PHYSICAL, CHEMICAL, AND BIOLOGICAL PROPERTIES OF THE INCOMPLETE PARTICLES OF HUMAN ADENOVIRUS TYPE 3

by

BETTY JEAN ROSE

B.A., Kansas State College, 1973

A MASTER'S THESIS

Submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Division of Biology

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1977

Approved by:

Byron T. Burlington

ACKNOWLEDGMENTS

I wish to thank Dr. Brian Spooner, Dr. Richard Consigli, Dr. Ronald Slesinski, and Dr. Vernon Bode for use of equipment necessary for this work. I am also grateful to the technicians who provided the numerous liters of medium and mounds of sterile glassware expended during my three years in the laboratory.

My sincerest thanks are extended to Dr. Byron Burlingham for maintaining faith in my ability to complete this degree, for providing insights into the directions of my research and into the world of science, and for returning to Kansas State University for the defense of this work.

Partial financial support for this work was provided by the Kansas Division of the American Cancer Society grant 1266, and by HEW grants CA 15620 and CA 15506.

PHYSICAL, CHEMICAL, AND BIOLOGICAL PROPERTIES OF THE INCOMPLETE PARTICLES OF HUMAN ADENOVIRUS TYPE 3

by

BETTY JEAN ROSE

B.A., Kansas State College, 1973

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Division of Biology

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1977

ABSTRACT

When KB cells are infected with adenovirus type 3 and the lysates of the infected cells are subjected to equilibrium density sedimentation in CsCl, several populations of particles are observed as light scattering bands by transillumination. These populations of virus particles have distinct buoyant densities. Analyses of these populations of particles in the ultracentrifuge indicate that the buoyant densities are 1.2787, 1.2913, 1.2945, 1.3030, 1.3139, 1.3315, and 1.3595 g/cm^3. The bands of particles are designated I, IIa, IIb, III, IV, V, and VI respectively. The virions are band V. The remaining bands are incomplete particles. The virions of adenovirus type 3 are less dense than the virions of adenovirus type 2.

Optical density determinations at 260 and 280 nm of CsCl equilibrium gradients indicates the presence of additional populations of incomplete particles which were not detected in the analytical ultracentrifuge. These bands are designated as IIIa and Va.

Buoyant densities and OD_{260}/OD_{280} ratios indicate that all of the incomplete particle populations contain nucleic acid. DNA is extractable from incomplete particles (II) and virions (V). The DNA from incomplete particles hybridizes better with adenovirion DNA than with KB cell DNA. Incomplete particle DNA hybridizes better with KB cell DNA than does adenovirus type 3 virion DNA suggesting either that host cell DNA may be present or that incomplete particle DNA contains sequences which are complementary to KB cell DNA.

The incomplete particle populations are produced in constant proportions with repeated passages except for band Va which increases with passage number.

The DNA of incomplete particles and virions is present within the capsids. A portion of both types of particles is resistant to DNase digestion. However, more of the nucleic acid in incomplete particles than in virions is susceptible to digestion by this enzyme, and the digestion of the DNA in incomplete particles occurs more slowly. These observations suggest differences in packaging and/or structure of incomplete particles and virions. The specific radioactivity is lower for incomplete particles than it is for virions. This might indicate that parental viral DNA is incorporated into the incomplete particles.

The biological role(s) of the incomplete particles remains unspecified. These particles may be degradation products of the viral replication process. They may have a role in viral assembly serving as precursors to infectious virions. Incomplete particles may serve to determine transforming ability and oncogenic potential of a virus population.

TABLE OF CONTENTS

Physical, Chemical, and Biological Properties of the Incomplete Particles of Human Adenovirus Type 3

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES - continued

INTRODUCTION

I. The Adenoviruses

A. Isolation

Adenoviruses were originally isolated by Rowe et al. (1953) from human adenoid tissue grown in culture and by Hilleman and Werner (1954) from respiratory secretions of army recruits with primary atypical pneumonia. Since these initial isolations, over 80 different serotypes of adenovirus have been isolated from human, simian, bovine, porcine, canine, murine, and avian sources.

B. Classification

Each serotype of adenovirus (except for avian adenoviruses) has a group specific antigen which cross reacts with all other types of adenovirus within the group. The group specific determinants reside on the hexon subunits (Klemperer and Pereira, 1959). The type-specific antigenic determinants of the adenovirion are localized on the penton fiber (Klemperer and Pereira, 1959; Philipson, 1960).

Rosen (1960) divided the serotypes of adenovirus into three subgroups by their ability to agglutinate Rhesus monkey and/or rat red blood cells. Green and Pina (1963, 1964) divided the human adenoviruses into three subgroups by their ability to induce tumors in newborn hamsters. There is no apparent correlation between the subgroups distinguished by serotyping, hemagglutination, or oncogenic potential. However, the oncogenic potential does correlate with guanine-cytosine content of the viral DNA (Pina and Green, 1965). The members within a subgroup based on oncogenic potential are more closely related with

each other than with members of either other oncogenic subgroup (Garon et al., 1973).

C. Physical Properties

1. Morphology

The morphology of the adenovirus capsid and subunits are illustrated in Fig. 1. Adenovirus particles are icosahedrons approximated by a diameter of 60-80 nm (Horne et al., 1959). Adenovirions are composed of 11-13% DNA and 87-89% protein. The virion is subdivided into a capsid containing 252 capsomeres (Horne et al., 1959; Wilcox et al., 1963; Dales, 1962) and a dense central core (Morgan et al., 1956). The facets of the capsid have six-fold symmetry, each with 12 hexons and thus each capsid is composed of 240 hexon subunits (Ginsberg et al., 1966; Pettersson et al., 1967). The 12 penton subunits have five-fold symmetry, are found at the vertices of the icosahedron, and consist of a penton base and a fiber with a terminal knob (Valentine and Pereira, 1965; Norrby, 1966; Pettersson et al., 1968). The length of the penton fiber is a unique characteristic of each serotype of adenovirus (Pettersson et al., 1968).

2. DNA

Adenovirus DNA is a double stranded molecule with no single-stranded breaks (Green et al., 1967; Van der Eb et al., 1969; Doerfler and Kleinschmidt, 1970). The DNA extracted from many serotypes of adenoviruses were characterized according to buoyant density and sedimentation coefficients by Green (1962), Green and Pina (1963) and Green et al. (1967). The molecular weight of the various adenovirus genomes was calculated to be 20-25 x 10^6 daltons (Green et al., 1967; Van der Eb

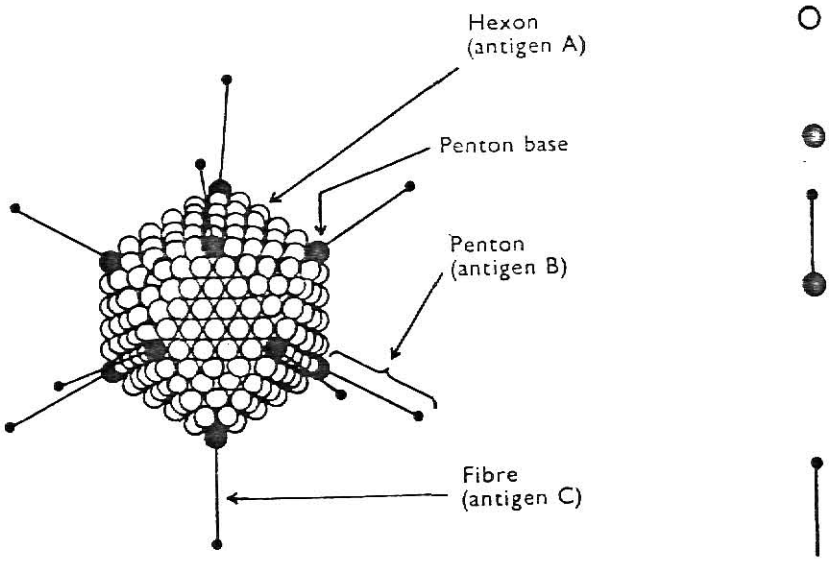

FIGURE 1. Morphology of the adenovirus capsid. The illustration shows the icosahedral structure of the capsid and shows the relationship of adenovirus antigens to the capsid.

From Russell et al. (1967).

et al., 1969; Green et al., 1970). This is sufficient genetic material to code for 25-50 average-sized proteins.

DNA released from virions under gentle conditions has a circular configuration (Robinson et al., 1973; Brown et al., 1975). Since these circular forms were readily converted to linear molecules by treatment with SDS, pronase, trypsin, or α-chymotrypsin, the ends of the linear DNA molecules are probably associated by a protein linker within the virion (Robinson et al., 1973).

Denaturation and self-annealing of highly purified adenovirus DNA molecules results in single-stranged, circular forms (Garon et al., 1972; Wolfson and Dressler, 1972; Roberts et al., 1974). These circles are due to inverted terminal repetition. Garon et al. (1972) reported that the length of the repeated sequence varied with the serotype of adenovirus.

Burnett and Harrington (1968) reported that DNA extracted from simian adenovirus 7 causes productive infection and oncogenic transformation. The DNA extracted from human Ad 1 is infectious (Nicolson and McAllister, 1972). Grahm and Van der Eb (1973a, b) reported that 1-10 plaque forming units and one transformed cell focus was caused per μg of DNA (1.5×10^9 molecules) extracted from Ad 5.

3. Proteins

The polypeptide composition of adenovirions has been analyzed by SDS-polyacrylamide gel electrophoresis. Maizel et al. (1968a, b) described 9 polypeptides ranging from 7,500 to 120,000 daltons for adenovirus types 2, 7 and 12. However, as many as 15 polypeptides have now been associated with virions of Ad 2 (Maizel, 1971; Everitt et al., 1973;

Anderson et al., 1973). Eight of these polypeptides are antigenically distinct and present in specific structures of the virion as shown in Fig. 2 (Everitt et al., 1973; Everitt and Philipson, 1974). Polypeptide II forms the hexon, III forms the penton base, and IV forms the fiber. Polypeptides VI and possibly VIII are hexon associated proteins. Polypeptide IX is specifically associated with groups of nine hexons. Polypeptides V and VII are associated with the core.

D. Productive Infection

1. Adsorption and penetration

The growth cycle of adenoviruses begins with adsorption to permissive cells. The virions attach to specific receptors located on the plasma membrane of the host cell (Philipson et al., 1968). There are about 1×10^4 adenovirus receptors per KB cell (Philipson et al., 1968). Levine and Ginsberg (1967) reported that Ad 5 fiber reduces the number of virions adsorbed to KB cells. Philipson et al. (1968) demonstrated that purified Ad 2 fiber specifically inhibits Ad 2 attachment to the cell receptors. Electron micrographs verified that the distance between adsorbed virions and the plasma membrane is roughly equivalent to the length of the fiber (Chardonnet and Dales, 1970a, b). These observations indicate that the fiber attaches the adenovirion to cell receptors.

The mechanism involved in adenovirus penetration of the cell membrane is controversial. Penetration is postulated to occur by pinocytosis (Dales, 1962; Chardonnet and Dales, 1970a), by direct penetration of the plasma membrane (Lonberg-Holm and Philipson, 1969; Morgan et al., 1969; Brown and Burlingham, 1973) or by a combination of the two processes (Morgan et al., 1969).

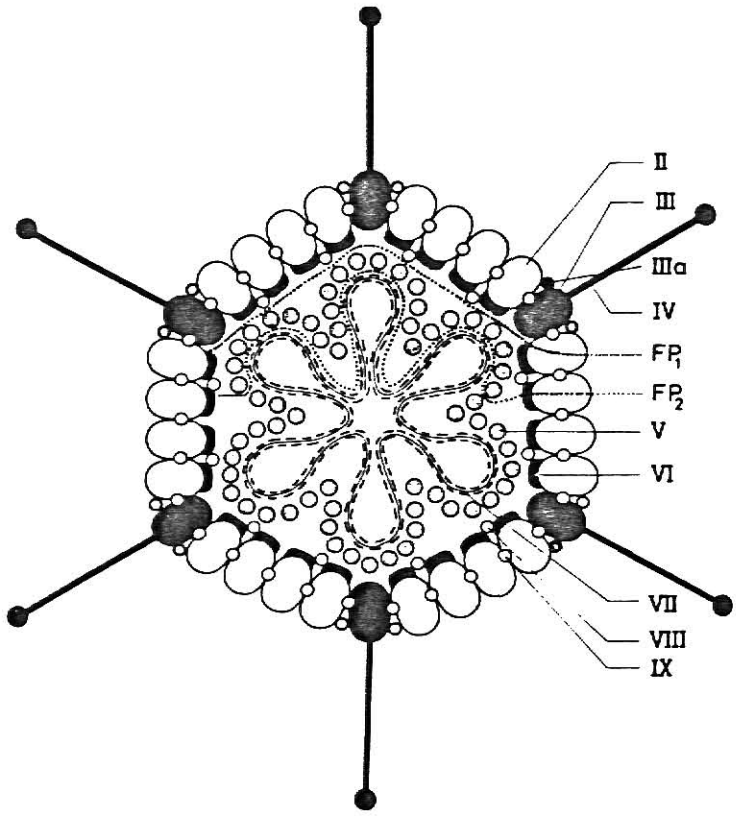

FIGURE 2. Location of structural polypeptides within the adenovirion. The Roman numerals refer to the viral polypeptides (Maizel, 1971). The locations of the fracture planes (FP_1 and FP_2) were derived from electron microscope data. The arrangement of the viral DNA molecule does not presuppose any definite organization and the loops extending into each of the substructures represent tightly packed coils of viral DNA.

From Brown et al. (1975).

2. Uncoating

Uncoating releases the DNA from the virion. Uncoating is usually measured by determining the susceptibility of the viral DNA to degradation by DNase. The mechanisms involved with adenovirion uncoating are not understood, although the process has been described by electron microscopy, physical, and chemical techniques. Within minutes after adsorption, virions which contain DNA susceptible to DNase appear in the cytoplasm (Sussenbach, 1967; Lonberg-Holm and Philipson, 1969; Morgan et al., 1969; Brown and Burlingham, 1973). Indirect arguments suggest that these cytoplasmic adenovirions have lost about 5% of their total protein. This loss is thought to be the penton subunits (Sussenbach, 1967). The hexon capsomeres remain at the nuclear membrane as the cores enter into the nucleus (Morgan et al., 1969; Chardonnet and Dales, 1972). Intact adenovirus DNA is freed into the nucleoplasm as the final event of uncoating (Lonberg-Holm and Philipson, 1969). The uncoating mechanism does not require DNA synthesis or protein synthesis and therefore must be mediated by pre-existing cellular or viral enzymes (Lawrence and Ginsberg, 1967; Philipson, 1967).

3. Synthesis

Two phases of virus controlled macromolecular synthesis occur during productive infection with adenovirions. The early phase precedes viral DNA replication. During the early phase only about 40% of the viral genome is transcribed (Tibbetts et al., 1974). The late phase begins with viral DNA replication and includes the synthesis of structural peptides of the adenovirion.

a. RNA. Viral RNA transcription begins immediately after the viral DNA reaches the nucleus (Lindberg et al., 1972). The adenovirus

RNA extracted from the nuclei is very heterogeneous in size (Parsons and Green, 1971; Wall et al., 1972) but the polysome associated viral RNA molecules are smaller and occur in discrete size classes (Parsons and Green, 1971; Lindberg et al., 1972; Craig and Raskas, 1974). Each of the size classes contain several species of mRNA (Craig et al., 1975; Philipson et al., 1974).

Adenovirus specific mRNA extracted from the cytoplasm during early and late phase contain polyadenylic acid sequences. Polyadenylation apparently occurs in the nucleus as part of post-transcriptional processing (Philipson et al., 1971). Inhibition of polyadenylation blocks transport of adenoviral mRNA into the cytoplasm (Philipson et al., 1971). Thus the addition of a poly-A sequence appears necessary for processing and transport of adenoviral mRNA into the cytoplasm (Darnell et al., 1971).

Adenovirus specific RNA is probably transcribed by cellular enzymes. RNA polymerase II, which synthesizes heterogeneous nuclear RNA in uninfected cells, appears to transcribe both early and late adenovirus specific RNAs. In addition, RNA polymerase III transcribes the 5.5 S adenovirus associated RNA (Weinmann et al., 1974).

About 10 times more viral mRNA accumulates in the cytoplasm during the late phase than during the early phase (Green et al., 1970; Philipson et al., 1973). Late transcription is blocked by either inhibitors of protein synthesis (Green et al., 1970) or RNA synthesis (Flanagan and Ginsberg, 1962). During late phase, a 5.5 S species of RNA is synthesized in large amounts. This "virus associated RNA" (VA RNA) is transcribed from adenoviral DNA (Ohe, 1972). Although its function is unknown, the 5.5 S RNA is intermittently associated with polyribosomes (Baum and Fox, 1974).

Hybridization mapping of the adenovirus mRNA extracted from productively infected cells has established that most of the late mRNA is complementary to the l-strand while the early mRNA hybridizes to both the h- and the l-strand (Landgraf-Leurs and Green, 1973; Patch et al., 1972). Tibbets et al. (1974) determined that 25-30 percent of early mRNA hybridizes to the l-strand and 10-15 percent hybridizes to the h-strand. But 65-70 percent of late mRNA hybridizes to the l-strand and 25 percent hybridizes to the h-strand. In contrast, Pettersson and Philipson (1974) determined that 85 percent of late nuclear RNA hybridized with the l-strand but only 65-70 percent of late cytoplasmic RNA hybridized with the l-strand. Therefore, not all of the transcribed viral RNA is transported to the cytoplasm.

Initiation and termination sites of RNA synthesis have not been determined in vivo. In vitro experiments indicate that E. coli RNA polymerase initiates synthesis at 5-10 preferred sites (Pettersson et al., 1974).

b. DNA. Viral DNA synthesis begins 6 to 8 hours after infection and reaches the maximum rate of synthesis at about 13 hours after infection (Ginsberg et al., 1967; Pina and Green, 1969). Nothing is known about initiation of adenovirus DNA replication or about the enzymes involved. Once adenovirus DNA synthesis starts, it is not affected by inhibitors of protein synthesis (Horwitz et al., 1973). No virus specific DNA polymerase has yet been isolated. Autoradiography suggests that the site of replication is in the nucleoplasm rather than at the nuclear membrane. The non-replicating adenovirus DNA molecules preferentially collect at the periphery of the nucleus (Vlak et al., 1976; Vlak et al., 1975; Shiroki et al., 1974). Two adenovirus DNA binding

proteins have been identified. These proteins have molecular weights of 72,000 and 48,000 daltons and both bind preferentially to single-stranded viral DNA (Van der Vliet and Levine, 1973). They suggest that these proteins bind to single-stranded DNA intermediates during viral replication.

