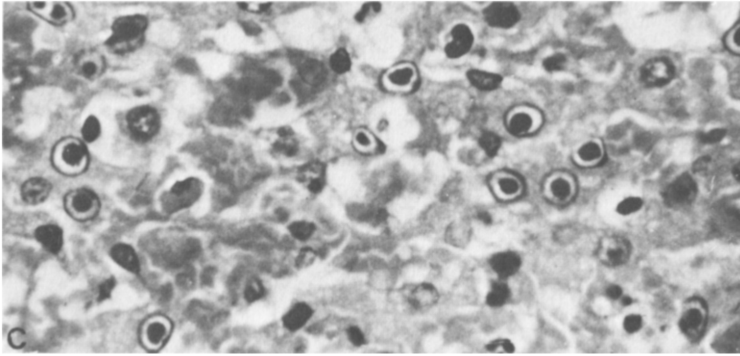


Fig. 5. Continued

The main lesions seen were pale, friable, swollen livers. Petechial or ecchymotic hemorrhages may be present in the liver and skeletal muscles (Howell, 1974; Macpherson *et al.*, 1974; McFerran *et al.*, 1976a). Some workers considered anemia the major sign (Stein, 1975), and German workers suggested the disease be renamed "hepatomyelopoietic disease" (Hoffmann *et al.*, 1975).

Eosinophilic inclusions were commonly seen in the hepatocytes of natural cases of IBH (Fig. 5) (Helmboldt and Frazier, 1963; Howell *et al.*, 1970; Bickford *et al.*, 1973; McFerran *et al.*, 1976a). Basophilic inclusions were rarely seen in field cases of IBH. In a study of the livers of 20 naturally infected birds, eosinophilic inclusions were seen in all, but basophilic inclusions were seen only in 6 birds (Itakura *et al.*, 1974, 1977). On electron microscopic examination of the livers in naturally occurring cases, these eosinophilic inclusions contained either very few or no adenovirus particles (Pettit and Carlson, 1972; Bickford *et al.*, 1973; McCracken and McFerran, unpublished observations; (Fig. 5). Itakura *et al.* (1977) found that virus particles were detected only in the cells with basophilic inclusions and that the eosinophilic inclusions corresponded to those cells consisting of fibrillar granular material, without viral particles. The lack of virus particles in the eosinophilic inclusions should be compared to the large numbers of particles associated with the basophilic inclusions in the livers of birds with a hepatitis following experimental infection (Figs. 5 and 6) (Bickford *et al.*, 1973; McCracken and McFerran, unpublished observations).

The naming of an isolate from IBH as the inclusion body hepatitis virus (Fadley and Winterfield, 1973) was premature because this virus was classified as a type 5 fowl adenovirus (McFerran *et al.*, 1975), and it is clear that if adenoviruses are associated with the etiology of IBH, then this affinity is not confined to one serotype. Thus F1 (Winterfield *et al.*, 1973), F2, F3, and F4 (McFerran *et al.*, 1976a; Grimes and King, 1977a), F5 (Fadly and Winterfield, 1973; McFerran *et al.*, 1976a), F8 (Macpherson *et al.*, 1974; McFerran *et al.*,