Several groups of investigators have observed that pulse labeled, replicating viral DNA molecules have a higher buoyant density than mature adenovirus DNA molecules. The labeled higher density DNA species could be chased into mature adenovirus DNA molecules (Pearson and Hanawalt, 1971; Sussenbach et al., 1972; Van der Eb, 1973; Pettersson, 1973). The density of the replicating DNA molecules was shifted to the density of mature DNA by treatment with nucleases specific for single-stranded DNA but not with ribonuclease (Pettersson 1973). These observations suggested that adenovirus DNA replicates through single-stranded intermediates.

Horwitz (1971) found no evidence of Okazaki fragments during adenovirus DNA replication. However, Bellett and Younghusband (1972) reported a 12 S "Okazaki-like" fragment during replication of CELO virus DNA. Winnacker (1975) described "Okazaki" fragments during replication of Ad 2 DNA in isolated nuclei from infected cells.

Electron microscopic studies of replicating adenovirus DNA have illustrated three types of adenovirus DNA molecules: (1) a Y-shaped molecule having an arm of single-stranded DNA of variable length, (2) linear molecules of unit length, either single-stranded or partially single-stranded, (3) double-stranded molecules of unit length (Sussenbach et al., 1972; Van der Eb, 1973).

Recently, extensive studies of adenovirus DNA replication have been undertaken in isolated nuclei. Under these experimental conditions, DNA molecules which have initiated prior to isolation of the nuclei continue to elongate, but no new initiation occurs. The results of experiments performed by Sussenbach and co-workers (Van der Vliet and Sussenbach, 1972; Sussenbach et al., 1972; Sussenbach et al., 1973; Ellens et al., 1974; Sussenbach and Van der Vliet, 1973) has led to the proposal of one model for adenovirus DNA replication. This model proposes that DNA replication begins at the right end of the molecule in the AT rich region. The parental l-strand is displaced by the new progeny strand which is replicating in the 5' → 3' direction. This gives rise to Y-shaped intermediates. Later, synthesis begins on the displaced strand detached from the replicating structure. The displaced strand serves as a template for discontinuous synthesis to form a double-stranded molecule. This model has difficulty in explaining how the 5' end of the daughter strand is completed after degradation of an RNA primer. Bellett and Younghusband (1972) have proposed that the 5' end of the daughter strand may be completed by formation of concatemers followed by staggered cleavage of both strands at genome intervals.

More recent experimental data disagrees with the model proposed by Sussenbach. Single-stranded DNA isolated from infected cells hybridizes equally well with both strands of DNA, indicating that both strands are exposed during replication (Lavelle et al., 1974; Tolun and Pettersson, 1975; Schilling et al., 1975). Each strand replicates in the 5' → 3' direction at similar rates but starting at opposite ends of the molecule. These observations support a model in which DNA synthesis is initiated at either end of the DNA molecule. Initiation could occur

simultaneously at both ends of a given molecule or randomly at either end in different molecules. Since no initiation occurs in isolated nuclei, the in vitro models must be interpreted with caution.

c. Proteins. The early virus coded proteins are difficult to identify. However, the T-antigen has been purified (Gilead and Ginsberg, 1968a, b; Tockstein et al., 1968) and characterized (Russel et al., 1967a; Shimojo et al., 1967; Gilead and Ginsberg, 1965). The P-antigen has also been detected (Russell and Knight, 1967) and may be a complex of the T-antigen and core protein (Russell and Skehel, 1972). Bablanian and Russell (1974) studied early peptides while host cell protein synthesis was suppressed with polio virus in medium containing guanidine. They described an early peptide with a molecular weight of 64,000 which may be the major component of the P-antigen. No virus specific enzymes have been definitely identified as early proteins. An early non-structural virus specific protein (E2) has been reported (Ishibashi and Maizel, 1974b).

Late protein synthesis begins 8-10 hrs after infection (Wilcox and Ginsberg, 1963a). DNA synthesis is required since cytosine arabinoside, hydroxyurea, or 5-fluorodeoxyuridine inhibit late protein synthesis (Green, 1962b; Flanagan and Ginsberg, 1962; Wilcox and Ginsberg, 1963b). The capsid subunits are found in large excess while the core proteins appear in more restricted amounts (White et al., 1969; Everitt et al., 1971). After synthesis in the cytoplasm, the viral peptides are transferred to the nucleus for assembly (Velicer and Ginsberg, 1970; Horwitz et al., 1969). Only 5-20% of the capsid proteins are incorporated into mature virions (Green et al., 1962b; Horwitz et al., 1969).

Two glycosylated adenovirus proteins have been described, the fiber polypeptide and E2 (Ishibashi and Maizel, 1974b). Glycosylation probably occurs subsequent to translation. The fiber protein is also phosphorylated, but the major phosphorylated species is the 64,000 molecular weight protein described by Russell and Skehel (1972), and Russell et al., 1972b).

Recent evidence indicates that some of the structural proteins are formed by post-translational cleavage of larger precursor molecules after synthesis or during assembly (Anderson et al., 1973). Polypeptide VII (Anderson et al., 1973), and polypeptides VI and VIII (Anderson et al., 1973; Everitt and Philipson, 1974; Ishibashi and Maizel, 1974a; Öberg et al., 1975) may be formed this way.

Five to seven polypeptides are induced by adenovirus as late, non-structural proteins (Russell and Skehel, 1972; Anderson et al., 1973). One of these appears identical with the messenger ribonucleoprotein peptide of infected cells (Lindberg and Sundquist, 1974) and others may be precursors to mature virion polypeptides (Walter and Maizel, 1974; Ishibashi and Maizel, 1974a).

d. Host cell effects. Adenovirus infection prevents cell division by generalized interference with host cell DNA and protein synthesis. Cellular DNA synthesis declines at 6-8 hrs after infection and by 10-13 hrs about 90% of the newly synthesized DNA is viral (Ginsberg et al., 1967; Pina and Green, 1969). Host cell protein synthesis declines at the same time (Ginsberg et al., 1967). After 12 hrs of infection almost all of the newly synthesized protein is virus-coded or virus-induced (Russell and Skehel, 1972; Anderson et al., 1973). Some

host cell mRNAs, such as the messenger ribonucleoprotein particles, seem to be translated late in infection (Lindberg and Sundquist, 1974).

As host cell protein synthesis is reduced, the accumulation of ribosomal RNA in the cytoplasm is suppressed (Raskas et al., 1970; Philipson et al., 1973). This effect may be due to decreased processing or to suppression of synthesis of the rRNA precursor (Eliceiri, 1973). Cellular tRNA synthesis is normal throughout infection (Ginsberg et al., 1967) and no virus specific tRNA has been identified (Raskas et al., 1970; Kline et al., 1972).

4. Assembly

The newly synthesized viral components accumulate within the nucleus as inclusion bodies (Martinez-Palomo and Granboula, 1967; Martinez-Palomo et al., 1967). Studies by Ginsberg and Dixon (1959) and Green (1962b) indicate only 10-15% of the viral DNA and protein subunits synthesized are incorporated into infectious virions. However, the yield of virions is apparently determined genetically by the virus strain (Mak, 1969).

The maturation of adenovirions requires the normal cytoplasmic concentration of arginine early in infection (Everitt et al., 1971). If arginine starvation begins 14 hrs after infection, almost normal yields of virus are obtained. If the medium is depleted of arginine, viral titers are reduced 3-4 orders of magnitude even though the arginine rich structural proteins are produced in normal amounts (Everitt et al., 1971). Starvation reduces viral DNA synthesis but late viral mRNA apparently is synthesized (Raskas et al., 1972). Winters and Russell (1971) reported that viral DNA and peptides extracted from cells grown in arginine deficient medium will assemble into virions if mixed with

extracts from infected cells in normal medium.

Elevated temperature reduces viral yields by about 2 orders of magnitude. Viral DNA and mRNA are synthesized at a faster rate at 42°C but virion assembly is defective at this temperature (Warocquier et al., 1969; Okubo and Raskas, 1972). Perlman et al. (1972) reported that translation is altered at 40°C and that the ratio between the rate of synthesis of hexon and fiber is increased.

Recent studies of the polypeptides of incomplete adenovirus have led to models of viral assembly which require structural intermediates (Sundquist et al., 1973b; Ishibashi and Maizel, 1974a; Edvardsson et al., 1976). These models propose that DNA containing cores are inserted into empty capsids composed of precursor polypeptides. The polypeptides are then cleaved to their mature forms. This model is supported by the following observations: (1) Incomplete particles and infectious virions appear simultaneously (Sundquist et al., 1973; Rosenwirth et al., 1974). (2) Radioactive amino acids are first incorporated into incomplete particles before appearing in infectious virions (Sundquist et al., 1973; Ishibashi and Maizel, 1974; Edvardsson et al., 1976). (3) Pulse-chase experiments show that radioactivity is first found in incomplete particles and is then chased into infectious particles (Ishibashi and Maizel, 1974; Sundquist et al., 1973; Edvardsson et al., 1976). The incomplete particles have altered amounts of core proteins and contain some polypeptides not found in mature complete virions (Edvardsson et al., 1976; Ishibashi and Maizel, 1974; Rosenwirth et al., 1974; Wadell et al., 1973; Prage et al., 1972). These polypeptides apparently are precursor forms of polypeptides found in the mature virion.

E. Transformation

Cell transformation by adenoviruses usually occurs in non-permissive hamster and rat cells. High multiplicities of infection are required and the incidence of transformation is low. Transformed cells can be readily distinguished from normal cells by their morphological appearance (Strohl et al., 1967). These cells grow to higher saturation densities, grow in disoriented arrays, and have an altered plasma membrane (Martinez-Palomo and Brailovsky, 1968). Infectious virus cannot be recovered from, detected in, or induced from these cells (Burns and Black, 1969; Weber, 1974). However, viral DNA is integrated into host cell DNA during non-permissive infection (Doerfler, 1970) and has been detected in transformed cells (Green et al., 1970; Pettersson and Sambrook, 1973; Sharp et al., 1974). This integrated DNA is incomplete portions of the total genome (Weber, 1974). The viral DNA is transcribed into RNA similar in size to HnRNA of uninfected cells (Green et al., 1970; Wall et al., 1973) and this RNA hybridizes with cell DNA suggesting a covalent linkage of viral and cellular DNA sequences (Tseui et al., 1972; Wall et al., 1973).

Adenovirus transformed cells have an increased DNA content (Kusano and Yamane, 1967) and an increased amount of membrane bound mucopolysaccharides (Martinez-Palomo and Brailovsky, 1968). They contain adenovirus specific T-antigen (Huebner et al., 1963; Pope and Rowe, 1964; Huebner, 1967).

Naked adenovirus DNA will transform cells (Grahm and Van der Eb, 1973b). Grahm et al. (1974) have shown that a DNA fragment from the left-hand end of Ad 5 will transform cells.

F. Tumor formation

The human adenoviruses have been divided into subgroups based on their oncogenicity for newborn hamsters (Huebner, 1967). However, tumors induced by weakly oncogenic viruses are indistinguishable from those induced by highly oncogenic types. Adenovirus induced tumors are described as undifferentiated sarcomas (Trentin et al., 1968), lymphosarcomas (Larson et al., 1965), undifferentiated fibrosarcomas (McAllister et al., 1966), and undifferentiated mesenchymal neoplasms (Huebner et al., 1962). All of these tumors show identical morphology (Slifkin et al., 1968; Yabe et al., 1963), suggesting that the viral genome controls the morphological appearance of the tumor cell (Strohl et al., 1967).

The tumor cells contain no detectable infectious virus and attempts to induce infectious virions have been unsuccessful (Burns and Black, 1969; Weber, 1974). The T-antigen is expressed (Huebner et al., 1963; Pope and Rowe, 1964; Huebner, 1967) and specific transplantation antigens occur on the surface of the transformed cells (Trentin and Bryan, 1964; Sjögren et al., 1967).

Cells transformed in culture by highly oncogenic and weakly oncogenic adenoviruses are tumorigenic in host animals (McAllister et al., 1969a, b). Hamster kidney cells transformed by Ad 2 cause tumors in hamsters (Lewis et al., 1974). In addition, naked DNA from SA 7 (Burnett and Harrington, 1968b) and fragments of SA 7 DNA (Mayne et al., 1971) are tumorigenic.

II. The Incomplete Particles Associated with Adenovirus Infected Cells

A. Isolation

The incomplete particles of adenoviruses are virion-like structures which differ from infectious virions in their buoyant densities. This physical property allows the incomplete particles to be separated from complete particles by equilibrium density gradient ultracentrifugation. Smith (1965a) first observed incomplete particles (top components) of adenovirus types 2 and 12 when infected KB cell lysates were purified by equilibrium sedimentation in CsCl gradients. Three bands of Ad 2 particles having densities of 1.348, 1.337, and 1.292 g/cm^3 were present. While the particles from each band were morphologically indistinguishable under the electron microscope, only the two heaviest bands were infectious. Since these initial observations, incomplete particles have been associated with many types of adenoviruses. They have been studied and characterized in the following types of human adenoviruses: Ad 2 (Burlingham et al., 1974; Rosenwirth et al., 1974; Ishibashi and Maizel, 1974; Sundquist et al., 1973); Ad 3 (Prage et al., 1972; Sundquist et al., 1973; Edvardsson et al., 1976); Ad 12 (Mak, 1971; Burlingham et al., 1974; Schaller and Yohn, 1974); Ad 16 (Wadell and Hammarskjöld, 1970; Wadell et al., 1973). In addition, incomplete particles from simian Ad 7 (Schaller and Yohn, 1974) and bovine Ad 3 (Igarashi et al., 1975; Niiyama et al., 1975) have been studied.

B. Physical Properties

1. Morphology

Under the electron microscope the external morphology of the incomplete particles is identical with the complete, infectious virions (Smith, 1965a; Prage et al., 1972; Wadell et al., 1973; Niiyama et al., 1975). However, the incomplete particles are more easily penetrated by negative stain. Often these preparations show more collapsed and broken particles indicating that the incomplete particles may be more fragile than complete virions (Prage et al., 1972; Wadell et al., 1973; Burlingham et al., 1974).

2. Buoyant density

The incomplete particles are identified by their buoyant densities (Table 1). Most bands of incomplete particles are less dense than the band of infectious virions, however more dense incomplete particles have been characterized for human adenovirus types 2, 12, and 16 (Burlingham et al., 1974; Wadell et al., 1973).

3. DNA content

Smith (1965a, b) reported that DNA could be extracted from incomplete particles of Ad 2, 7, and 12. However Maizel et al. (1968) reported that there was little or no DNA in the incomplete particles of Ad 2, and Prage et al. (1972) reported similar results for the incomplete particles of Ad 3. Wadell et al. (1973) found that ^{3}H-thymidine was incorporated into the incomplete particles of Ad 16 and that the ratio of radioactivity to hemagglutinating activity increased with increased buoyant density of the bands (Table 2). Burlingham et al. (1974) reported that the relative specific radioactivity of ^{3}H-thymidine

Table 1

Characterization of Incomplete Particles of Adenovirus by Buoyant Density in CsCl

Adenovirus Type 2[a]	
Band Designation	Buoyant Density (g/cm^3)
Ia	1.289
Ib	1.292
II	1.299
III*	1.334
IV	1.344
V	1.353

Adenovirus Type 3[b]	
Band Designation	Buoyant Density (g/cm^3)
Incomplete	1.298
Incomplete	1.307
Complete*	1.355

Adenovirus Type 12[a]	
Band Designation	Buoyant Density (g/cm^3)
I	1.289
II	1.301
IIIa	1.325
III*	1.327
IV	1.338
V	1.35

Table 1 (continued)

Adenovirus Type 16[c]

Band Designation	Buoyant Density (g/cm^3)
I	1.298-1.307
II	1.310-1.323
III	1.326
IV	1.330-1.338
V*	1.344
VI	1.349-1.353
VII	1.363
VIII	1.38-1.39

Simian Adenovirus Type 7[d]

Band Designation	Buoyant Density (g/cm^3)
Incomplete	1.30 $\pm$ 0.02
Complete*	1.34 $\pm$ 0.02

Bovine Adenovirus Type 3[e]

Band Designation	Buoyant Density (g/cm^3)
IV	1.300
III	1.302
II	1.338
I*	1.340

*designates complete infectious particles.

[a]Burlingham et al. (1974).

[b]Prage et al. (1972).

[c]Wadell et al. (1973).

[d]Schaller and Yohn (1973).

[e]Niiyama et al. (1975).

Table 2

Incorporation of ^{3}H-thymidine into Incomplete Particles of Adenovirus

Adenovirus Type 2[a]		
Band	Density (g/cm^3)	Relative Specific Radioactivity[b]
Ia	1.289	0.23
Ib	1.292	0.33
II	1.299	0.42
III	1.334	1.00
IV	1.344	1.24
V	1.353	1.49

Adenovirus Type 12[a]		
Band	Density (g/cm^3)	Relative Specific Radioactivity[b]
I	1.289	0.23
II	1.301	0.623
IIIa	1.325	0.96
III	1.327	1.00
IV	1.338	0.75
V	1.35	0.73

Adenovirus Type 16[c]		
Band	Density (g/cm^3)	cpm/HAU x 10^{-3}[d]
I	1.298-1.307	0.5-2.0
II	1.310-1.323	6-15
III	1.326	25
IV	1.330-1.338	27
V	1.344	40

Footnotes to Table 2

[a]Burlingham et al. (1974).

[b]The relative specific radioactivity = (cpm in band I-V/A280 of band I-V) (cpm in band III/A280 of band III).

[c]Wadell et al. (1973).

[d]1 HAU = the highest dilution of virus which gives clear-cut agglutination when incubated with green monkey erythrocyte

increased in a similar manner in Ad 12 and Ad 2 incomplete particles (Table 2).

Specific sized pieces of DNA were isolated and purified from the particles of each incomplete band of Ad 2 and Ad 12 (Burlingham et al., 1974). These pieces of DNA were characterized by sedimentation coefficient and buoyant density (Table 3). The DNA has the density of adenovirus DNA and hybridizes more efficiently with viral than with cellular DNA. The DNA fragments from bands IV and V of Ad 2 each yielded two species on zonal sedimentation. One of these species cosedimented with DNA from complete virions while the second species sedimented much slower. The data from the more dense incomplete particles of Ad 12 suggested that the larger sized DNA may be parental DNA while the smaller DNA fragment may be newly synthesized progency DNA.

4. Protein composition

The polypeptide composition of incomplete particles of adenovirus has been determined using SDS-polyacrylamide gel electrophoresis (Edvardsson et al., 1976; Ishibashi and Maizel, 1974; Rosenwirth et al., 1974; Wadell et al., 1973; Prage et al., 1972). The structural polypeptides of the capsid (hexon, penton, and fiber) were present in the same relative amounts in incomplete particles and infectious virions (Prage et al., 1972; Rosenwirth et al., 1974; Wadell et al., 1974). However, the polypeptides of the core were different. Prage et al. (1972) reported that the incomplete particles of Ad 3 lacked the major core polypeptide V and polypeptides VI-VII which were present in infectious virions. This observation was verified by Sundquist et al. (1973), who also showed that these polypeptides were absent in incomplete particles

Table 3

Characterization of DNA Extracted from Incomplete Particles of Adenovirus[a]

Adenovirus Type 2		
Band	S[b]	Density (g/cm^3)
Ia	8.9	1.714
Ib	9.8	1.715
II	15.6	1.716
III	31.7	1.714
IV	19.4	1.715
V	29.4	1.713
Adenovirus Type 12		
Band	S	Density (g/cm^3)
I	9.6	1.706
II	15.5	1.706
IIIa	28.1	1.704
IV	18.5	1.706
V	22.0	1.705

[a] Burlingham et al. (1974).

[b] S = sedimentation coefficient determined by zonal sedimentation in 5 to 20% sucrose gradients relative to a viral marker DNA.

of Ad 2 (Figure 3). Similar results were reported for Ad 16 by Wadell et al. (1973).

In addition to missing certain major core polypeptides, the incomplete particles contained new polypeptides not present in complete virions (Prage et al., 1972; Wadell et al., 1973; Rosenwirth et al., 1974; Ishibashi and Maizel, 1974; Sundquist et al., 1973). Sundquist et al. (1973) found five polypeptides in incomplete particles of Ad 2 and Ad 3 (Figure 3) which are not present in complete particles. Rosenwirth et al. (1974) found polypeptides in incomplete particles of Ad 2 which corresponded with virion polypeptides V, VI/VII, and VIII/IX except for higher molecular weights. Amino acid analysis of the Ad 3 particles indicates that the incomplete particles contain greater amounts of leucine, glutamic acid, and aspartic acid but lesser amounts of arginine and alanine than complete virions (Table 4; Prage et al., (1972).

C. Biological Properties

1. Infectivity

Complete virions are readily distinguished from incomplete particles on the basis of infectivity. On the basis of mass, incomplete particles have infectivity titers which are four or more orders of magnitude lower than complete particles (Table 5). In each case it has been impossible to determine whether the low infectivity of incomplete particles was due to the presence of contaminating complete virions. Contamination could be due to physical trapping of complete virions in the incomplete particle populations or to the tailing of complete particles as the gradients are dripped from the bottom of the centrifuge tube. Burlingham et al. (1974) monitored purity of incomplete particle

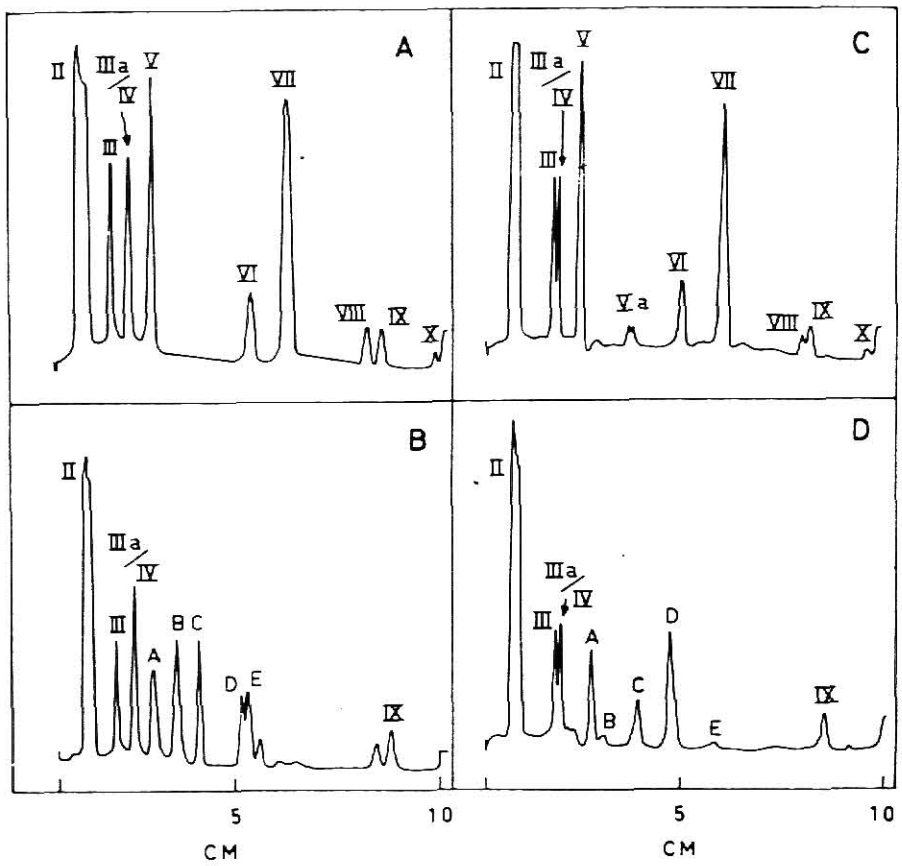

FIGURE 3. Polypeptide patterns of incomplete and complete particles of adenovirus type 2 and 3. Incomplete and complete particles from adenovirus type 2 and 3 were purified and analyzed on 13% SDS-polyacrylamide gels. The gels were stained with Coomassie blue and scanned in a Gilford spectrophotometer at 550 nm. The anode is to the right, and the absorbance is given in arbitrary units. The peaks are numbered according to the nomenclature of Maizel (1971). A, Polypeptide pattern of adenovirus type 2-complete particles. B, Adenovirus type 2-incomplete particles. C, Adenovirus type 3-complete particles. D, Adenovirus type 3-incomplete particles.

From Sundquist et al. (1973).

Table 4

Amino Acid Composition of Ad 3 Particles[a]

Amino Acid	Ad 3 Complete Virion[b]	Ad 3 Incomplete Particles[b]
Lys	3.8 ± 0.1	3.9 ± 0
His	1.4 ± 0.1	1.6 ± 0.1
Arg	8.5 ± 0.2	5.6 ± 0.2
Asp	12.3 ± 0.2	13.2 ± 0.2
Thr	8.0 ± 0.1	7.5 ± 0
Ser	6.6 ± 0	7.2 ± 0.1
Glu	7.7 ± 0.2	9.3 ± 0.2
Pro	6.5 ± 0.1	6.4 ± 0
Gly	7.8 ± 0.1	7.0 ± 0
Ala	8.7 ± 0	7.7 ± 0.1
Cys	Trace	0.05 ± 0.05
Val	6.3 ± 0.3	6.1 ± 0.3
Met	2.9 ± 0.2	3.0 ± 0.2
Ile	3.5 ± 0	3.6 ± 0
Leu	7.2 ± 0	8.5 ± 0.1
Tyr	4.9 ± 0	5.0 ± 0.1
Phe	3.8 ± 0	4.3 ± 0

[a]Prage et al. (1973).

[b]Results are expressed as residues per 100 residues.

Table 5

Infectivity of Adenovirus Particles

Ad 2[a]

Band	Specific Infectivity (PFU/OD_{280})[d]
Ia	9.2×10^7
Ib	1.5×10^8
II	1.0×10^7
III	1.2×10^{14}
IV	3.6×10^9
V	2.3×10^7

Ad 3[b]

Band	FFU/μg[c]	PFU/μg[e]
Incomplete	8.3×10^2 - 1.2×10^3	1.7×10^3 - 3.1×10^3
Complete	1.8×10^7 - 5.1×10^7	1.8×10^7 - 2.0×10^7

Ad 12[a]

Band	Specific Infectivity (PFU/OD_{280})[d]
I	3.0×10^4
II	2.6×10^4
IIIa	2.1×10^6
III	5.2×10^{14}
IV	2.6×10^3
V	4.7×10^6

Table 5 (continued)

Ad 16[f]	
Band	TCD_{50}/HAU[g]
I	2
II	7-45
III	450
IV	1000
V	22,000

[a]Burlingham et al. (1974).

[b]Prage et al. (1973).

[c]Fluorescent focus-forming units per μg of protein, determined on KB cells using adenovirus type 2 anti-hexon serum. Protein was quantitated by the method of Lowry et al. (1951).

[d]Plaque forming unit determined from plaque assays on HEK cells and adjusted to an A_{280} = 1.00.

[e]Plaque forming units per μg of protein, determined by plaque assay on HEK cells. Protein was determined by the method of Lowry et al. (1951).

[f]Wadell et al. (1972).

[g]50% tissue culture infectious dose per hemagglutination unit. TCD50 was determined on human fibroblasts by the method of Reed and Muench (1938).

preparations in the Model E analytical ultracentrifuge and could detect no contaminating complete virions by this procedure. However they reported that the incomplete preparations could contain a maximum of 0.01% contamination with infectious virions. Prage et al. (1972) noted that incomplete particle preparations purified in preparative CsCl equilibrium density gradients sometimes contained small amounts of infectivity at the density of complete virions. Wadell et al. (1973) reported similar observations for incomplete particle preparations of Ad 16.

Dose response experiments indicated that Ad 3 and Ad 16 complete virions follow single hit kinetics but the incomplete particles deviate from this pattern (Prage et al., 1972; Wadell et al., 1973). Interpretation of these results was impossible since: (1) contamination with complete particles could not be eliminated; (2) aggregation of complete and incomplete particles was not determined and (3) interference and competition effects could not be determined.

2. T-antigen induction, cell killing, and inclusion body formation

Mak (1971) studied the biological properties of T-antigen induction, cell killing, and inclusion body formation by incomplete particles of Ad 12. He found that incomplete particles and complete particles induced T-antigen and cell killing to about the same extent (Table 6). The cell killing property could be diminished by treating both complete and incomplete populations with UV, suggesting that this property was the result of a genome function. Incomplete particles did not induce inclusion bodies as readily as complete virions. However, incomplete

particles could induce inclusion bodies more readily than they could form plaques (Table 6).

3. Transformation

Schaller and Yohn (1974), compared the ability of incomplete particles to transform embryonic hamster cells with that of infectious virions. Transforming activity correlated with viral particles in both Ad 12 and simian Ad 7 populations (Table 7). In the simian Ad 7 preparation, the incomplete particles with a density of 1.31 g/cm^3 contained 49% of the total viral particles and had 45.7% of the total transformation activity. The population containing 91% of the total infectivity represented only 29.6% of the total particles and had 19.3% of the transforming activity. Therefore in these studies the transforming ability of the incomplete particles appears similar to that of the complete virions.

4. Tumorigenicity

Incomplete particles apparently induce tumors in newborn hamsters. Igarashi et al. (1975) reported that bovine Ad 3 incomplete particles were as efficient in tumor induction as the infectious, complete particles (Table 8). Yohn (1973) observed that male hamsters were less susceptible to tumor formation by oncogenic adenoviruses than female hamsters. Schaller and Yohn (1974) found that Ad 12 complete particles produced a higher incidence of tumors in the total hamster population, than did the incomplete particles. The female subpopulation was equally susceptible to tumor development when inoculated with complete or incomplete particles (Table 8). Incomplete particles of simian Ad 7 induced tumors in both male and female hamsters (Table 8).

Table 6

Induction of T-antigen, Cell Killing, and Inclusion Body Formation by Ad 12 Particles[a]

Band	Infectivity[b] (PFU/ml)	Inclusion Body[c] Induction	T-antigen[d] Induction	Cell Killing[e] Ability
Iab	8.6×10^6	77	38	5.9
III	0.77×10^6	93.7	42	5.9

[a]Adapted from Mak (1971).

[b]Determined by plaque assay on KB cells.

[c]Expressed as % without inclusion bodies. Greater than 1600 HEp2 cells were fixed with acetic acid-alcohol (1:3) and stained with oricin.

[d]Expressed as % without T-antigen based on greater than 1600 HEp2 cells infected for 48 hr with Ad 12. The cells were fixed in CCl_4 stained with fluorescein-labeled antiserum from hamsters bearing Ad 12 induced tumors, and washed extensively.

[e]Expressed as % of cells surviving based on clone forming ability. Survival is expressed as the percent of noninfected control cells which clone at an efficiency of about 70%.

Table 7

Correlation of Transforming Ability with Particle Content and Infectious Activity in Adenovirus Particles[a]

Virus	Density (g/cm^3)	% Viral[b] Particles	% Infectious[c] Activity	% Transformation[d] Activity
Ad 12	1.274 ± 0.012	1.2	3.7	0.4
	1.294 ± 0.009	66.3	3.7	39.2
	1.311 ± 0.008	1.8	5.6	2.4
	1.336 ± 0.018*	30.7	87.0	58.0
Simian Ad 7	1.289 ± 0.011	0.1	1.0	3.7
	1.310 ± 0.011	49.0	0.8	45.7
	1.330 ± 0.009	20.3	7.1	31.3
	1.347 ± 0.008*	29.6	91.1	19.3

[a]Modified from Schaller and Yohn (1974).

[b]Determined from optical density at 260 nm.

[c]Determined by fluorescent focus assay on HEp2 cells.

[d]Determined at input multiplicities of 64 or 160 viral particles per cell.

*Designates complete virions.

Table 8

Tumor Formation in Newborn Hamsters Inoculated with Adenovirus

Virus	Particles Injected	Bovine Ad 3[a] Total	Ad 12[b] Male	Ad 12[b] Female	Ad 12[b] Total	Simian Ad 7[b] Male	Simian Ad 7[b] Female	Simian Ad 7[b] Total
Incomplete[c]	10^7	2/2						
	10^6	4/8						
	10^5	2/3						
	$10^{9.8}$		0/5	1/2	1/7			
	10^9		0/4	1/1	1/5	1/2	2/4	3/6
Complete	10^7	5/5						
	10^6	9/10						
	10^5	0/7						
	$10^{9.8}$		3/6	2/4	5/10			
	10^9		0/3	1/3	1/6	1/5	-	1/5

[a]From Igarashi et al. (1975).

[b]From Schaller and Yohn (1974).

[c]Incomplete particles of bovine Ad 3 had a density of 1.338 g/cm^3 and contained less than 0.1% contamination with complete virus as measured by plaque assay.
Incomplete particles of Ad 12 and SA 7 had a mean density of 1.30 g/ml and represented pooled sections of CsCl equilibrium density gradients containing adenovirus.

D. Incomplete Particle Production

The relative proportion of incomplete particles produced varies with the type of adenovirus used for the infection. Adenovirus type 3 produces about 30% incomplete particles (Prage et al., 1972) while adenovirus type 12 incomplete particles represent about 10% of the total viral particles (Burlingham et al., 1974). For a given type of adenovirus the incomplete particles are produced in constant relative amounts regardless of the multiplicity of infection (MOI) or the passage number (Rosenwirth et al., 1974; Burlingham et al., 1974). The relative proportion of incomplete particles is not altered by changing the serum concentration of the medium, increasing the vitamin or amino acid composition of the medium, or by increasing the concentration of arginine. The physical properties of the incomplete particles are not altered by replication in different host cells (Burlingham et al., 1974).

The time course of incomplete particle production indicates that the incomplete particles are produced exponentially, concurrently and proportionally with infectious virions (Sundquist et al., 1973; Rosenwirth et al., 1974). Figure 4 indicates that the incomplete particles and virions are first detectable about 13 hours after infection when an MOI of 25 is used. The same result was also reported by Sundquist et al. (1973).

E. Role of Incompletes in Viral Assembly

The role of the incomplete particles during infection has generated considerable interest. Protein labeling studies indicate that radioactivity first appears in incomplete particles. Following a

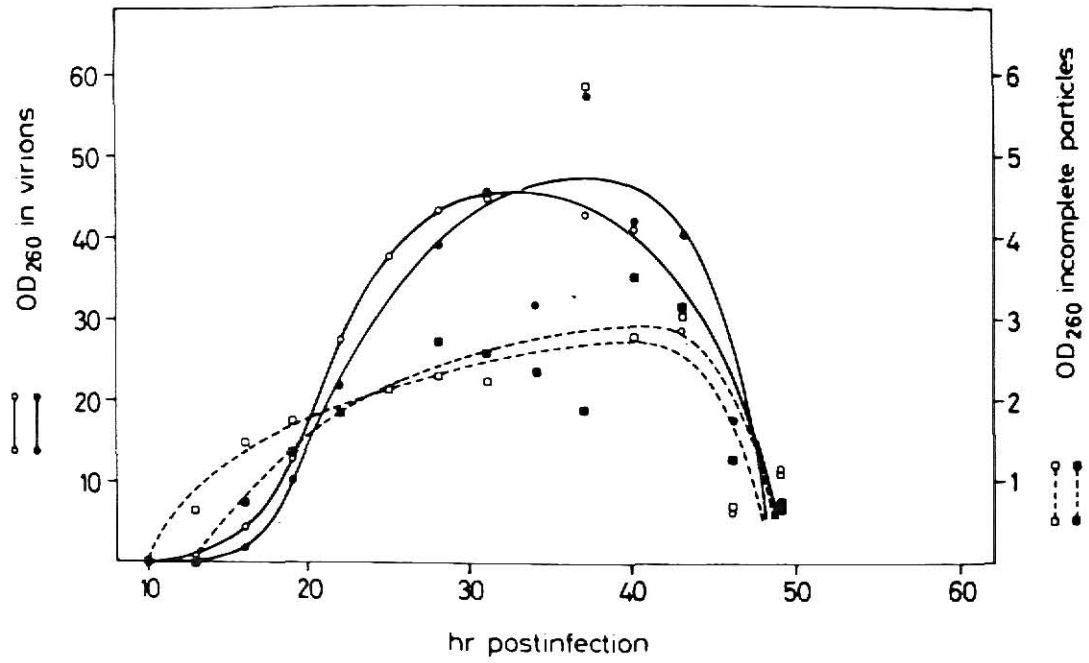

FIGURE 4. Time course of appearance of Ad 2 virions and incomplete particles in Ad 2-infected KB cells. KB cells growing in suspension culture were infected with 25 or 100 PFU/cell of complete virions of Ad 2 purified by three cycles of equilibrium centrifugation in CsCl density gradients. At time intervals after infection, as indicated, virions and incomplete particles were isolated from 500-ml portions of the infected cultures and purified. Virions and incomplete particles were quantitated after purification by their absorbance at 260 nm. The absorbance values at 260 nm were measured in suitable dilutions and were not corrected for light scattering. Note the different scales used for the quantitation of the complete virions (ordinate on the left) and the incomplete particles (ordinate on the right). □---□ , Incomplete particles, multiplicity 100 PFU/cell; ■---■ , incomplete particles, multiplicity 25 PFU/cell; ○—○ , complete virions, multiplicity 100 PFU/cell; ●—● , complete virions, multiplicity 25 PFU/cell.