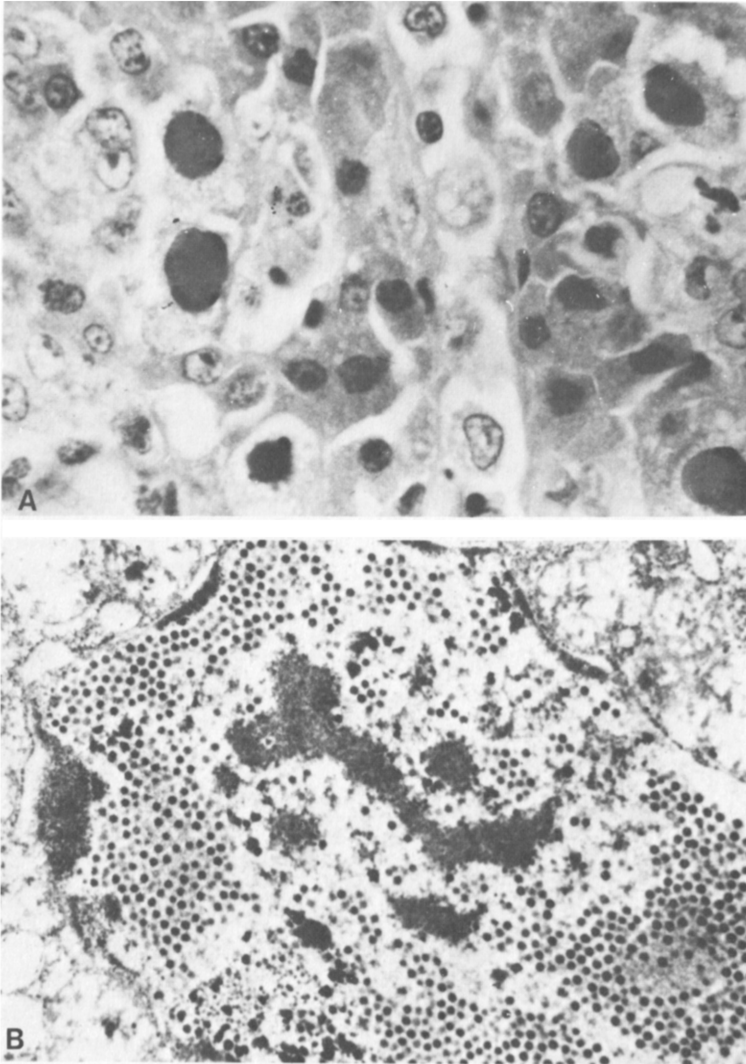


Fig. 6. Experimental inclusion body hepatitis of fowl (a) Liver section. Note large basophilic intranuclear inclusion bodies. (b) Thin section of affected nucleus showing numerous virus particles.

1976a; Grimes *et al.*, 1977b), and F9 (Grimes *et al.*, 1978) have all been isolated from clinical outbreaks of IBH. In addition, unclassified adenoviruses have been found (Di Franco *et al.*, 1974; Wells and Harrigan, 1974). It should be noted that reoviruses have also been isolated from some outbreaks (Hoffmann *et al.*, 1975; McFerran *et al.*, 1976a).

A number of workers have been able to reproduce the syndrome, but usually

only by parenteral inoculation of very young chicks (Rosenberger *et al.*, 1974; Grimes *et al.*, 1977b). One group has reproduced it both by natural routes of infection and in older birds (Fadly and Winterfield, 1973). Other workers could not reproduce typical lesions even by unnatural routes (Sharpless and Jungherr, 1961; Kawamura and Horiuchi, 1964; McDougall and Peters, 1974; Macpherson *et al.*, 1974). McCracken *et al.* (1976) produced a hepatitis when type strains and strains isolated from birds with IBH were inoculated into 5-day-old chicks. These changes occurred with seven fowl and two turkey adenovirus serotypes, but not with F1 strains. It is difficult to reconcile these conflicting findings. It may be that some other agent is also required, a suggestion given substance by the development of a hemorrhagic–aplastic anemia syndrome in one trial which was not associated with adenoviruses (Grimes and King, 1977b). Some workers have claimed that prior infection with infectious bursal disease virus enhances the pathogenicity of the adenovirus in IBD (Rosenberger *et al.*, 1975; Fadly *et al.*, 1976), and regression of the bursa of Fabricius in IBD has been described (Pettit and Carlson, 1972; Bickford *et al.*, 1973). However, results from both natural cases (MacPherson *et al.*, 1974) and experimental infection (Grimes *et al.*, 1977b) suggest that IBD virus is not necessary to produce the IBH syndrome.

b. Depressed Egg Production. Winterfield *et al.* (1973) recorded the isolation of an F1 (Indiana C) virus from a flock with poor eggshell quality. Other workers have attributed a 10% decline in production to adenovirus infection (Berry, 1969; Cook, 1972). Adenoviruses were also associated, on the basis of the development of group antigen, with depressed egg production linked to soft-shelled and shell-less eggs in the Netherlands (Van Eck *et al.*, 1976). McFerran *et al.* (1978a), investigating the same syndrome, isolated adenoviruses belonging to serological groups from birds 26 to 40 weeks of age. However, they did not feel that these isolates were of major importance in the syndrome. Workers in the United States (Cowen *et al.*, 1978b) experimentally infected birds with four isolates, including F1 (Indiana C). They had no effect on egg quality, and only one F5 (IBH-2) had a minimal effect on egg production. Furthermore, it is quite common to isolate adenoviruses from uninoculated eggs or cell cultures derived from flocks with normal production (Cook, 1968; McFerran, unpublished observations).

c. Poor Food Conversion and Poor Growth. A growing complaint from meat chicken producers is poor food conversion or failure to achieve estimated weights for a given age. Schwartz (1975) has reported increased food consumption associated with adenovirus infection, while other workers (Cowen *et al.*, 1978a) found that birds infected with F5 (IBH-2) had decreased food intake. Grimes and King (1977b) recorded that an F8 (AAG5 (2a)) virus given intra-abdominally depressed body weight, and Cook (1974), using the same route, found that F2 caused a 70% mortality and a significant depression of weight gain in the survivors. Surveys of normal and affected broiler flocks in Northern

Ireland during the growing period yielded many isolates of adenovirus, but no differences in the types of serotypes or in the numbers isolated could be established.