From Rosenwirth et al. (1974).

lag of 60-80 minutes, the labeled protein becomes detectable in complete virions (Sundquist et al., 1973). These results, illustrated in Figure 5 suggested the possibility of a precursor-product relationship between the incomplete particles and the infectious virions. Sundquist et al. (1973) found that inhibiting protein synthesis immediately following the addition of radioactive label drastically reduced the synthesis of both incomplete and complete particles. However, the effect seemed to be greater on the incomplete particles.

Protein labeling, pulse-chase experiments by Ishibashi and Maizel (1974) indicated that the label was first incorporated into the incomplete band Ia of Ad 2. After 17.5 hours of chase the label was predominantly in band Ib (Figure 6). Five polypeptides were shared between bands Ia,b and the virions. These polypeptides did not accumulate in the complete virions but were apparently precursor molecules which were further processed.

III. The Incomplete Particles of Other Viruses

Incomplete or defective virus particles have been described for many DNA and RNA animal viruses. These incomplete particles differ from incomplete particles of adenovirus since they exhibit the Von Magnus phenomenon. Von Magnus (1954) first descri' a biological property associated with defective particles of influenza virus. He observed that following inoculation of fertilized eggs with high MOI or with virus of undiluted passage, the titer of the resulting virus decreased. This decrease was due to particles which interfered with the replication of infectious progeny virus. These interfering particles were identified

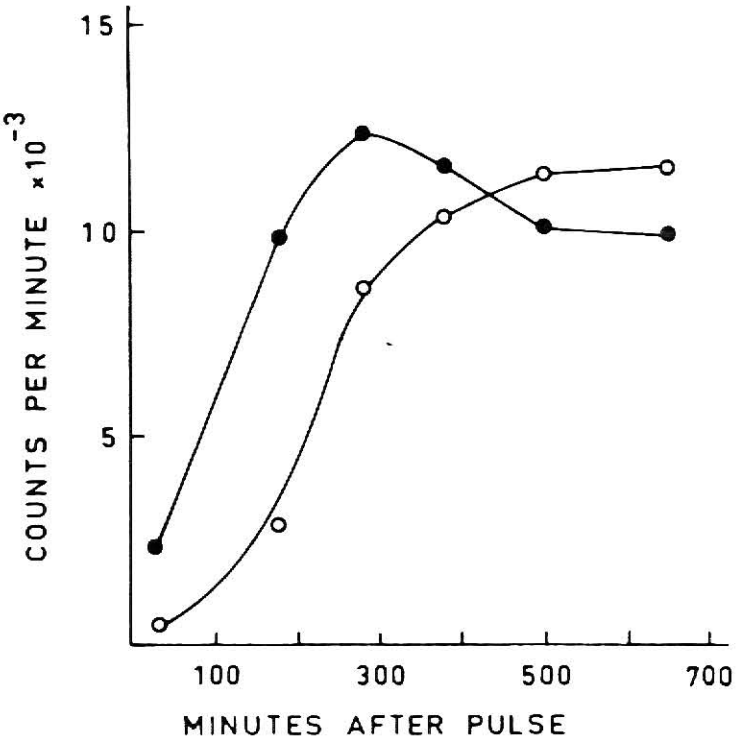

FIGURE 5. Precursor-product relationship between incomplete and complete particles of adenovirus type 2. ^{3}H-amino acids were used to pulse label Ad 2 infected KB cells for 5 min at 15 hr following infection. Following a chase with medium enriched with excess unlabeled amino acids, nuclear extracts of the cells were prepared and centrifuged at 71,000 x g for 85 min in 5-20% high salt-sucrose gradients. The radioactivity in the peaks of incomplete particles (●) and complete particles (○) was determined and is plotted here as a function of time after pulsing.

From Sundquist et al. (1973).

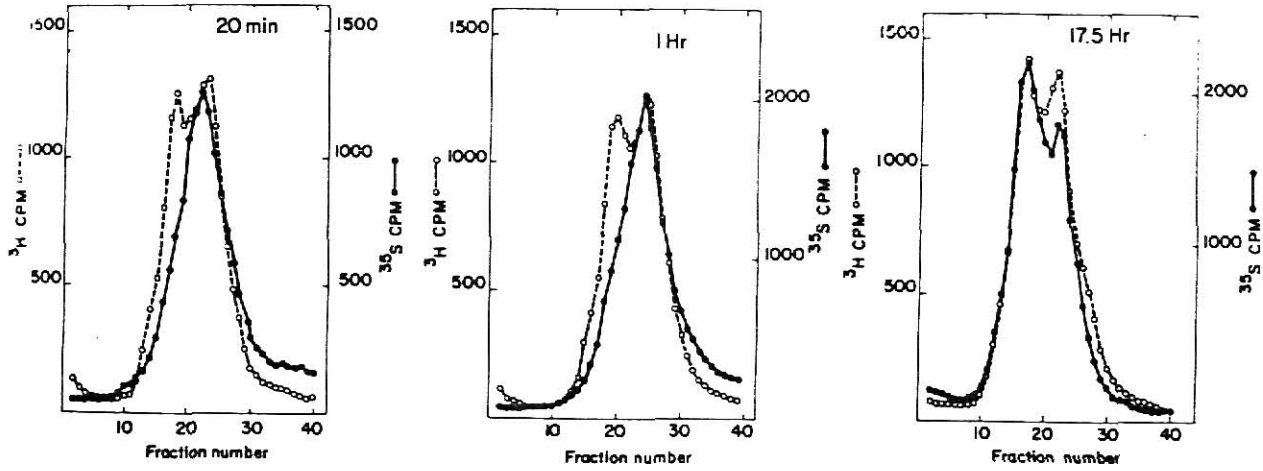

FIGURE 6. Radioactive labeling of Ad 2 incomplete particle bands Ia and Ib. HeLa cells infected with Ad 2 for 23 hr were pulse labeled with ^{35}S-methionine for 25 min and chased with medium containing excess unlabeled methionine for 20 min, 1 hr, or 17.5 hr. After CsCl equilibrium density gradient centrifugation, the radioactive peak fractions representing incomplete particle were mixed with purified incomplete Ad 2 particles which had been labeled with ^{3}H-methionine from 23 to 41 hr after infection. The mixture was layered on preformed CsCl gradients ranging in density from 1.2 to 1.34 g/cm^3. After centrifugation for 3 hr at 25,000 rpm in an SW 27 rotor, twenty drop fractions were collected from the bottom of the tubes. The sedimentation profiles of ^{35}S-activity in top components at 20 min, 1 hr, and 17.5 hr of chase with that of ^{3}H-labeled reference top components are shown above.

From Ishibashi and Maizel (1974).

by their low infectivity to hemagglutination ratio. The number of interfering particles increased with further undiluted passage and were termed defective particles. The Von Magnus phenomenon has been reported in VSV (Vesicular Stomatitis Virus) (Bellett and Cooper, 1959), Rift Valley Fever virus (Mims, 1956), fowl plague virus (Rott and Scholtissek, 1963), Sendai virus (Kingsbury et al., 1970), Polyoma virus Blackstein et al., 1969), and SV 40 virus (Uchida et al., 1966).

The physical properties of incomplete particles from other viruses are similar to the incomplete particles of adenovirus. Defective particles of Shope papilloma virus (Breedis et al., 1962), Polyoma virus (Blackstein et al., 1969), ECHO virus (Halperen et al., 1964), Sendai virus (Kingsbury et al., 1970), Reovirus (Smith et al., 1969), and SV 40 virus (Yoshiike, 1968) have been separated from the infectious particles by isopycnic banding in the ultracentrifuge. Under the electron microscope the morphology of these defective particles appears identical with virions. An exception, the defective VSV particles have a spherical shape while the infectious virions have the characteristic bullet shape (Huang et al., 1966).

Defective particles of influenza contain small fragments of RNA but they lack the large species of RNA found in infectious virions (Duesberg, 1968). Incomplete particles of Sendai virus also lack a major species of RNA, but they contain small fragments of RNA (Kingsbury et al., 1970). The RNA fragment contained in incomplete particles of VSV was one-third the size of the infectious genome (Huang and Wagner, 1966). In contrast, no RNA could be detected in incomplete particles of REO (Smith et al., 1969) or ECHO (Halperen et al., 1964).

Incomplete particles of polyoma virus contain one-half and three-fourth genome fragments of supercoiled DNA (Blackstein et al., 1969). Defective particles of SV 40 contain supercoiled DNA which is 12% shorter than DNA from infectious particles. This shortened DNA is not infectious (Yoshiile, 1968; Uchida and Watanabe, 1969). Brockman et al. (1973), showed that the composition of DNA from detective particles of SV 40 changed with serial undiluted passage. The first four passages resulted in defective particles which contain only viral DNA, however following the 13th passage almost all of the DNA was cellular with just a fraction of viral DNA attached.

Although protein composition has not been studied on most of these particles, incomplete particles of influenza (Ada and Perry, 1958; Lief and Henle, 1956) and VSV (Huang et al., 1966) have been shown to be antigenically similar to their corresponding infectious particles. The complete and incomplete particles of reovirus were shown to have identical polypeptide compositions (Smith et al., 1969). Maizel et al., 1967), showed that incomplete particles of polio lacked a major polypeptide but contained a different peptide not present in infectious particles.

Kingsbury and Portner (1970) reported that irradiated incomplete particles of Sendai virus could not replicate even in the presence of complete particles indicating that the incomplete genomes played a role in their replication. Huang and Wagner (1966) described similar results with irradiated incomplete particles of VSV. Defective particles of SV 40 transform cells as efficiently as complete particles. T-antigen production was equivalent in cells transformed by complete or

defective particles of SV 40 (Uchida and Watanabe, 1969), and defective particles of SV 40 are tumorigenic (Uchida and Watanabe, 1968).

Huang and Baltimore (1970) proposed that incomplete particles which result from the Von Magnus phenomenon have an integral role in viral disease processes. They proposed a model for defective particle replication which would allow for acute and persistent viral infection (Figure 7). The model proposes that one infectious virion gives rise to more infectious virions. Occasionally a defective particle is produced. The defective particle reproduces along with the virions, but it interferes with virion production. Eventually the defective particles become the predominant species. However, as the infectious virion population decreases, the incomplete or defective virus population must also decrease. As this decrease in population occurs, immunological reactions are able to eliminate the infection. If, however, the concentration of infectious particles remains very low and is not eliminated by antibodies, the defective particles will not be able to replicate. In this instance, the infectious virion population can build up and allow a new cycle of infection.

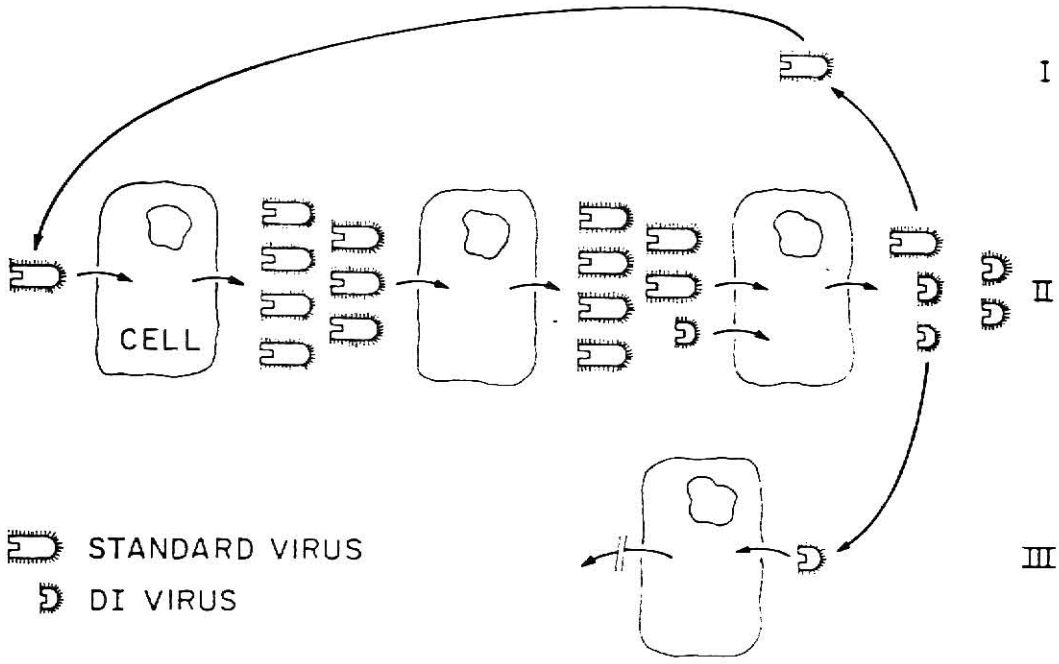

FIGURE 7. A model for the role of defective (DI) particles in viral infections. The model proposes that defective particles arise randomly during infection of cells with standard virus. A defective particle cannot be replicated unless a standard virus particle is also present in the same cell.

From Huang and Baltimore (19).

THE INCOMPLETE PARTICLES OF HUMAN ADENOVIRUS TYPE 3

I. Characterization of Physical and Chemical Properties

Abstract

When human adenovirus type 3 is replicated in KB cells and is purified by equilibrium density gradient centrifugation, several populations of virus particles can be observed as light-scattering bands. Six discrete populations of Ad 3 particles have been identified in the analytical ultracentrifuge and have mean densities of 1.2787, 1.2913, 1.2945, 1.3030, 1.3139, 1.3315, and 1.3595 g/cm^3, respectively. The buoyant density of the particles and the incorporation of tritiated thymidine into the particles indicates that all of these particles contain DNA. Most of the DNA within the incomplete particles and the complete virions is resistant to degradation by pancreatic DNase. The nucleic acid was extracted from highly purified incomplete and complete virions. The nucleic acid from both types of virions hybridized better to viral DNA than to cellular DNA. The nucleic acid extracted from both types of particles were readily degraded by DNase. When the DNA from either the complete or the incomplete particles were incubated with pronase about 5% of the ^{3}HTdR label was solubilized.

Introduction

Incomplete virus particles were first associated with adenovirus by Smith (1965). These particles had virion-like morphology but differed from complete infectious virions by buoyant density. Thus the

various types of virus particles could be separated by equilibrium sedimentation in CsCl density gradients. Incomplete particles have been associated with adenovirus types 2, 3, and 12 (Prage et al., 1972; Burlingham et al., 1974; Wadell et al., 1973; Schaller and Yohn, 1974; Niiyama et al., 1975). The physical and chemical properties of adenovirus types 2 and 12 incomplete particles and virions have been well characterized by Burlingham et al. (1974).

Prage et al. (1972) first described the incomplete particles of adenovirus type 3. They noted that incomplete particles with a low buoyant density were produced in large proportions. About 30% of the total viral particles were isolated as two discrete populations of incomplete particles. They reported that the buoyant density of these two populations of incomplete particles was 1.307 and 1.298 g/cm^3 respectively and the buoyant density of the complete Ad 3 particles was 1.355 g/cm^3.

We find that Ad 3 purified by equilibrium density gradients contain additional discrete bands of incomplete virions. When complete Ad 3 virions are co-sedimented in the analytical ultracentrifuge with purified complete Ad 2 virions, the Ad 3 virions are less dense. From the reported densities, one would expect the Ad 3 particles to be more dense. These two observations l ' to a critical analysis of the physical and chemical properties of the incomplete particles associated with Ad 3.

Materials and Methods

Cells. KB cells were obtained from the American Type Culture Collection. They were grown as monolayers in prescription bottles with Eagle's minimum essential medium (MEM) supplemented with 5% calf serum.

Virus. Human adenovirus types 2 and 3 (Ad 2 and Ad 3) were obtained from the American Type Culture Collection. Virus was produced by infecting monolayers of KB cells at a multiplicity of infection of approximately 100 diluted into 0.25% bovine serum albumin (BSA) in phosphate buffered saline (PBS). Each monolayer was inoculated with 10 ml of virus stock and incubated at 37°C for 2 hr with rocking every 15 min. Following adsorption, Eagle's MEM containing 10% calf serum was added and the cells were incubated at 37°C for 48 hr or until the cells had detached from the glass. Any cells remaining attached were shaken loose and all of the detached cells were sedimented by centrifugation, resuspended in 5 ml of 0.25% BSA in PBS, and transferred to a sterile vaccine vial. Following three cycles of freeze-thawing, the cell lysate was homogenized by forceful expulsion through a 20 gauge needle with a 10 ml syringe. Cell debris was sedimented by centrifugation at 1000 x g for 15 min. Supernatant fluid was collected for virion purification.

Purification of complete and incomplete adenovirions. The supernatant fluid containing the adenovirus was extracted three times with equal volumes of 1,1,2-trichlorotrifluoroethane (Genetron 113). The virions were purified from the aqueous phase by zonal sedimentation in a discontinuous CsCl gradient. The discontinuous gradient was prepared by placing 0.5 ml of CsCl in 10 mM Tris, pH 7.2, 1 mM EDTA (TE),

with a density of 1.50 g/cm^3 in a 5/16" x 2 3/8" cellulose nitrate centrifuge tube. Then 2.5 ml of CsCl solution in TE with a density of 1.20 g/cm^3 were carefully layered on the denser CsCl solution. About 1.5 ml of virus suspension was layered on top of each tube and sedimented at 4°C and 50,000 x g for 1.5 hr. The complete and incomplete virus particles collected on the cushion of CsCl with a density of 1.50 g/cm^3 and were visualized by light scattering from trans-illumination. The virions were withdrawn directly through the tube by puncturing the side of the tube with a 25 gauge needle attached to a 1 cc tuberculin syringe.

The partially purified virus was transferred to a new cellulose nitrate centrifuge tube. This tube was then filled with a CsCl solution with a density of 1.34 g/cm^3 for density gradient sedimentation. Equilibrium was established by centrifugation at 4°C, 100,000 x g for a minimum of 12 hours. The banded particles were visualized by trans-illumination and collected from the side of the tube with a tuberculin syringe. The purified particles were dialyzed against five changes of PBS for cell experiments or TE for biochemical experiments.

Equilibrium sedimentation in the analytical ultracentrifuge. Analytical equilibrium sedimentation was done as described by Vinograd and Hearst (1962). The refractive indices of the solutions and viral suspensions were determined with an Abbe Refractometer at 25°C. From 0.015 to 0.020 optical density units of the respective populations of virus particles was diluted in solutions of optical grade CsCl dissolved in TE. The mixtures were injected into 12 mm cells fitted with aluminum filled Epon single sector centerpieces. Sedimentation was carried out

in a Beckman Model E Analytical ultracentrifuge at 20°C and 75,000 x g. When equilibrium was reached, photographs were taken on Kodak electron image plates with the monochromator set at 265 nm. The photographs were traced on a Joyce-Loebl scanning microdensitometer, and the distance between the rotor reference mark and the points of maximum concentration were measured. The actual densities of the samples were determined by direct pycnometry. The methods and equations of Ifft et al. (1961) were used to calculate the buoyant densities.

Sedimentation of adenovirus particles in CsCl equilibrium density gradients. Radioactively labeled Ad 3 was prepared by adding Eagle's medium containing 5 μCi/ml ^{3}H-thymidine (^{3}HTdR) to the virus infected cells following adsorption. A cell free extract from one 32 oz bottle was treated with DNase at 37°C for one hour. Following dialysis against PBS the sample was extracted with Genetron and mixed with purified complete virions of Ad 2 labeled with 2000 cpm of ^{14}C-formate. CsCl solution with a density of 1.34 g/cm^{3} was added and equilibrium centrifugation was carried out at 4°C and 100,000 x g in a Type 40 rotor. Ten drop fractions of the gradient were collected from the bottom into scintillation vials. Ten milliliters of Bray's fluor was added to each vial and the radioactivity determined at 4°C in a Packard liquid scintillation spectrometer.

Optical density determinations. The optical density of virus suspensions or DNA was determined in microcuvettes at 260 or 280 nm with a Zeiss spectrophotometer.

Preparation of DNA. Adenovirus DNA was extracted according to the methods of Doerfler (1968) and Burlingham et al. (1971).

Purified virus particles were dialyzed extensively against TE. The dialyzed virus was maintained at 4°C for 10 days or until the particles precipitated. SDS was added to a final concentration of 0.5% and the sample was incubated at 37°C for 30 min. One-tenth volume of pronase was added and the mixture was incubated for 30 min at 37°C. Fresh pronase was added and the incubation was repeated. The digested sample was then extracted three times with an equal volume of phenol, three times with an equal volume of ether, and dialyzed against five changes of TE.

Cellular DNA was prepared by lysing KB cells in STE (0.5% SDS, 10 mM Tris, 1 mM EDTA). The lysate was digested twice with pronase, extracted with phenol and ether and dialyzed against TE.

DNA-DNA hybridization. DNA-DNA hybridization was carried out according to the procedure of Denhardt (1966) with the washing procedure of Packman and Sly (1968). Schleicher and Schuell B-6 filters were heated to 80°C for 15 min in 6 x SSC (SCC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). The filters were then washed with 5 ml of 6 x SSC. The DNA in 6 x SSC was denatured by heating in a boiling water bath for 10 min. It was immediately diluted into 100 volumes of ice cold 6 x SSC with swirling to insure rapid chilling. The denatured DNA suspension was adsorbed by pa ing through a filter at not more than 5 ml/min. The filters were washed with 10 ml of cold 6 x SSC, placed in glass scintillation vials and vacuum desiccated overnight, and then heated in an 80°C oven for 2 hrs. Approximately 96% of the DNA adhered to the filters and less than 1% could be eluted during the remaining procedures. One ml of preincubation mixture (PM) containing

0.05% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin in 3 x SSC was added to each filter and incubated at 80°C for 2 hr. The PM was poured off and 5 ml of 3 x SSC were added. After incubation with PM, filters with no bound DNA would absorb less than 0.05% of DNA in the incubation solution.

The labeled DNA in 6 x SSC to be hybridized to the filter-bound DNA was sonicated with a Branson Sonifier for one minute with the output control set to 4. This fragmented DNA in 6 x SSC was then denatured in a boiling water bath for 10 minutes and quickly cooled in an ice bath. The 3 x SSC was poured off the filter and 1 ml of the denatured, labeled DNA was added to the vials containing the filters. After incubating the vials at 65°C overnight, the filters were washed 6 times with 20 ml of 0.01 M Tris HCl, pH 8.1 and transferred to scintillation vials. They were dried overnight in a vacuum desiccator and then counted in toluene fluor.

Enzymatic and KOH digestion. Radioactively labeled virus particles or DNA were treated with one-tenth volume of DNase or RNase at 37°C for up to 16 hours, or they were treated with KOH at a final concentration of 0.3 M overnight. At various intervals, 100 µl samples were removed and acid precipitated in 5% cold trichloroacetic acid (TCA). Salmon sperm DNA (100 µg/sample) was included as a co-precipitate. The acid precipitable material was collected on GF/C glass fiber filters. The radioactivity on the dried filters was determined in toluene fluor.

Preparation of radioactive labeled virus. Following adsorption of Ad 3 to KB cells, Eagle's medium was added which contained 5 µCi ^{3}H-TdR/ml or 20 µCi ^{32}P/ml. The infected cells were incubated at 37°C and harvested at 48 hours.

Results

KB cells were infected with AD 3 labeled with ^{3}H TdR, lysed by freeze-thawing and mechanical shear, digested with DNase, and extracted with Genetron. This Ad 3 virus preparation was mixed with highly purified Ad 2 virions labeled with ^{14}C-formate. The mixture was centrifuged to equilibrium in a Beckman type 40 rotor at 100,000 x g in 13.5 ml CsCl with a mean density of 1.34 g/cm^{3}. The bottom of the centrifuge tube was punctured, ten drop fractions were collected into scintillation vials, suspended in 10 ml Bray's fluor and the radioactivity was determined. The radioactive profile is shown in Figure 8. Purified Ad 2 virions are banded four fractions lower in the gradient than the bulk of the ^{3}H TdR-Ad 3 radioactivity. This indicates that the major population of ^{3}H-Ad 3 particles has a lower buoyant density than complete Ad 2 virions.

The profile of ^{3}H TdR radioactivity suggests the presence of several populations of Ad 3 particles with distinct densities. These populations are better detected by scanning the optical density of CsCl equilibrium density gradients of Ad 3 lysates. After centrifugation to equilibrium, the bottom of the tubes are punctured and seven drop fractions are collected. The optical density of each fraction is determined at 260 and 280 nm. Figure 9 shows these optical density profiles. One peak is more dense than the major peak and at least four peaks are less dense than the major peak. The peaks are designated as bands I-VI beginning with the least dense material. The OD_{260}/OD_{280} ratios of the material in all the peaks is greater than 0.6, the ratio value of material consisting only of protein. This suggests the presence of

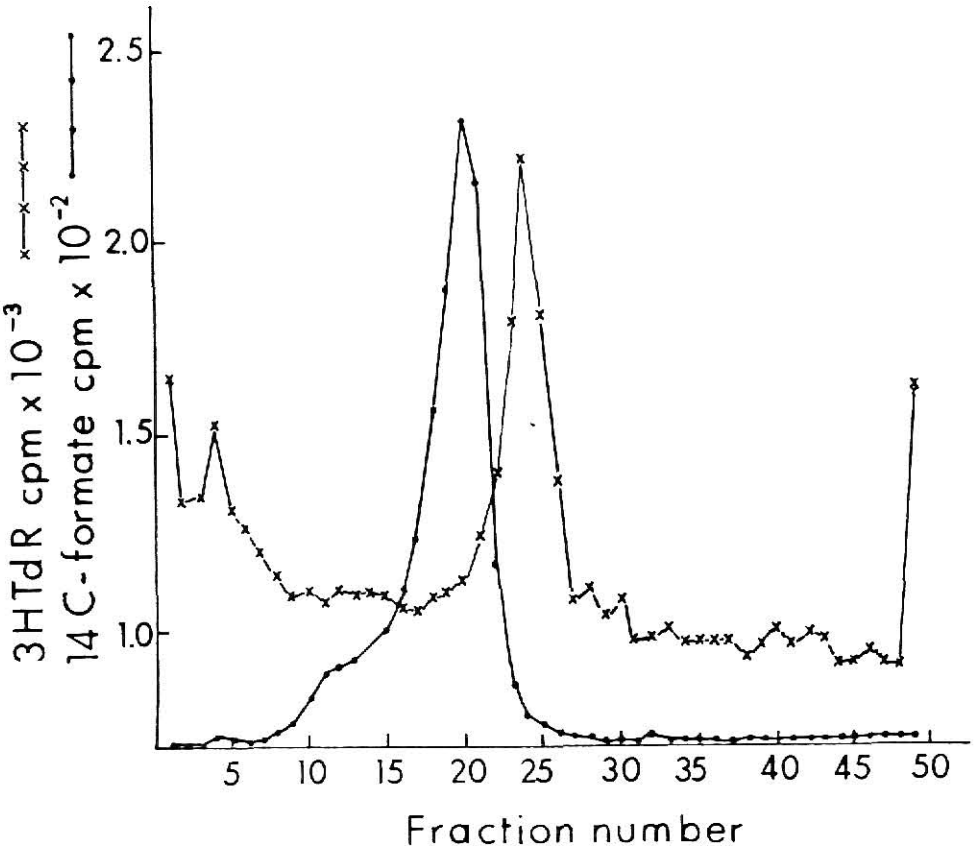

FIGURE 8. Cosedimentation of Ad 2 and AD 3 in a CsCl equilibrium density gradient. A cell free extract containing AD 3 particles labeled with ^{3}HTdR (X-X-X) was centrifuged to equilibrium in CsCl having a mean density of 1.34 g/cm^{3}. Purified Ad 2 virions labeled with ^{14}C formate (•-•-•) were included as an internal marker. The radioactivity in counts per minute has been corrected for background and radioactivity spillover.

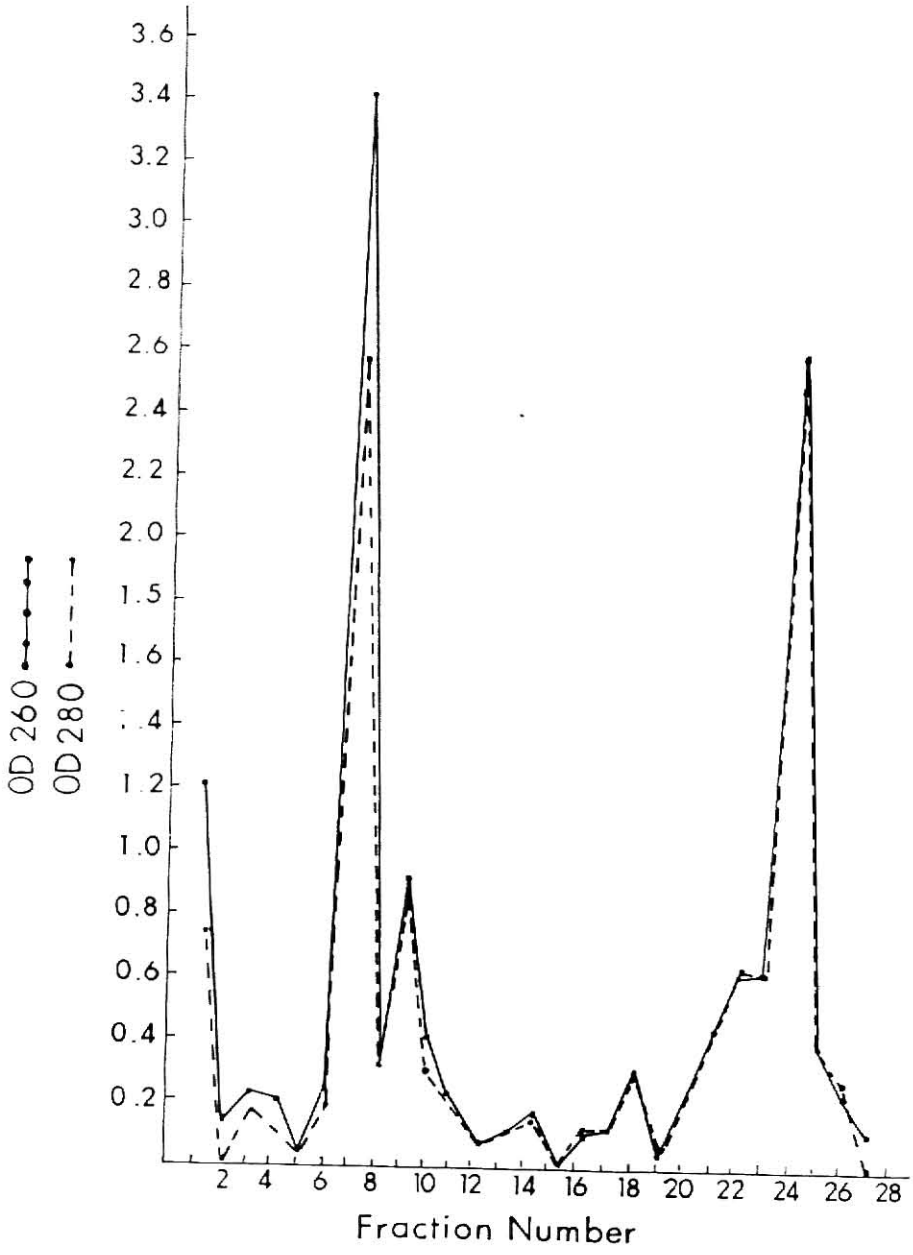

FIGURE 9. Optical density profiles of a CsCl equilibrium density gradient containing a lysate of Ad 3 infected KB cells. A KB cell lysate containing Ad 3 particles was added to CsCl having a mean density of 1.34 g/cm^3 and centrifuged to equilibrium at 100,000 x g. The bottom of the tube was punctured and 7 drop fractions were collected. Optical densities at 260 and 280 nm were determined for each fraction with a Zeiss spectrophotometer.

nucleic acid in these particles.

Although several bands of light-scattering particles were visualized when CsCl equilibrium density gradients containing purified Ad 3 were transilluminated, the main band (V) and one denser or two closely spaced less dense bands (II) were most easily visualized. Two additional less dense bands (III and IV) were routinely observed. Bands were purified by three cycles of CsCl equilibrium density sedimentation from seven different Ad 3 preparations. The buoyant densities of these bands in CsCl were determined in the analytical ultracentrifuge using the method published by Ifft et al. (1961). Figure 10 illustrates the frequency distribution of the buoyant densities. The histogram indicates that six discrete populations of particles, designated I-VI, are present. However, microdensitometer tracings of photographs from the analytical centrifuge indicate that incomplete particles (II) in the 1.2850-1.2900 g/cm^3 range are distributed into two distinct bands (IIa and IIb). Purified band II was centrifuged to equilibrium in the analytical ultracentrifuge at 75,000 x g and photographed. The centrifugal force was then reduced to 30,000 x g. After equilibrium was re-established another photograph was made. Figure 11 illustrates the microdensitometric tracings. At a force of 75,000 x g a shoulder on the less dense side of the major peak can be seen. When the force is reduced to 30,000 x g the shoulder separates from the major peak (IIb) as a separate less dense peak (IIa). Electron microscopic analysis of material from bands IIa and IIb indicates the presence of particles which have the morphological appearance of adenovirions.

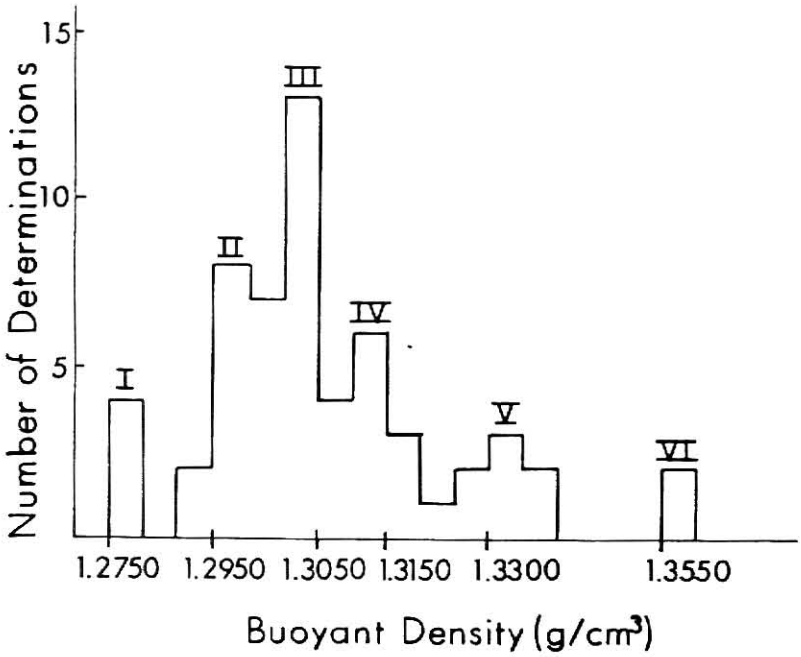

FIGURE 10. Frequency distribution of buoyant densities of purified Ad 3 viral particles. The buoyant densities were determined by CsCl equilibrium sedimentation in the analytical ultracentrifuge.