d. Tenosynovitis. This condition is usually associated with reovirus infection (Olsen and Weiss, 1972). However, during routine isolations, both adenoviruses and reoviruses have been isolated from these affected joints, and many of these have been F1, which is not a common isolate in Northern Ireland (McFerran, unpublished observations). Grimes and King (1977a) also isolated an F1 from synovitis. Mackenzie and Bains (1976) isolated an adenovirus and *Staphylococcus aureus* from an outbreak of tenosynovitis, and Jones *et al.* (1978) studied 52 cases and isolated viruses from 20. Twelve of these isolates were reovirus, seven were adenovirus, and one was a mixture of both.

e. Respiratory Tract Disease. A number of groups have recorded the isolation of adenoviruses from birds with respiratory tract disease (Kawamura *et al.*, 1963; Rinaldi *et al.*, 1968; McDougall and Peters, 1974). In a study of 94 flocks with respiratory tract disease, adenoviruses were isolated from 44%, whereas 74 flocks without respiratory tract disease produced only a 15% recovery. Also, adenovirus was isolated from the lungs of the birds with respiratory tract disease (McFerran *et al.*, 1971a). Attempts at experimental reproduction of disease have given equivocal results. Some workers have produced respiratory tract disease or microscopic lesions only following natural routes of infection (Kawamura *et al.*, 1963; Aghakhan and Pattison, 1974). Other workers have been successful using only intratracheal inoculation (Lim *et al.*, 1973; McDougall and Peters, 1974) or have not reproduced disease (Kawamura and Horiuchi, 1964).

Following the major outbreak of respiratory tract disease in Northern Ireland, a successful program of infectious bronchitis vaccination was introduced. The number of cases of respiratory tract disease has fallen dramatically, while adenoviruses are still widespread. However, examination of the autopsy and virus records suggests that where adenoviruses were isolated from trachea, lung, or air sacs, signs, lesions, and mortality were more severe (McFerran, unpublished observations).

f. Disease Produced in Fowl by a Duck Adenovirus. In 1976 a new condition affecting laying hens was described in the Netherlands. This was characterized by a fall in egg production and by the production of soft-shelled and shell-less eggs, and the albumin quality was affected. Most cases were in birds around 30 weeks of age, and while the falls could be severe, they returned to normal production in 6–8 weeks (Van Eck *et al.*, 976).

A similar condition was described in Northern Ireland except that no effect was seen in the internal quality of the egg (McFerran *et al.*, 1977, 1978a). As in the Dutch birds, no illness was evident and all cases occurred between 26 and 35 weeks, with most between 29 and 31 weeks. Epidemiological evidence suggested that the virus was egg transmitted but otherwise had limited powers of lateral

spread. Viruses which agglutinated fowl erythrocytes and 17 fowl adenoviruses were isolated from affected flocks. One agent, 127, was selected for further study. The hemagglutinating agents were classified as adenoviruses on the basis of their biochemical properties (Todd and McNulty, 1978) and their physical, chemical, and growth characteristics (Adair et al., 1979b).

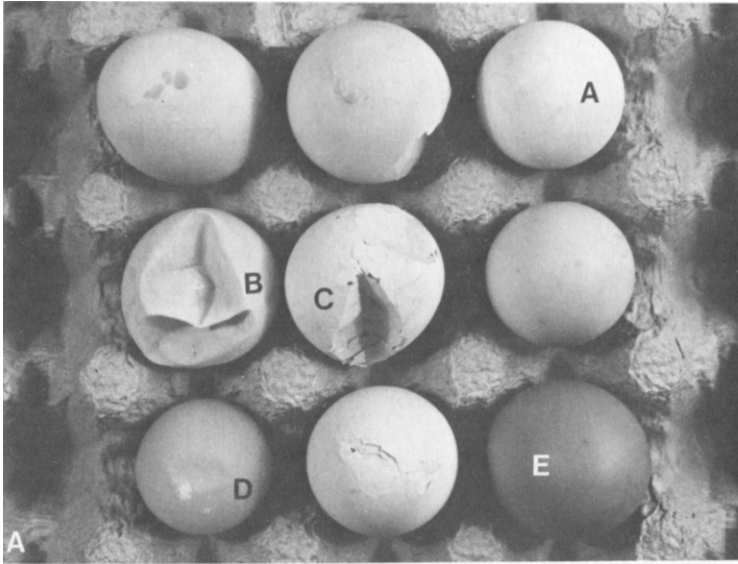
Although these viruses did not apparently share a group antigen with other avian adenoviruses using immunodiffusion techniques, the group antigen could be demonstrated by infecting birds previously infected with adenovirus with 127 virus and eliciting a recall of the group antigen (McFerran *et al.*, 1978b). This would explain the Dutch findings, which on the basis of the DID test indicated that fowl adenoviruses were associated with this syndrome (Van Eck *et al.*, 1976). A similar virus, BC14, was isolated in England (Baxendale, 1978). The disease can be experimentally reproduced using BC14 or 127 virus (Baxendale, 1978; McCracken and McFerran, 1978). The first changes were seen 7 days after oral infection with 127 virus, when loss of shell pigment occurred. This was followed by thinning of the eggshell and then on the ninth day by soft-shelled and shell-less eggs. Eggshell quality was worst at 13–16 days after infection, and the majority of birds had returned to normal egg quality by day 24 (Fig. 7a).

The origin of this virus is of interest. It grows better in cells of duck origin than in fowl cells, and it grows to very high titers in embryonated duck eggs but not in embryonated fowl eggs (Adair *et al.*, 1979c). Fowl sera prior to the importation of the infected birds did not have antibody to 127 virus (McFerran *et al.*, 1977). Duck flocks in England have antibody, and this virus has been isolated from them (Baxendale, 1978). Duck flocks but not fowl flocks in the United States also have antibody to 127 virus, and there was evidence that infected ducks did not readily infect fowl (Calnek, 1978). It seems reasonable to postulate that these viruses are duck adenoviruses. The problem of the widespread distribution of this virus in different breeds in Western Europe and its apparently poor ability to spread from flock to flock is best explained by contamination of a vaccine. It is not related to the prototype fowl or turkey adenovirus.

5. *Diagnosis*

It is preferable to use cell culture to isolate fowl adenoviruses. Liver and kidney cells are equally sensitive, but chick embryo fibroblasts are much less sensitive. Chick tracheal organ cultures are also not sensitive (Butler *et al.*, 1972). In addition to examining the organ with lesions, it is advisable to test the pharynx and feces because virus persists in these specimens longer and to higher titers. Normally, two passages of 6 days each are sufficient for isolation, although a third pass may be given (McFerran *et al.*, 1971a).

Embryonated eggs, although widely used, cannot be recommended for adenovirus isolation. Thus Burke *et al.* (1959) made only 3 isolations in embryonated eggs compared to 45 in cell culture. Kawamura *et al.* (1964), using their eight



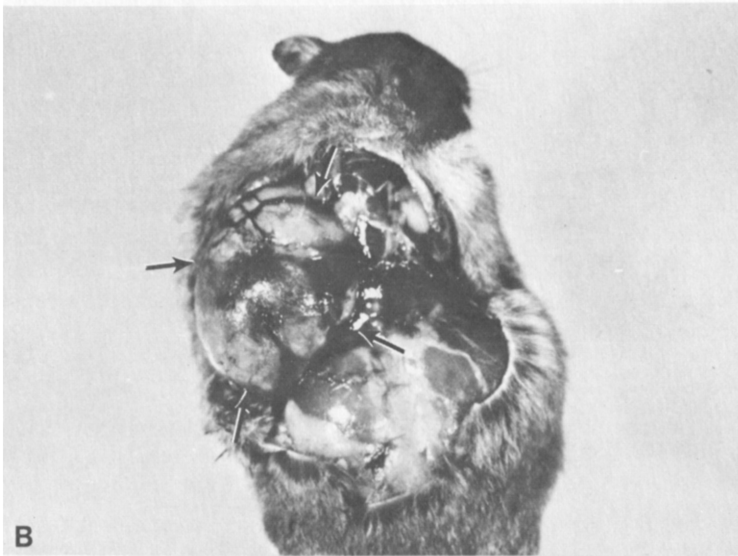


Fig. 7. (a) Abnormalities in eggshells following experimental infection of hens with 127 virus. (A) Loss of pigmentation. (B) Thin-shelled. (C) Cracked shell. (D) Shell-less. A normal egg is shown for comparison in E. (b) Tumor in hamster following subcutaneous inoculation with fowl adenovirus type 1.

serotypes, found that when high concentrations of virus were inoculated into the allantoic cavity only F1 (OTE), F5 (TR-22), and F6 (CR-119) killed embryos, and if more dilute suspensions were used only F1 killed embryos.

The DID test has been used for diagnosis and monitoring of specific pathogen-free (SPF) flocks for infection. It has the advantage of being inexpensive and quick, and it detects group antibody. McFerran *et al.* (1975) drew attention to the failure of SPF birds experimentally infected by a natural route with one serotype always to develop detectable antibody. This observation has been confirmed by others (Grimes *et al.*, 1977a; Yates *et al.*, 1977). Cowen *et al.* (1978b) found, when studying naturally infected flocks, that in spite of isolating many adenoviruses especially from the 8–14-week age group, the percentage of birds wtih detectable precipitin antibody rose steadily from 28% in 8–14-week-old birds to 97% in the 30–43-week age group. Apart from the inherent lack of sensitivity of the test, it is probable that more sensitivity could be achieved by using more than one antigen. Thus, although the fowl and turkey adenoviruses tested have a common immunoprecipitin antigen (McFerran *et al.*, 1975), it is clear from CF studies that this group antigen is not shared to the same extent by all strains (Kawamura *et al.*, 1964).