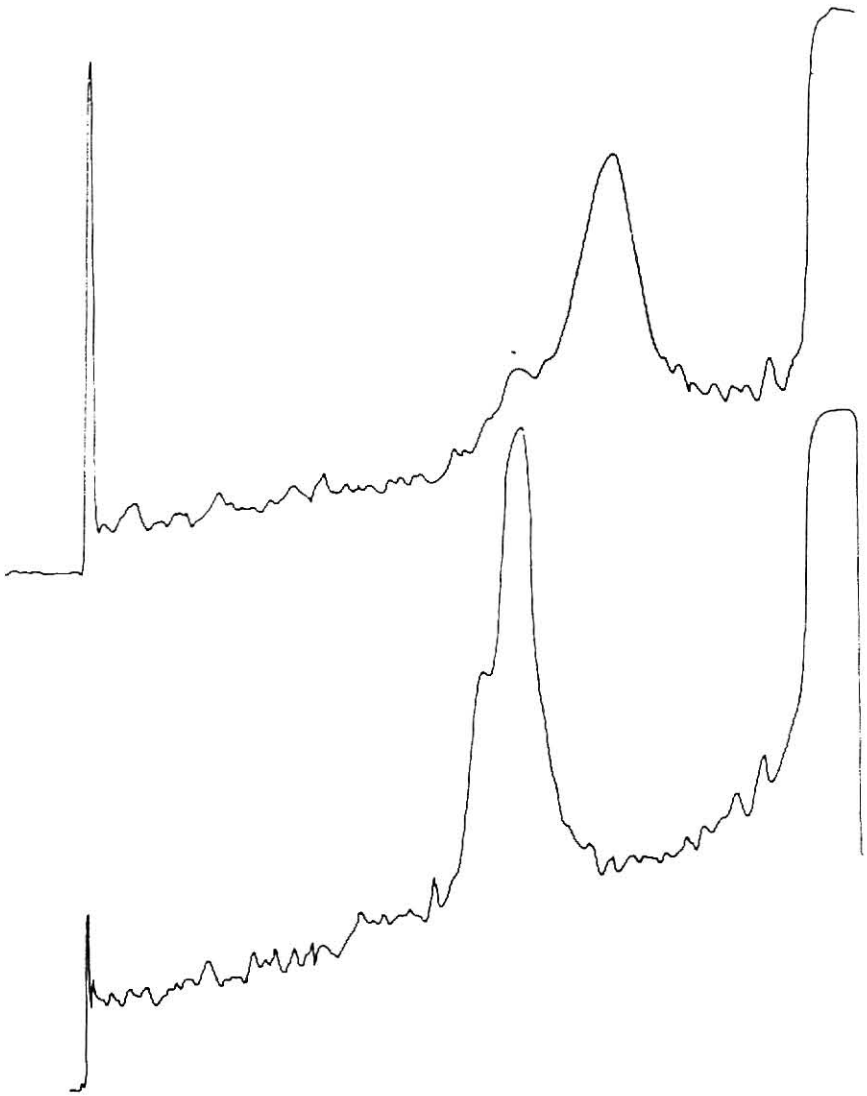

FIGURE 11. Resolution of Ad 3 incomplete particle bands IIa and IIb in the analytical ultracentrifuge. The bottom tracing represents purified Ad 3 band II centrifuged to equilibrium in CsCl and 75,000 x g. The top tracing represents the same virus particles at equilibrium in CsCl at 35,000 x g.

The mean buoyant densities of the Ad 3 particles are presented in Table 9. Band I has a mean density of 1.2787 g/cm^3. This value is greater than 1.25, the density of an empty adenovirus capsid (Burlingham et al., 1974). The incomplete particles in band I probably represent a very small percentage of the total particle yield since visualization of this band is exceedingly difficult by trans-illumination of preparative CsCl gradients. The difficulty in detecting this band in preparative gradients accounts for the low number of density determinations represented in the histogram.

The particles in band IIa have a mean density of 1.2913 g/cm^3. Band IIb usually represents a larger percentage of the total particle yield than band IIa. The particles in band IIb have a mean density of 1.2945 g/cm^3. These two bands are always produced in large quantities and are easily visualized in preparative gradients. The particles in band III have a density of 1.3030 g/cm^3 and those in band IV have a density of 1.3139 g/cm^3. The particles in band V are complete, infectious virions. The mean density of the virions is 1.3315 g/cm^3. The particles in band VI have a density of 1.3595 g/cm^3 and were purified and analyzed twice in the analytical ultracentrifuge. This low number of determinations reflects the difficulty in visualizing the band in preparative g lients during purification.

The incomplete particles of Ad 2 and Ad 12 are produced in constant proportion with repeated passage. An exception is Ad 12 band IIIa which increases in yield with repeated passage (Burlingham et al., 1974). The effect of passage number on the production of Ad 3 incomplete particles was examined. Lysates of KB cells containing Ad 3 virus

Table 9

Buoyant Densities of Banded Virus Particles of Human Adenovirus Type 3

Band	Mean Density (g/cm^3)	SEM[a]	Number of Determinations
I	1.2787	0.0009	4
IIa	1.2913	0.0019	5
IIb	1.2945	0.0026	9
III	1.3030	0.0025	15
IV	1.3139	0.0043	10
V	1.3315	0.0038	8
VI	1.3595		2

[a]SEM = standard error of the mean.

of passages 3, 4, and 5 from plaque purification were prepared by freeze-thawing and mechanical shearing. Cell debris was sedimented and the supernatant fluid was collected and extracted with Genetron. Each sample was layered on to a discontinuous CsCl gradient for zonal sedimentation. After centrifugation at 50,000 x g for 1.5 hr, the virus particles were visualized by light scattering from trans-illumination and were withdrawn directly through the side of the tube. The optical density of each sample was determined at 280 nm. Equal optical density units of each sample were added to separate cellulose nitrate centrifuge tubes and the tubes were filled with CsCl having a density of 1.33 g/cm^3. The samples were centrifuged to equilibrium in a SW 56 rotor at 100,000 x g. The bottom of the tubes were punctured and 8 drop fractions were collected into conical centrifuge tubes. The absorption of each fraction was determined at 260 and 280 nm.

Figures 12, 13, and 14 show the profiles of absorbance at 280 nm for the gradients containing particles from passes 3, 4, and 5 respectively. The profiles representing the different passage numbers are similar. The largest peak in the lower third of the gradient represents band V, the complete virions. The shoulder appearing on the less-dense side of band V has been designated as Va. The large broad peak in the top third of each profile probably represents bands IIa and IIb. Three peaks are present between the two major peaks of pass 3 and possibly again in pass 5. Three peaks were observed in similar positions in the optical density profile shown in Figure 9. With respect to these three bands, the one nearest the top of the gradient probably represents band III while the one nearest the virion peak is probably band IV. The

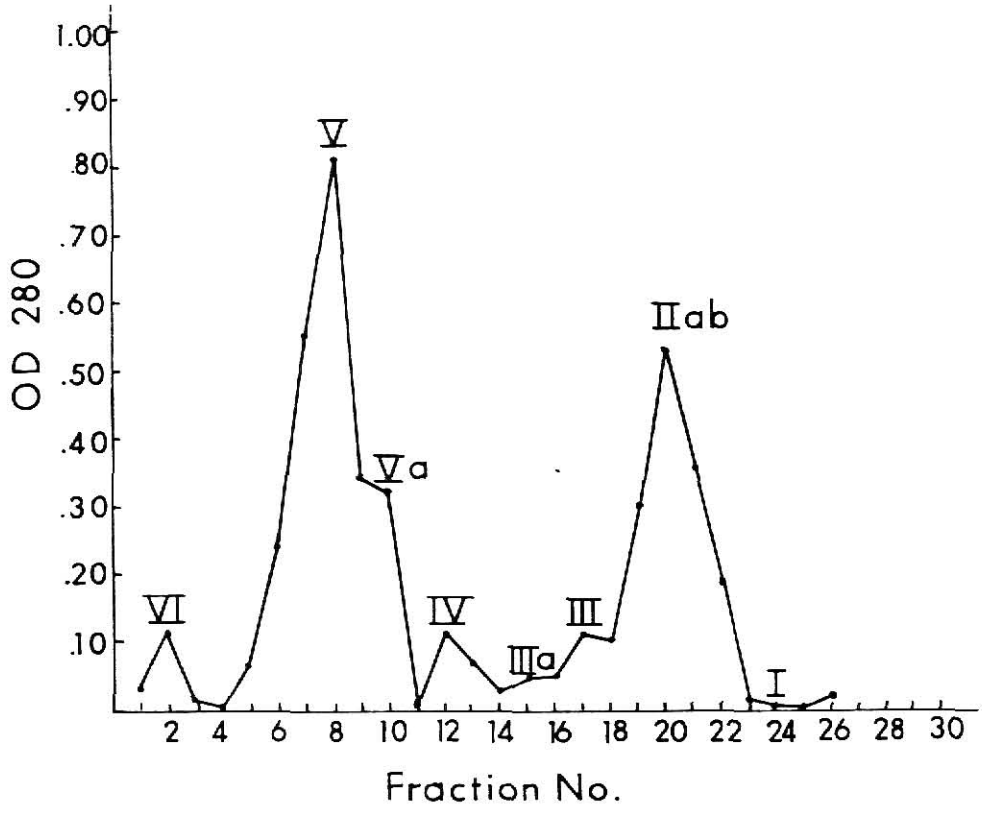

FIGURE 12. Optical density profile of a CsCl equilibrium density gradient containing purified Ad 3 particles from passage #3. Virus particles were removed from a CsCl step gradient and added to CsCl having a mean density of 1.33 g/cm^3. The particles were then centrifuged to equilibrium at 100,000 x $\underline{g}$ in an SW56 rotor. The bottom of the centrifuge tube was punctured and 8 drop fractions were collected for optical density determination.

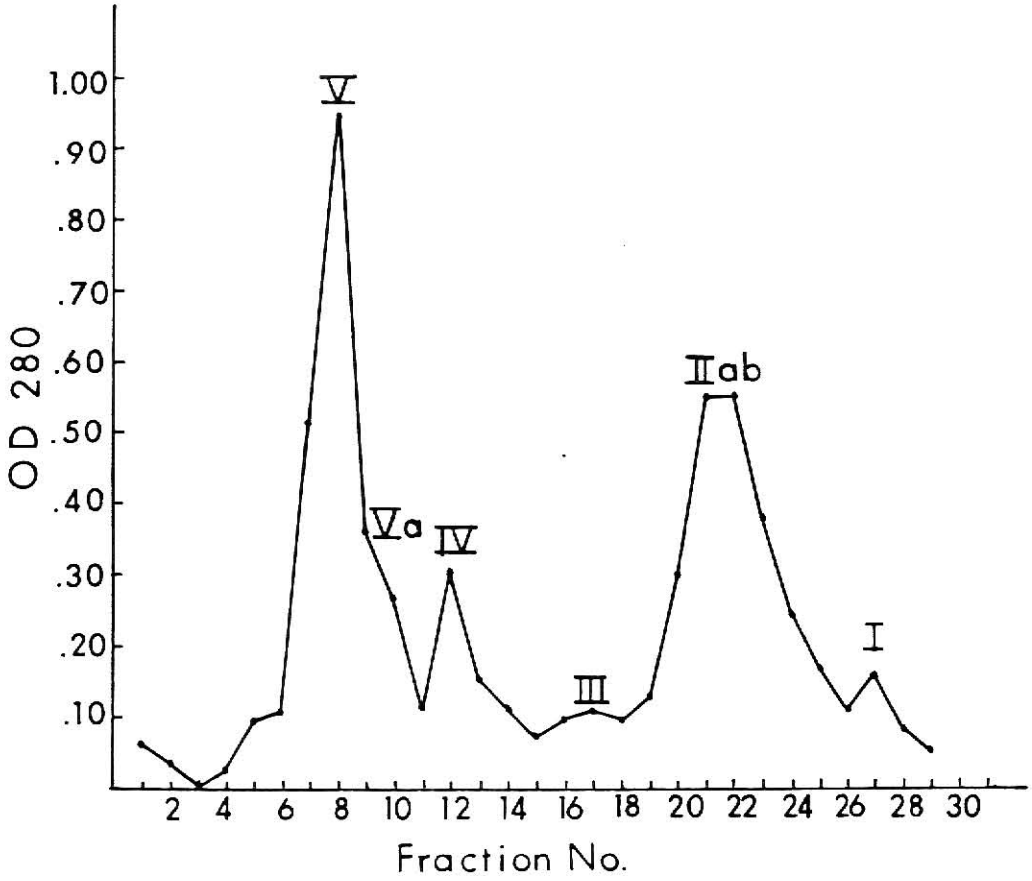

FIGURE 13. Optical density profile of a CsCl equilibrium density gradient containing purified Ad 3 particles from passage #4. Virus particles were removed from a CsCl step gradient and added to CsCl having a mean density of 1.33 g/cm^3. The particles were then centrifuged to equilibrium at 100,000 x g in an SW56 rotor. The bottom of the centrifuge tube was punctured and 8 drop fractions were collected for optical density determination.

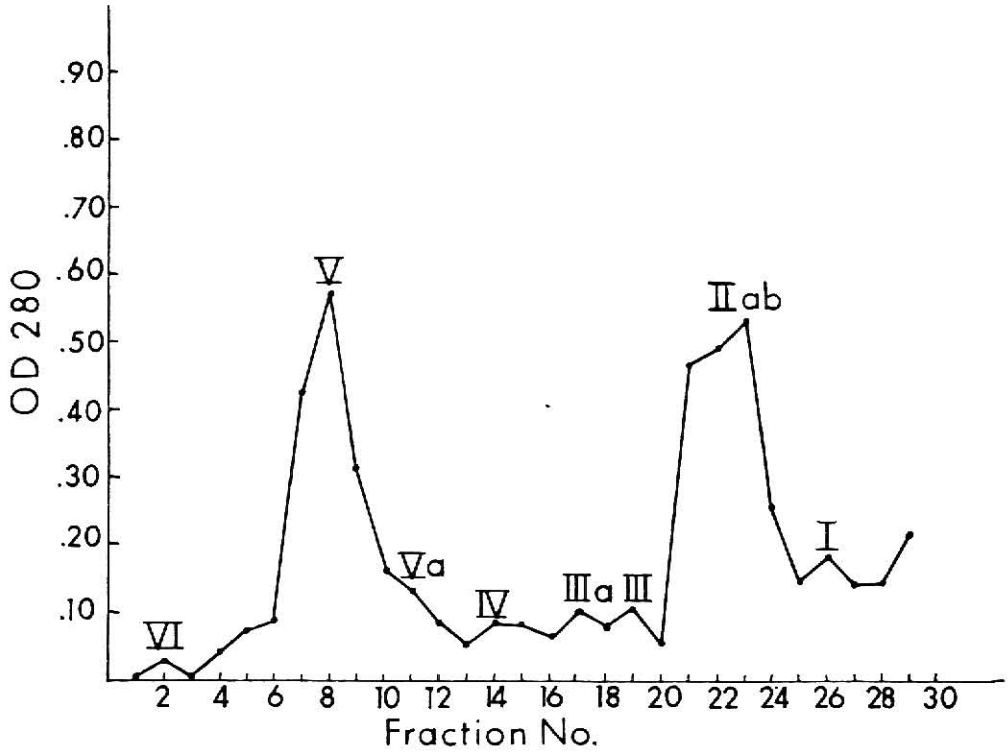

FIGURE 14. Optical density profile of a CsCl equilibrium density gradient containing purified Ad 3 particles from passage #5. Virus particles were removed from a CsCl step gradient and added to CsCl having a mean density of 1.33 g/cm^3. The particles were then centrifuged to equilibrium at 100,000 x g in an SW56 rotor. The bottom of the centrifuge tube was punctured and 8 drop fractions were collected for optical density determination.

middle peak (IIIa) probably represents an additional population of incomplete particles which was not detected previously. This population represents only a very small percentage of the total particles produced and is not detectable as a light scattering band by transillumination.

The effect of passage number on the production of Ad 3 particles is presented in Table 10. The incomplete particles in bands IIa and IIb are produced in approximately equivalent amounts to the virion band V. The particles in these bands apparently are produced in constant proportions in the three passages examined. The particles in bands III, IIIa, and IV are produced in roughly equal amounts. They are also produced in constant proportions in the three passages examined. Only band Va appears to increase consistently with passage number. Band Va is believed to be similar to band IIIa of Ad 12 described by Burlingham et al. (1974) and exhibits an increasing concentration of defective particles with passage number at high multiplicity.

The OD_{260}/OD_{280} values for Ad 3 particles are presented in Table 11. These values suggest the presence of nucleic acid in all the populations of particles. In general, the OD_{260}/OD_{280} values increase with increasing density of the bands indicating larger amounts of nucleic acid, presumably DNA, in the more dense populations. The optical density ratio for each band remains constant with passage number.

Adenovirus type 3 particles from bands IIa and IIb are produced in large quantities. Therefore these particles were used as representative incomplete particles in all of the further work. Nine purified preparations of Ad 3 complete virions (V) and incomplete

Table 10

Effect of Passage Number on Production of Ad 3 Particles

Passage Number	% of total OD_{280} contained in band number							
	I	IIaIIb	III	IIIa	IV	Va	V	VI
3	9.13	37.67	4.83	4.98	5.68	8.43	30.35	1.20
4	5.53	39.16	6.02	--	9.21	11.99	31.34	1.50
5	0.32	33.75	5.85	2.69	4.61	15.20	44.14	3.48

Table 11

Optical Density Ratios of Ad 3 Incomplete Particles and Virions

Passage Number	OD_{260}/OD_{280} of band number							
	I	IIaIIb	III	IIIa	IV	Va	V	VI
3	0.92	1.02	0.96	0.95	0.84	1.01	1.04	1.59
4	0.94	1.02	1.02	--	1.01	1.06	1.12	1.17
5	--	1.04	1.11	1.12	1.14	1.17	1.18	1.27
$\bar{X}$	0.93	1.03	1.03	1.03	1.02	1.08	1.11	1.34

particles (II) were used for optical density measurements at 260 and 280 nm. The purified incomplete particles had a mean OD_{260}/OD_{280} of 1.022 ± 0.033. The mean OD_{260}/OD_{280} ratio for virions was 1.180 ± 0.135. These values indicate the presence of nucleic acid associated with both types of particles and that the nucleic acid to protein ratio is higher for virions than for incomplete particles. Some of the virion preparations had optical densities greater than 1.0 and these were not diluted. Therefore the large standard error in the OD_{260}/OD_{280} determination is probably a result of light scattering effects.

Since the virions are incorporating ^{3}H-thymidine, the nucleic acid is presumably DNA. Adenovirus DNA packaged within virions is resistant to degradation by DNase but DNA outside the virion is susceptible. The DNA associated with incomplete virions was examined for resistance to DNase degradation. Ad 3 labeled with $^{32}PO_4$ was incubated with DNase at 37°C. At intervals, samples were withdrawn, acid precipitated, and the acid precipitable counts were determined. The results of this experiment are shown in Figure 15. Purified DNA extracted from virions was almost totally degraded during the first five minutes of incubation while the DNA associated with the virions remained acid precipitable. After 180 minutes of incubation 82% of the total radioactivity in the virion preparation remained acid precipitable while 56% of the total radioactivity in the incomplete particle preparation was precipitable. Therefore the DNA is within the virions but the DNA packaged within incomplete particles is more susceptible to degradation by DNase than DNA packaged within complete virions. It is also possible that the incomplete particle preparation may have contained more

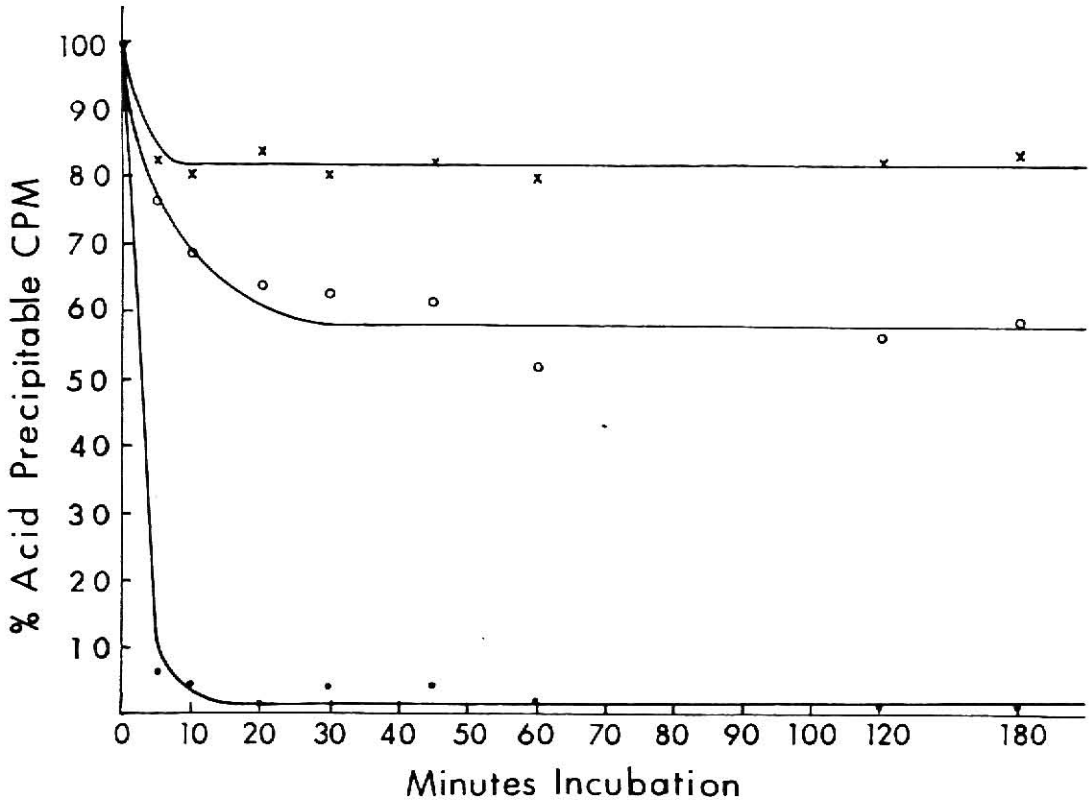

FIGURE 15. Effect of DNase on DNA associated with AD 3 viral particles. Purified ^{32}P-labeled Ad 3 complete (V), (X-X-X), or incomplete particles (II), (0-0-0), or ^{3}HTdR-labeled Ad 3 DNA (•-•-•) were incubated at 37°C with pancreatic DNase. At intervals, 100 µl samples were withdrawn, acid precipitated and counted. Similar results were obtained when the virus particles were labeled with ^{3}HTdR.

contaminating DNA than the virion preparation although the kinetic data do not support this alternative. The data also indicate that the limited degradation reaction occurs more quickly in the incomplete virions than in the complete particles. This suggests that the DNA is more accessible in the incomplete particles. The DNase resistant label within virions reached a constant level within the first ten minutes of incubation while the DNase resistant label within the incomplete particles did not become constant until 40 minutes of incubation. This may be due to the reported fragility of incomplete virions.

The DNA was extracted from purified ^{3}H TdR labeled Ad 3 incomplete and complete virions. The DNA from each type of particle was characterized by OD_{260}/OD_{280}, specific radioactivity, and hybridization to KB cell and Ad 3 virion DNA. These results are presented in Table 12. The OD_{260}/OD_{280} ratio of DNA extracted from incomplete particles is lower than that of virion DNA. This result may indicate either greater protein contamination in the incomplete particle DNA sample or a smaller molecular size of the DNA. The specific radioactivity of the incomplete particle DNA was about 7% of the specific radioactivity of virion DNA. The DNA from complete and incomplete virions hybridized better to Ad 3 virion DNA than to KB cellular DNA. The DNA extracted from complete Ad 3 virion and incomplete particles hybridized equally well to Ad 3 DNA however, twice as much DNA from incomplete particles hybridized with KB cell DNA than did DNA from complete virions. This result suggests that incomplete particles might contain some host cell DNA or that the viral DNA in incomplete particles are enriched for some sequences complementary to KB cell DNA.

Table 12

Characterization of DNA Extracted from Complete Virions and Incomplete Virions of Ad 3

DNA	OD_{260}/OD_{280}	Specific[a] Radioactivity	% Input Hybridized to Ad 3	% Input Hybridized to KB	% Hybridized to Control
Incomplete (II)	1.49	1583.74	17.00	6.8	96.4
Complete (V)	1.77	11299.00	17.63	3.25	100

[a]Specific radioactivity = total cpm/total OD_{260}.

The DNA extracted from purified complete (V) and incomplete (II) particles were further examined for resistance to degradation by DNase, pronase, and KOH. The radioactively labeled DNA samples were incubated at 37°C with DNase, pronase, or KOH. At intervals aliquots were withdrawn, acid precipitated, and counted. The results of these experiments are illustrated in Figures 16 and 17. Pancreatic DNase rapidly digested all but 3-4% of the radioactive DNA of both the complete and incomplete particles within the first twenty minutes of incubation. Pronase digested 7-8% of the radioactivity associated with the DNA from either complete or incomplete particles. This decrease in counts could be due to contaminating nucleases but the possibility of protein linkers or binding proteins associated with the DNA cannot be eliminated. Incubation of incomplete particle DNA with KOH indicated that no RNA was present. However KOH digested 12% of the radioactivity associated with DNA from the complete virions. Since a sample was taken at only one time point, this decrease is most probably due to experimental error.

Discussion

Lysates of Ad 3 infected KB cells contain populations of viral particles which have distinct buoyant densities. Analysis of purified Ad 3 particles in the analytical and preparative centrifuge indicates that eight distinct populations may be present. Complete Ad 3 virions have a buoyant density of 1.3315 g/cm^3 and are designated as band V. Prage et al. (1972) reported that the density of Ad 3 virions was 1.355 g/cm^3 and Sundquist et al. (1973) reported a value of 1.34 g/cm^3. However, these determinations were made from preparative gradients.

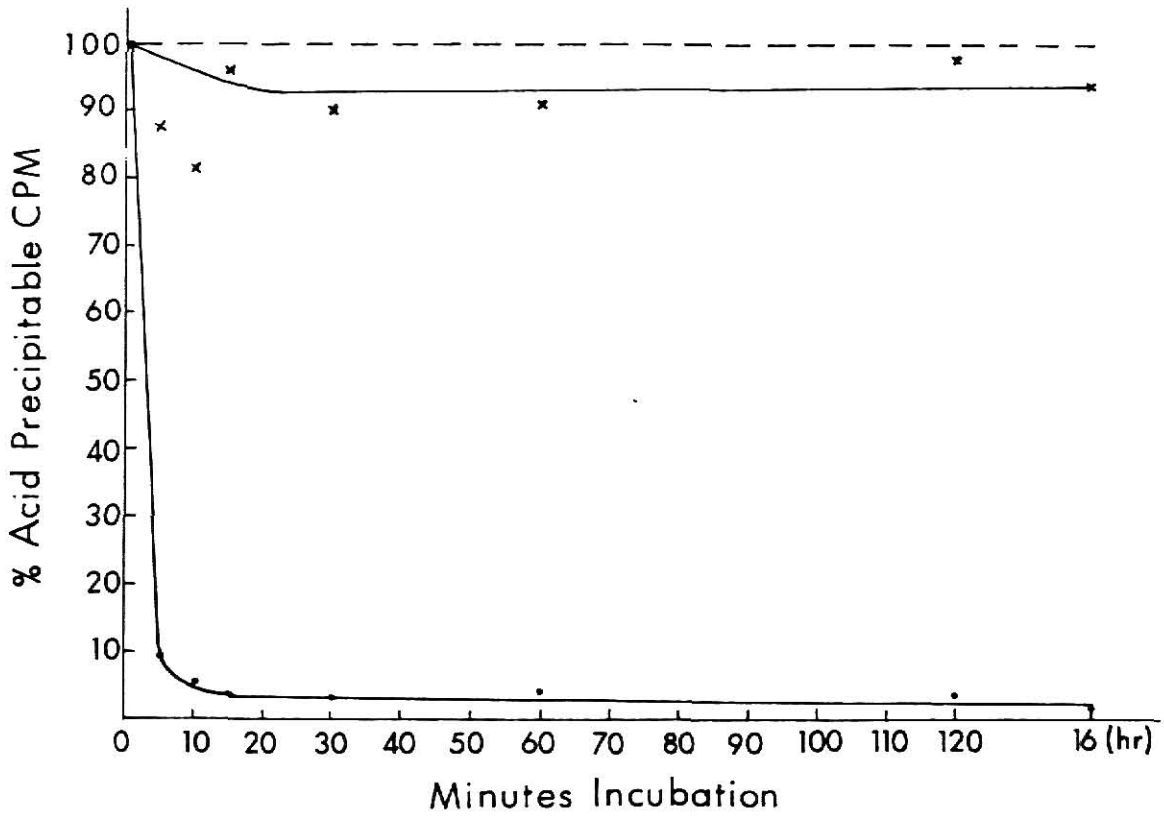

FIGURE 16. Effects of enzymes and KOH on the nucleic acid extracted from Ad 3 incomplete particles. 5 µg ^{3}DNA extracted from Ad 3 bands IIa and IIb labeled with ^{3}HTdR was incubated at 37°C with pancreatic DNase ( ), pronase (X-X-X) or 0.3 M KOH (---). At intervals 100 µl samples were removed, acid precipitated, and the acid insoluble radioactivity was determined by liquid scintillation counting.

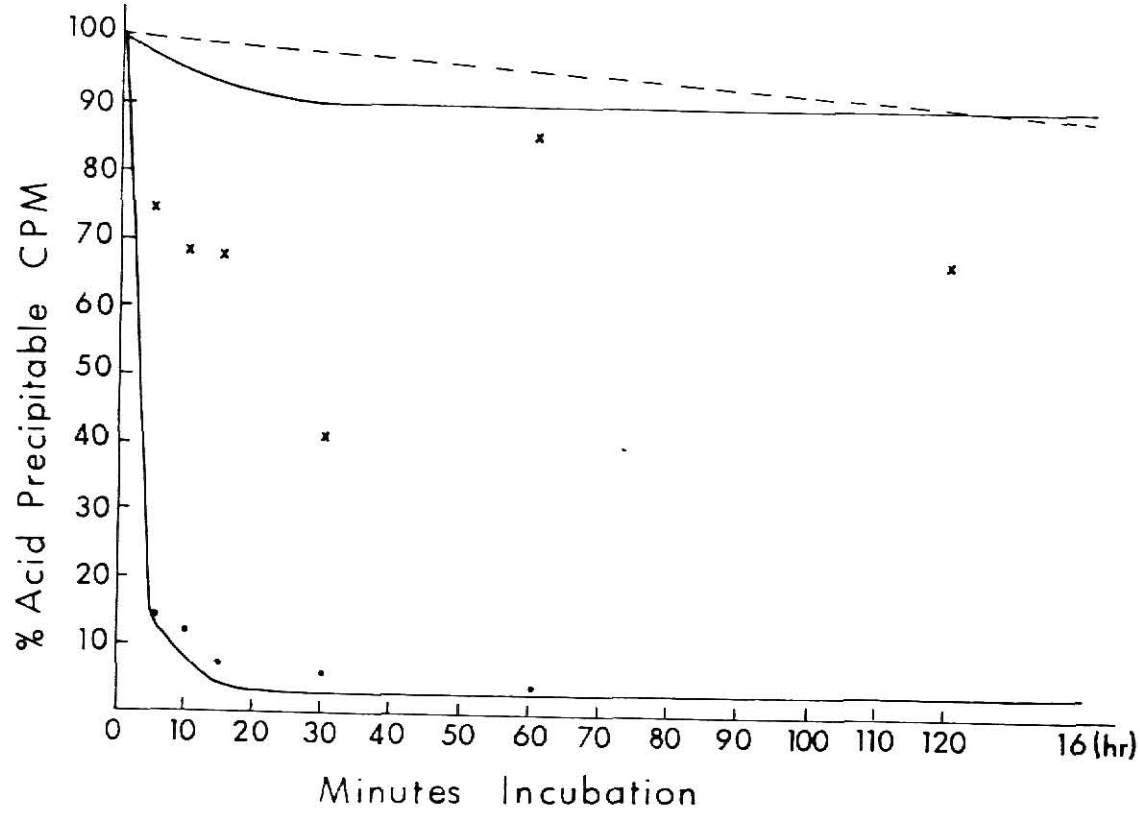

FIGURE 17. Effects of enzymes and KOH on the nucleic acid extracted from Ad 3 complete virions. 5 µg DNA extracted from Ad 3 band V labeled with ^{3}HTdR was incubated at 37°C with pancreatic DNase ( ), pronase (X-X-X) or 0.3 M KOH (---). At intervals, 100 µl samples were removed, acid precipitated, and the acid insoluble radioactivity was determined by liquid scintillation counting.

The most predominant incomplete bands, IIa and IIb, are present in large quantities and have densities of 1.2913 and 1.2945 g/cm^3 respectively. Band IIb usually contains more particles than band IIa. These two bands are probably the same as the incomplete bands characterized by Prage et al. (1972). Band III with a buoyant density of 1.3030 g/cm^3 and band IV with a buoyant density of 1.3139 g/cm^3 are usually present in quantities large enough to be visualized by light scattering. However band I with a buoyant density of 1.2787 g/cm^3 and band VI with a buoyant density of 1.3595 g/cm^3 are not easily seen. More analyses are necessary to verify the densities of the particles in these two bands.

The buoyant densities of all the incomplete bands are greater than the buoyant density predicted for a capsid composed only of protein. Significant differences in OD_{260}/OD_{280} were noted between complete and incomplete virions. The lower value of this ratio for incomplete particles supports the presence of a small amount of nucleic acid in these particles. The mean OD_{260}/OD_{280} value for Ad 3 bands IIa and IIb reported in this paper is slightly higher than the value reported for Ad 2 bands Ia and Ib (0.94 and 0.98 respectively), and for Ad 12 band I (0.81) by Burlingham et al. (1974). The mean OD_{260}/OD_{280} value for complete Ad 3 virions reported here (1.18) is lower than the value determined for complete Ad 2 virions (1.34) and complete Ad 12 virions (1.31) by Burlingham et al. (1974). This lower value is probably due to light scattering effects since concentrated samples were not diluted.

The labeling of incomplete particles with ^3H TdR supports the presence of DNA as the nucleic acid. The DNA within both complete and

incomplete particles was relatively resistant to DNase digestion. A greater percentage of the radioactive counts in the incomplete particle sample became soluble. The degradation reaction occurred more rapidly for the DNA within incomplete particles than the degradation reaction for the DNA within complete virions. These results might be due to differences in packaging of DNA in complete virions and incomplete particles or differences in the structure of the particles. Prage et al. (1972) reported that the major virion core polypeptides V and VI-VII are missing in Ad 3 incomplete particles. A number of other investigators have reported differences in the core polypeptide composition of adenovirus incomplete particles and complete virions (Rosenwirth et al., 1974; Ishabashi and Maizel, 1974; Wadell et al., 1974). Perhaps these differences in DNA packaging accounts for the greater amount of digestion of incomplete particle DNA. A major portion of the DNA from both complete and incomplete particles is protected from DNase action over an extended period of incubation. This protection is presumably due to a similar protein coating over the nucleic acid.

The DNA is extractable from complete and incomplete particles of Ad 3. While the DNA of incomplete particles hybridizes better with Ad 3 DNA than with KB cell DNA, it does hybridize with KB cell DNA to some extent. I appears to hybridize about 2 times better to KB cell DNA than does the DNA from complete virions. This could indicate that incomplete particles contain some KB cell DNA fragments or that the DNA in the incomplete particles is enriched for sequences complementary with KB cell DNA.

The biological role of the incomplete particles during infection is still not understood. The particles may be errors resulting from the assembly process. However, they do not arise from the virus inoculum and they appear simultaneously with progeny virions (Rosenwirth et al., 1974). Phillipson and co-workers have suggested that incomplete particles serve as precursor molecules to infectious virions (Sundquist et al., 1973; Ishibashi and Maizel, 1974; Edvardsson et al., 1976). They propose a model in which empty capsids are first assembled containing precursor polypeptides. Cores are later inserted and modifications of the precursor polypeptides convert the intermediates to infectious virions. This model is based solely on protein labeling pulse-chase experiments. DNA pulse-chase labeling experiments would further substantiate or refute this model. Schaller and Yohn (1974) have suggested that the incomplete particles may have a biological role in determining oncogenic and transformation potentials of adenovirus populations. This role has not been supported by experimental evidence and therefore requires additional investigation.

APPENDICES

I. Supplementary Information for Analytical Ultracentrifugation

A. Calibration of the Rotor Temperature Indicator Control (RTIC)

A precision mercury thermometer was calibrated against 6 physical constants for five chemicals (Figure 18). This calibrated thermometer was placed in the thermometer holder and silicon oil was added to provide good thermal conduction. The holder and thermometer were then inserted into rotor ANF #386, and the rotor was chilled to 4 °C or warmed to 37 °C.