The most sensitive test in use is the SN test, and in its microtiter form (Grimes *et al.*, 1977a) it is very useful. Other tests, such as the passive hemagglutination test (Trewick and Lang, 1971; Moreau, 1974) and ELISA (Todd and McFerran, unpublished observations), have shown promise vut still need to be fully evaluated. The radial immunodiffusion test (Pereira *et al.*, 1972; McFerran *et al.*, 1975) is useful for surveys but detects only group antibody.

One of the main requirements for a sensitive test is to monitor SPF flocks producing eggs for vaccines and research. Often the first indication of infection is the degeneration of control cell cultures on first or second passage. In these circumstances, both cloacal swabbing of the flock and serological testing using the SN test have failed to detect the infected birds, but the use of the indirect immunofluorescence test has been successful (Adair and McFerran, unpublished observations).

J. Infection in Turkeys

1. Classification

Three classes of adenovirus infect turkeys. These are fowl adenoviruses, turkey adenoviruses, and the virus of turkey hemorrhagic enteritis.

a. Fowl Adenoviruses. Fowl adenoviruses type 1 have been isolated from turkeys. These viruses grow in embryonated egg and cell cultures of both fowl and turkey origin (Blalock *et al.*, 1975; Cho, 1976; King, 1977). An isolation made in Germany may also be F1 but was not fully characterized (Ahmed, 1971).

b. Turkey Adenoviruses. A number of workers have reported the isolation of one or more serotypes, but unfortunately comparative studies have not been carried out. Thus two serotypes (TAV1 and TAV2) were described in Northern Ireland (Scott and McFerran, 1972; McFerran *et al.*, 1975). Two serotypes (WTAV and AMK49) were described in the United States, and WTAV is similar to TAV1 (King and Hanson, 1975; King, 1977). The NC-120 isolate (Simmons *et al.*, 1976), and NC-J and NC-K isolates (Sutjipto *et al.*, 1977), and the MST virus isolated by Domermuth (Easton and Simmons, 1977) were compared (Sutjipto *et al.*, 1977). Three serotypes were recognized. The NC-120 and MST viruses were identical and were different from the NC-J and NC-K viruses. The turkey viruses studied share a group antigen with the fowl adenoviruses, are not neutralized by the prototype fowl antisera (McFerran *et al.*, 1975), and either do not grow or grow poorly in cells of fowl origin (Scott and McFerran, 1972; King, 1977).

c. Turkey Hemorrhagic Enteritis Virus (HEV). HEV has not yet been grown in embryonated eggs or cell culture; therefore, neutralization tests have not been carried out with other avian adenoviruses. The HEV virus and the virus associated with marble spleen disease (MSV) of pheasants are very closely related. They share a common antigen (Domermuth and Gross, 1975; Iltis *et al.*, 1975; Silim *et al.*, 1978). Marble spleen disease virus (MSDV) will infect pheasants and turkeys, producing lesions. These lesions could be prevented by the use of HEV antiserum (Domermuth *et al.*, 1975; Iltis *et al.*, 1975).

These viruses can be classified as adenoviruses on the basis of their size of 70–90 nm and their morphology (Carlson *et al.*, 1973; Fujiwara *et al.*, 1975; Tolin and Domermuth, 1975; Iltis *et al.*, 1977). Also, they are resistant to chloroform (Domermuth *et al.*, 1975) and have a density of 1.32–1.34 gm/ml in CsCl (Carlson *et al.*, 1974; Iltis, 1975). MSV contains DNA (Iltis, 1975), and both HEV and MSDV replicate intranuclearly in the cells of the reticuloendothelial system, primarily in the spleen (Itakura and Carlson, 1975; Fujiwara *et al.*, 1975). There are conflicting reports in the literature on whether they share a group antigen with avian and mammalian adenoviruses. Iltis *et al.* (1977) claimed that MSDV shares an antigen with TA1 and TA2 turkey adenoviruses. However, other workers have failed to show this relationship with fowl adenoviruses (Jakowski and Wyand, 1972; Silim *et al.*, 1978).

2. *Distribution*

Antibody to turkey isolates is widespread in the United States (Simmons *et al.*, 1976), and in Northern Ireland 70% of 20-week-old birds had antibody to TAV1 (Scott, 1974).

HEV has been recorded in the United States, Canada, Japan, Australia, India, and Israel (Domermuth and Gross, 1978), and antibody has been found in all flocks tested, although only half had signs of disease (Beasley and Wisdom, 1978).

3. *Epidemiology and Pathogenesis*

Scott (1974) infected turkeys by a combined oral–intranasal route. Virus was reisolated from most organs on day 4, suggesting a viremia. Virus persisted in the respiratory tract until the seventh day and in the alimentary tract and feces until the eleventh day after infection. From experience gained in growing kidney cell cultures derived from embryos and young poults, obtained from commercial sources, it is clear that turkey adenoviruses can be transmitted in the embryo and can remain latent in kidney cells.

Although viruses were isolated from turkeys aged 10 days to 6 months (Scott and McFerran, 1972), antibody studies indicate that birds 4–6 weeks of age are fréquently devoid of antibody. However, this was present in 10-week and older birds (Scott, 1974).

HEV occurs in 6–12-week-old birds and is most common in 10–12-week-old birds (Domermuth and Gross, 1978), but it has been seen in 2.5-week-old birds (Harris and Domermuth, 1977). It appears that the resistance of young poults to infection is not due to maternal antibody (Beasley and Wisdom, 1978). Turkeys are susceptible to oral infection with HEV (Gross and Moore, 1967). Following infection there is a viremia, and the highest virus titers are found in the spleen (Domermuth *et al.*, 1972). The turkey is susceptible to oral infection (Gross and Moore, 1967). There is no evidençe of egg transmission or vectors, and it is considered that infection persists in the litter between crops of birds (Domermuth and Gross, 1978). HEV will infect fowl if given intraperitoneally or orally, and fowl kept in contact with infected fowl also become infected. Lesions comparable to those seen in turkeys develop (Silim *et al.*, 1978).

4. *Association with Disease*

a. None. Conventional fowl and turkey adenoviruses have been isolated from clinically normal turkeys (Scott and McFerran, 1972; Cho, 1976). Using turkey strains to infect turkeys, no evidence of disease could be obtained (Scott, 1974; Simmons *et al.*, 1976; Sutjipto *et al.*, 1977). Similar results were obtained with F1 virus (Yates and Fry, 1957).

b. Respiratory Disease. Viruses have been isolated from the respiratory tract of turkeys with respiratory tract disease (Scott and McFerran, 1972; Blalock *et al.*, 1975; Simmons *et al.*, 1976; Sutjipto *et al.*, 1977). Blalock *et al.* (1975) elicited a respiratory response when their F1 isolate was inoculated into turkeys. Cho (1976), using another F1 isolate, claimed to have produced hepatitis, respiratory tract disease, bursal atrophy, and growth depression in both chicks and poults. However the possibility of infectious bursal disease (IBD) virus infection was not eliminated since the affected organs were not examined by immunofluorescence or electron microscopy. Therefore the possibility exists that adenoviruses alone were not responsible for these lesions. Adenoviruses were isolated from a severe upper respiratory tract disease of young turkeys. But attempts to repro-

duce this condition have failed, although mild disease was produced with some strains (Simmons *et al.*, 1976; Dillman and Simmons, 1977; Sutjipto *et al.*, 1977). Page *et al.* (1978) have shown that these birds had antibody to IBD virus, which had not previously been recognized in turkeys, and suggested that turkeys might be infected with IBD, which could interfere with the immune response. This suggestion was opportune, as IBD virus has now been isolated from turkeys (McNulty *et al.*, 1979).

c. Other Conditions. Isolates have been made from birds with diarrhea and depressed egg production, but their significance is unknown (Scott and McFerran, 1972; King, 1977).

d. Hemorrhagic Enteritis. This condition was first recognized in turkeys in Minnesota (Pomeroy and Fenstermacher, 1937). Following a 5–6-day incubation period, there was sudden depression and thirst, and blood was seen in the feces. Mortality varied from nil to 60% (Gross and Moore, 1967). Experimental infection resulted in mortalities of 0–80%, depending on the virulence of the virus (Domermuth and Gross, 1978). Death occurred in a few hours or the birds recovered. The pathology has been described by a number of authors (Gross, 1967; Itakura and Carlson, 1975; Fujiwara *et al.*, 1975). The main lesions were splenomegaly, congestion of the small intestine, and hemorrhagic contents of the intestine. Microscopically, there was an acute hemorrhagic enteritis, degenerative changes of the lymphoid tissue, and proliferation of reticuloendothelial cells in all areas. Intranuclear inclusions were seen in the large mononuclear cells in visceral organs and in reticular cells around sheathed arteries of the spleen. Death was probably due to bleeding from damaged capiallaries in the tips of the villi. The HEV or a similar virus of chickens has been isolated from the enlarged spleens of birds condemned for Marek's disease (Domermuth, 1979).

4. *Diagnosis*

To isolate turkey adenoviruses, as opposed to fowl adenoviruses affecting turkeys, it is necessary to use turkey embryo kidney or liver cells or turkey kidney cells. Turkey fibroblasts are not sensitive (McFerran, unpublished observations). Some turkey adenoviruses can be isolated on the first or second passage (Scott, 1974). But other viruses required up to nine passages in cell culture before isolation (Simmons and Gray, 1976). Although initial isolations were made in turkey kidney cells (Simmons *et al.*, 1976), in a comparative study, turkey embryo liver cells but not turkey kidney cells detected virus on initial isolation. Both cells were sensitive on subsequent passage (Simmons and Gray, 1976). With these slow-growing adenoviruses, turkey adenoviruses had to be neutralized with antibody. Acridine orange was found to be helpful in detecting viral presence (Simmons and Gray, 1976). As suggested for the bovine adenoviruses, FA staining might be of major help.

HEV has not been grown in cell culture or embryonated eggs (Domermuth *et*

al., 1972; Carlson *et al.*, 1974). Therefore virus isolation involves inoculation of turkeys. Turkeys 6–10 weeks old are infected orally with feces or intravenously with spleen extract of affected birds. Deaths should occur 3 days after intravenous or 5–6 days after oral infection. Birds not dying should have enlarged spleens. These spleens or spleens from dying birds can be used as an antigen in a DID test (Domermuth *et al.*, 1973). Birds not dying will develop precipitating antibody after 2 weeks, and peak titers are reached around 4 weeks.

K. Diseases of Pheasants

1. Classification

An adenovirus has been isolated from pheasant chicks and identified as an adenovirus by the presence of group antigen (Cakala, 1966). The relationship of MSDV to the adenovirus group and to turkey hemorrhagic enteritis has already been considered.

2. Distribution

MSD has been recognized in Italy (Mandelli *et al.*, 1966), Canada (Carlson *et al.*, 1973), England (Bygrave and Pattison, 1973), and the United States (Wyand *et al.*, 1972).

3. Epidemiology and Pathogenesis

In view of its close relationship to HEV, it is likely that MSD behaves in a similar fashion. Pheasants 4–8 months old are commonly affected, but MSD can occur in younger birds (Wyand *et al.*, 1972; Bygrave and Pattison, 1973).

4. Association with Disease

The disease in pheasants produced by MSDV resembles the disease in turkeys produced by HEV, except that MSDV does not produce gut lesions in pheasants or turkeys and death appears to be due to pulmonary edema. Usually birds are found dead. At autopsy, splenomegaly with varying confluent gray foci of necrosis (marble spleen) and intensely congested and edematous lungs were observed. Focal necrosis around the tertiary bronchi of the lungs and of the germinal follicles of the spleen was observed. Masses of pale eosinophilic fibrinoid-type material, not amyloid, was seen around blood vessels and the areas of necrosis (Wyand *et al.*, 1972; Bygrave and Pattison, 1973; Carlson *et al.*, 1974). Eosinophilic and basophilic inclusions were seen in the lung and spleen and eosinophilic inclusions in some liver cells. It is interesting that in experimental transmission studies, neither death nor the full spectrum of MSD lesions was found. This led Iltis *et al.* (1975) to suggest that some synergistic factor must be required.

5. *Diagnosis*

The history and pathological signs are usually sufficient. However, the techniques suggested for HEV are suitable.

L. Infection in Other Avian Species

Quail bronchitis was described in bobwhite quail *(Colinies virginianus)* by Olson (1950). This condition has a short incubation period and sudden onset in young birds. Mortality can reach 100%, and it is highly contagious. The signs are rales, coughing, depression, nervous signs, and in some birds conjunctivitis (Olson, 1950; Dubose *et al.*, 1958). The virus isolated from this condition has been shown to be identical to F1 (Yates and Fry, 1957; Dubose and Grumbles, 1959).

F1 has also been isolated from guinea fowl with pancreatitis (Pascucci *et al.*, 1973). F2 and F8 have been isolated from budgerigars, F5 from a mallard duck, and F8 from pigeons (McFerran *et al.*, 1976b).

It is interesting that fowl adenoviruses have been isolated from a number of avian species. It is not clear if this means that they share one group of viruses or if the failure to detect other avian viruses simply reflects the failure to use homologous cell cultures. The second reason appears more feasible, because turkeys are infected with turkey adenoviruses in addition to fowl adenoviruses. The turkey viruses do not grow in fowl cells and are unrelated to fowl adenovirus serotypes.

V. PROPHYLAXIS

Vaccines are mainly used in canine and to a lesser degree in bovine and avian medicine. Both inactivated and attenuated vaccines are used for the control of ICH in dogs. The attenuated vaccines have proved very effective. However, not only is the vaccine virus excreted in the urine, but it may result in focal interstitial nephritis (Appel *et al.*, 1973). In addition, it may cause corneal edema, which is usually transient, provided corticosteroids are not used. One study suggests that over 0.4% of vaccinated dogs may have "blue eye" following vaccination (Curtis and Barnett, 1973). The immunity following inactivated vaccine appears less effective, and this has led to some manufacturers to recommend repeated revaccination. This procedure could lead to the development of circulating immune complexes and glomerulonephritis, as suggested by Murray and Wright (1974), but there is no direct evidence for this. Because A26/61 has been associated only with lesions of the respiratory tract, it has been suggested that an attenuated strain would be suitable for protection of dogs against both ICH and ILT (Appel *et al.*, 1973). A commercial A26/61 vaccine is now in use.

Some work has been carried out using both inactivated and attenuated vaccines in the control of adenovirus infection in cattle (Tribe *et al.*, 1969; Burki, 1973; Bartha, 1974). Bartha (1974) has suggested that immunization with B1 and B8 would provide protection against the other members of the subgroups, but this remains to be confirmed. A temperature-sensitive mutant of B3 has also been produced (Zygraich *et al.*, 1976).

An inactivated vaccine prepared against the hemagglutinating adenovirus associated with falls in production has been prepared (Baxendale, 1978), and it appears to be effective.

VI. GENERAL CONSIDERATIONS

Although not enough information is available to make valid judgments, it would appear that some of the adenoviruses discussed tend to grow in the upper respiratory tract and others to colonize the intestines. Norrby *et al.* (1976) suggest that vertical transmission of adenoviruses does not occur. This may be true for human adenoviruses, but is not true for the animal viruses. In many species, and especially in the fowl, vertical transmission may be the main factor in the epidemiology.

Some of the adenoviruses discussed are clearly associated with disease, but the majority appear to be normally not associated with disease and require the presence of some as yet undefined factor(s) before they cause recognizable signs and lesions.

Surprisingly differences are found in the ease of isolating adenoviruses from different species. Thus it is easy to isolate fowl and canine adenoviruses and difficult to isolate bovine and porcine adenoviruses. Apart from inherent differences in the ability of adenoviruses to replicate in cell cultures, other factors may be important. It appears that they grow best in primary epithelial cells from the homologous species, and with some types (e.g., the group 2 bovines) these epithelial cells must be from certain organs. Some of the differences reported may be due to the percentage of epithelial cells in the cultures. Cells grown from bovine testes or embryo fowl liver can all too often end up as fibroblast cultures. Furthermore, the cells may contain latent adenovirus or, in the case of bovine cells, latent or noncytopathic bovine viral diarrhea (BVD) virus. Furthermore, BVD virus may be introduced into other cell lines through the use of contaminated fetal calf serum. As a first step, all cultures should be tested for the presence of BVD virus by immunofluorescence. It may also be advisable, especially where low titers of stock pools are found, to treat them with chloroform to remove possible contaminants. Adeno-associated parvoviruses can also cause a major reduction in the titer of pools and, where possible, should be removed.

One of the problems with adenoviruses of certain species is the time required to identify the adenovirus effect on cell cultures, as for example, the five blind

passages in calf-tested cells to isolate a B8 strain (Rondhuis, 1973). It is probable that the use of immunofluorescence would help to reduce the time. Furthermore, in investigating major syndromes, convalescent serum can be used in the indirect immunofluorescence test to aid in viral detection. Convalescence or pooled serum collected from old animals can also be used most successfully for immuno-electron microscopy (McFerran and McNulta, 1978c [Fig. 8]). Possibly not enough attention has been paid to the metabolism of the cells, since for the growth of a number of strains, actively dividing cells are required.

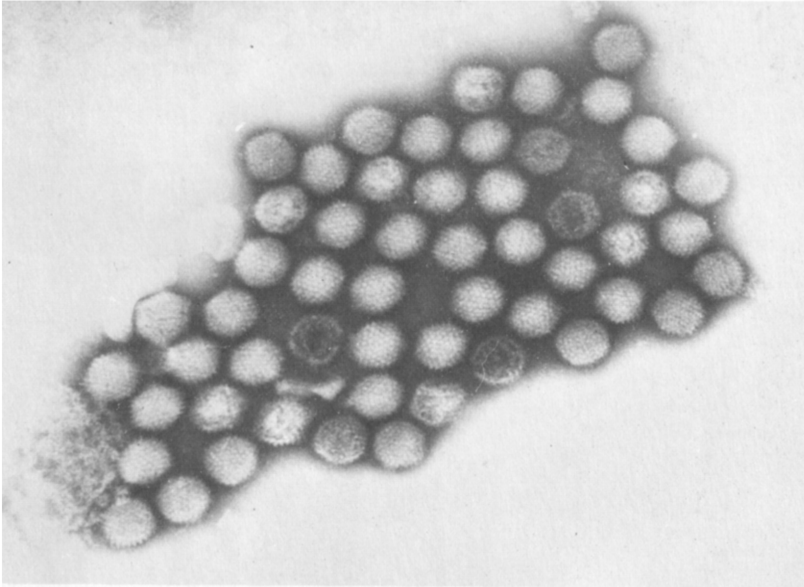


Fig. 8. Immune electron microscopy. Ovine adenovirus clumped by "old animal serum."

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