The RTIC needle was balanced to zero, then a 10^6 Ω resistance was inserted to insure that the circuitry was operating properly. The reading of the test resistance was 5394 and has remained constant throughout the use of the machine. The test resistance was removed and the thermal calibration stand was inserted. The chilled or warmed rotor was placed in the stand. At intervals, the RTIC was rebalanced, the range and balance were determined, and these values were recorded along with the corresponding temperature. A plot of range-balance readings as a function of temperature is shown in Figure 19. Within a single range, all balance readings were linear. However the equations of the lines for different ranges were not equivalent. The shifts which occurred with the changes in range are due to inherent properties of the instrument's electronic circuits.

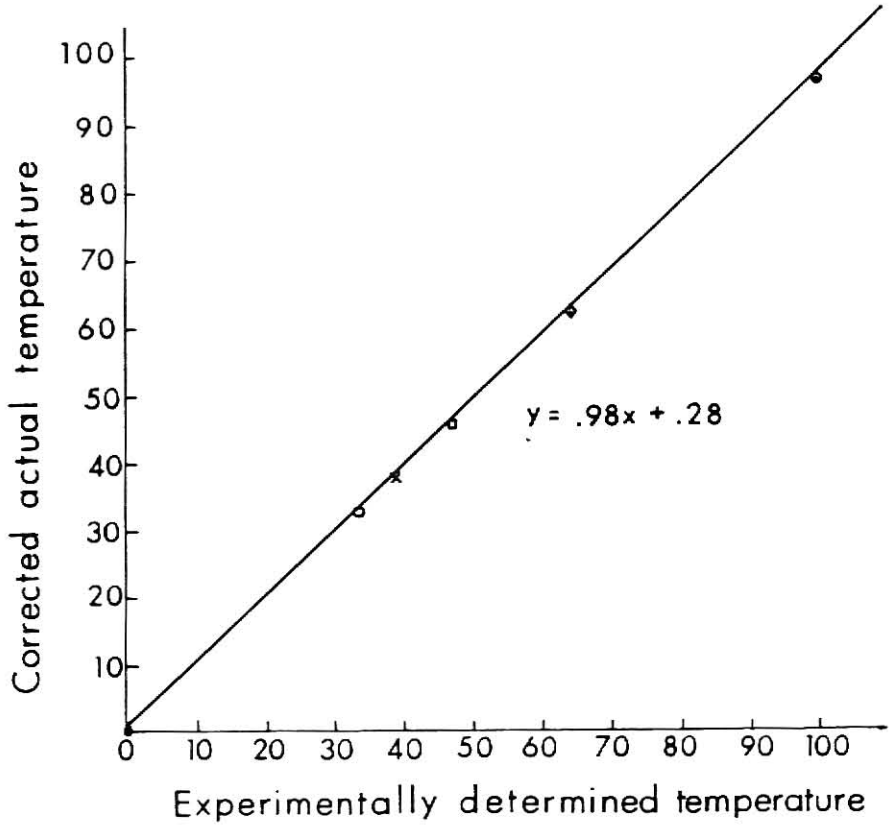

FIGURE 18. Temperature calibration of precision mercury thermometer NBS 61663. Boiling points were determined with mercury thermometer NBS 61663 for distilled H_2O (⊙), methanol (■), Genetron (□), dichloromethane (x), and diethyl ether (o). The melting point of distilled water ice (●) was also determined. The experimental values were corrected for barometric pressure.

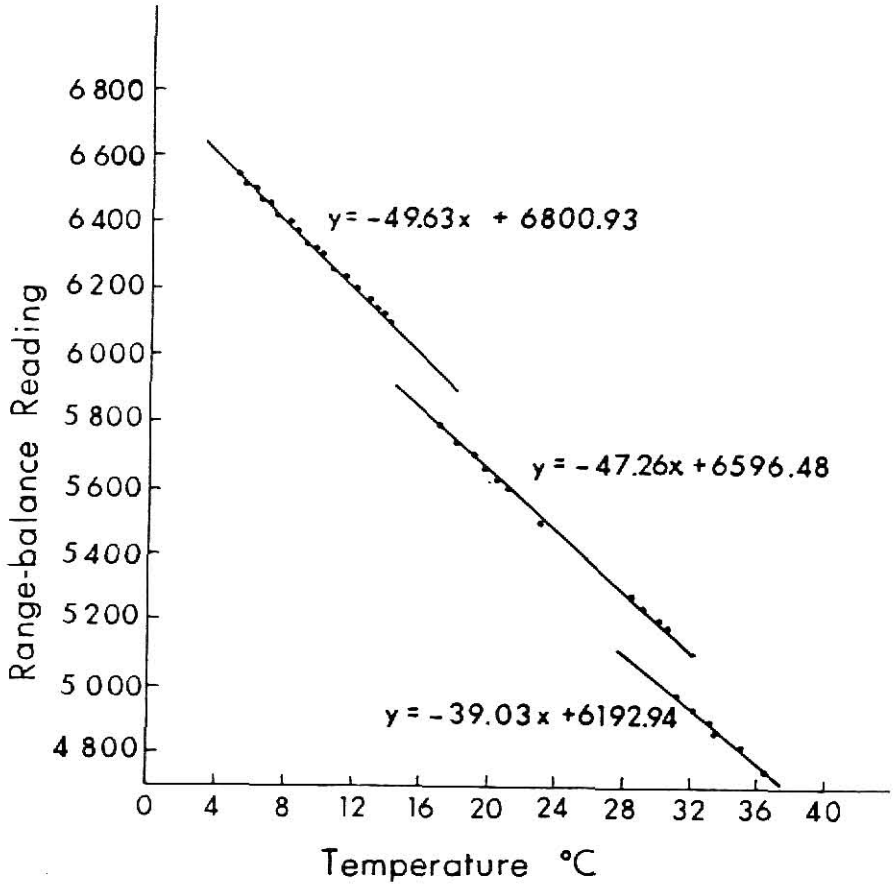

FIGURE 19. Temperature calibration of rotor ANF #386. A calibrated thermometer was placed in a holder and inserted into rotor ANF #386. The rotor was then chilled to 4 °C or warmed to 37°C. The rotor was placed in a calibration stand which was plugged into the analytical ultracentrifuge. At intervals the range and balance readings were determined and the corresponding temperature was recorded.

B. Calibration of the Counterbalance and Determination of the Optical Magnification Factor

The distance between the reference marks in the counterbalance was measured with a microcomparator. The mean distance was found to be 15.876 $\pm$ 0.001 mm. To determine the magnification factor of the photographic system, the counterbalance was positioned in the rotor opposite a balanced empty cell. The rotor reference hole was plugged. When a speed of 12,000 rpm was reached the monochromator was adjusted to 265 nm and a series of photographs was taken of the counterbalance. Beginning with a focal length of 3.0" the distance was increased by 0.5" for each successive photograph. The photographs were traced with the Joyce-Loebl microdensitometer, and the distances between the reference marks were measured for each picture. The optical magnification factor, defined as the measured distance between the reference marks on the tracing divided by the actual distance determined with the microcomparator, was calculated for each focal distance. A plot of magnification factor as a function of focal length is shown in Figure 20.

C. Determination of the Focal Point for Rotor ANF #386

A focal length series (5 1/8" to 6 7/8" at 1/8" intervals) was taken of a CsCl filled plane windowed cell with the monochromator set at 265 nm and tracings were made of the meniscus at each focal length. The result was a parabolic curve, shown in Figure 21. The theoretical curve for this type of parabola is represented by the equation: $y = p(x-a)^2 + b$ where (a,b) are the coordinates of the minimum point and p is a constant. Determination of the theoretical equation which best fit the experimental data was done iteratively. The best

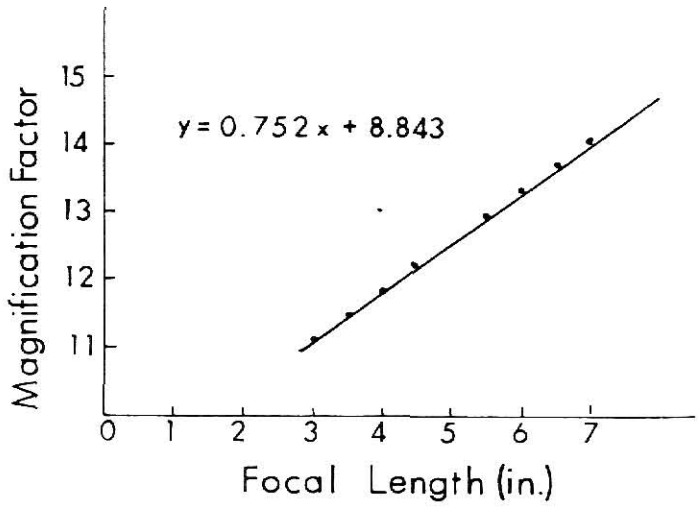

FIGURE 20. Optical magnification factor as a function of focal length. A focal series from 3.0 to 7.0" at 0.5" intervals was taken of the plane windowed cell at 265 nm. The photographs were traced with the Joyce Loebl microdensitometer. The optical magnification factor was determined by dividing the measured distance on the tracing by the actual distance between the reference holes in the counter balance which was determined with the microcomparator.

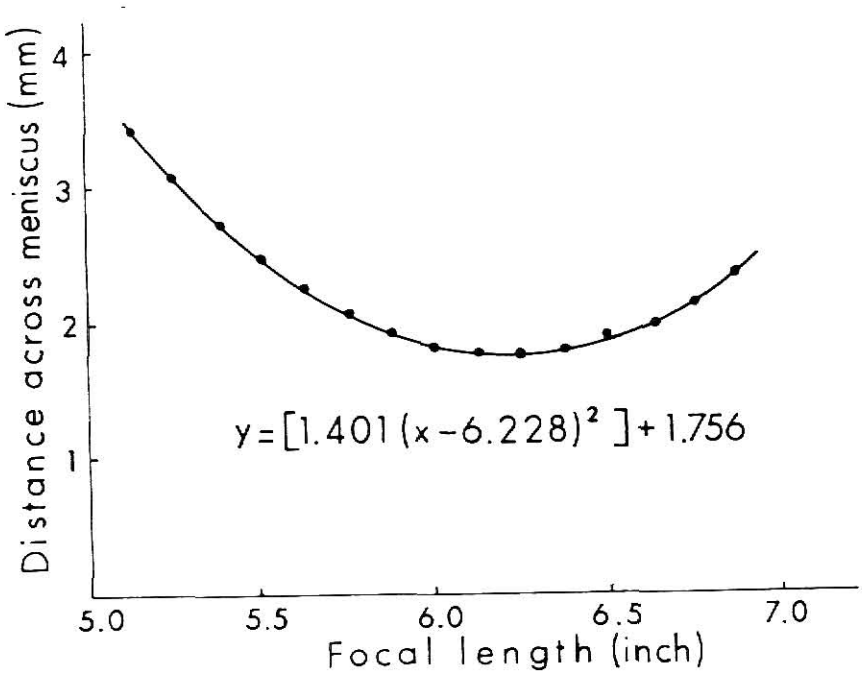

FIGURE 21. Distance across the meniscus as a function of focal length at 265 nm. A focal series from 5 1/8 to 6 7/8" at 1/8" intervals was taken of the plane windowed cell at 265 nm. The photographs were traced with the Joyce Loebl microdensitometer and the width of the meniscus was determined for each focal length.

theoretical curve for the actual data was determined to be: $y = 1.401\ (x - 6.228)^2 + 1.756$. The focal point was determined by setting the first derivative of the theoretical equation equal to zero and solving for the X value. The focal point for rotor ANF #386 was calculated to be 6.228" at 265 nm.

D. Determination of the Distance from the Bottom of the Cell to the Reference Mark

The four cells were placed in ANF rotor #386 and were spun at 12,000 rpm. Two photographs were taken of each cell at 265 nm. The bottom portion of each photograph was traced on the Joyce Loebl microdensitometer a minimum of six times, and the distance between the bottom of the cell and the reference mark was measured. The average distances were found to be as follows: cell #1, 1.19 cm; cell #2, 1.19 cm; cell #3, 1.28 cm; cell #4, 1.15 cm.

E. Determination of CsCl Density by Direct Pycnometry

Following each run in the analytical ultracentrifuge the cells were removed from the rotor and inverted several times to insure mixing of the contents. The contents were withdrawn with a 1 cc tuberculin syringe and frozen in a small sealed tube until pycnometry was carried out.

A 100 µl pipette was carefully cleaned and weighed a minimum of five times on an analytical balance. The same pipette was then filled with distilled water and weighed five times. It was dried and filled with CsCl solution from the ultracentrifuge cell and weighed in the same manner. The volume of the pipette was determined by dividing the mass

of the water by the density of water as recorded in the International Critical Tables. The density of the CsCl solution was then calculated by dividing the mass of the CsCl solution by the determined volume of the pipette.

F. Determination of Buoyant Density of Adenovirus Bands from the Analytical Ultracentrifuge Data

The methods and equations of Ifft et al. (1961) were used to determine buoyant density of adenovirus bands. Measurements were made on the tracings as shown in Figure 22. The following quantities were determined:

R_r = radius of the reference = 7.30 cm for all cells.

D_m = mean density of cell contents determined by direct pycnometry.

R_m = radius of the meniscus = $R_r - (M_m)\left(\frac{1}{OMF}\right)$ where OMF = optical magnification factor.

R_x = radius of peak X = $R_r - (M_x)\left(\frac{1}{OMF}\right)$.

$\beta = [\beta_0 - \beta_1)(D_m) + \beta_2(D_m)^2 - \beta_3(D_m)^3] \times 10^9$

where $\beta_0 = 47.9726$

$\beta_1 = 85.3724$

$\beta_2 = 51.7764$

$\beta_3 = 10.4312$

R_{mid} = radius of the midpoint in the CsCl column = $R_r - \left(\frac{M_m - M_b}{2}\right)\frac{1}{OMF}$.

dD = difference from mean density = $\frac{\omega^2}{2\beta}(R_x^2 - R_{mid}^2)$

where ω = angular velocity in radians/second.

The buoyant density of peak X, ρ_x, was determined by the equation $\rho_x = D_m + dD$.

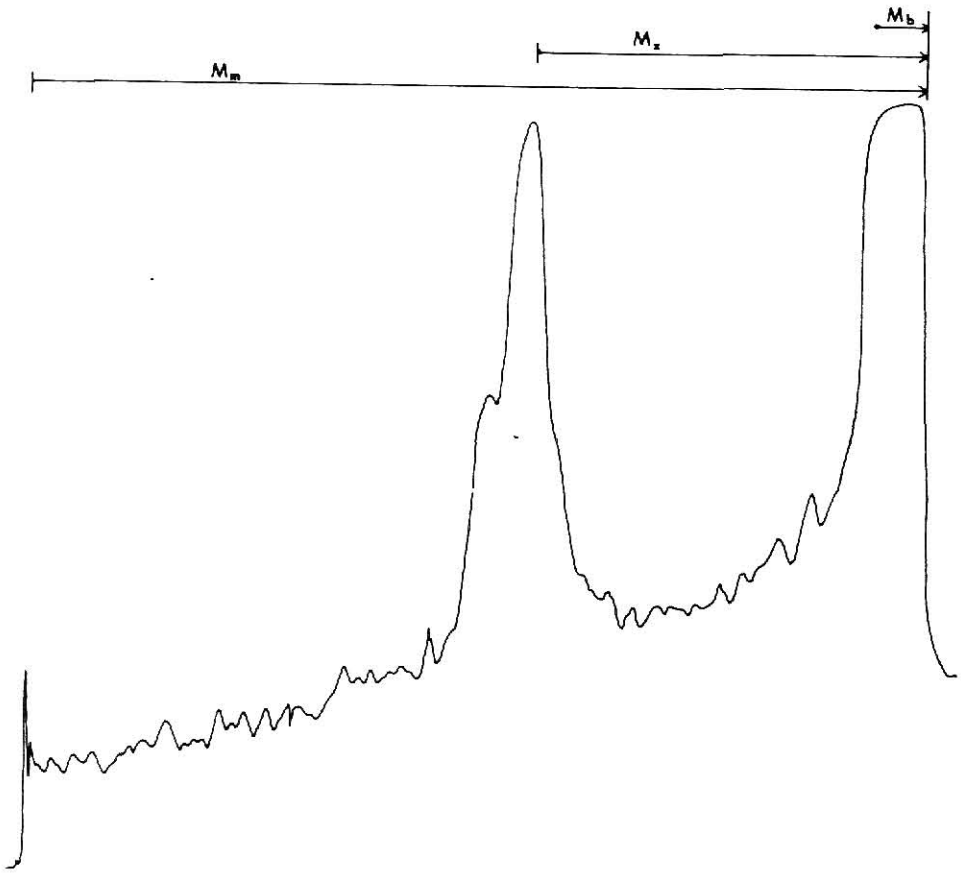

FIGURE 22. Tracing of a Model E photograph taken with UV optics. Mm = measurement from the meniscus to the reference mark, Mx = measurement from the maximum of peak X to the reference mark, Mb = measurement from the bottom of the cell to the reference mark.

II. Supplementary Information on DNA-DNA Hybridization

A. Burton DNA Assay on DNA bound to Nitrocellulose Filters

The amounts of DNA bound to Schleicher and Schuell type B6 filters was quantitated as described by Burton (1961). The acetaldehyde and the glacial acetic acid used in the Burton reagent were freshly distilled prior to the assay. Salmon sperm DNA was denatured and applied to filters in the usual manner. The filters were washed, placed in 13 mm test tubes, dried in a vacuum desiccator overnight, and then placed in an oven at 80°C for 2 hours. A 1 ml volume of TE was added to each tube followed by the addition of 1 ml of 1.0 M perchloric acid. After mixing, 4 ml of Burton reagent was added to each tube, and all tubes were incubated at 30°C overnight. The samples were mixed well and the absorbance at 600 nm was determined. The standard curve for the determination of DNA concentration is shown in Figure 23. The results indicate that the Burton DNA determination is quantitative for DNA bound to nitrocellulose filters.

B. Saturation of Nitrocellulose Filters with DNA

Each lot of nitrocellulose filters differed in its capacity to bind DNA. The saturation point of Schleicher and Schuell type B6 filters, lot #42S5 (72975) was determir . Increasing amounts of denatured salmon sperm DNA were applied to separate filters. The filters were washed, and dried as usual. Burton DNA determinations were made of the DNA bound to each filter and the absorbance at 600 nm was recorded. The μg of DNA bound was determined from the standard curve. Figure 24 shows that the saturation point of lot #42S5 (72975) filters

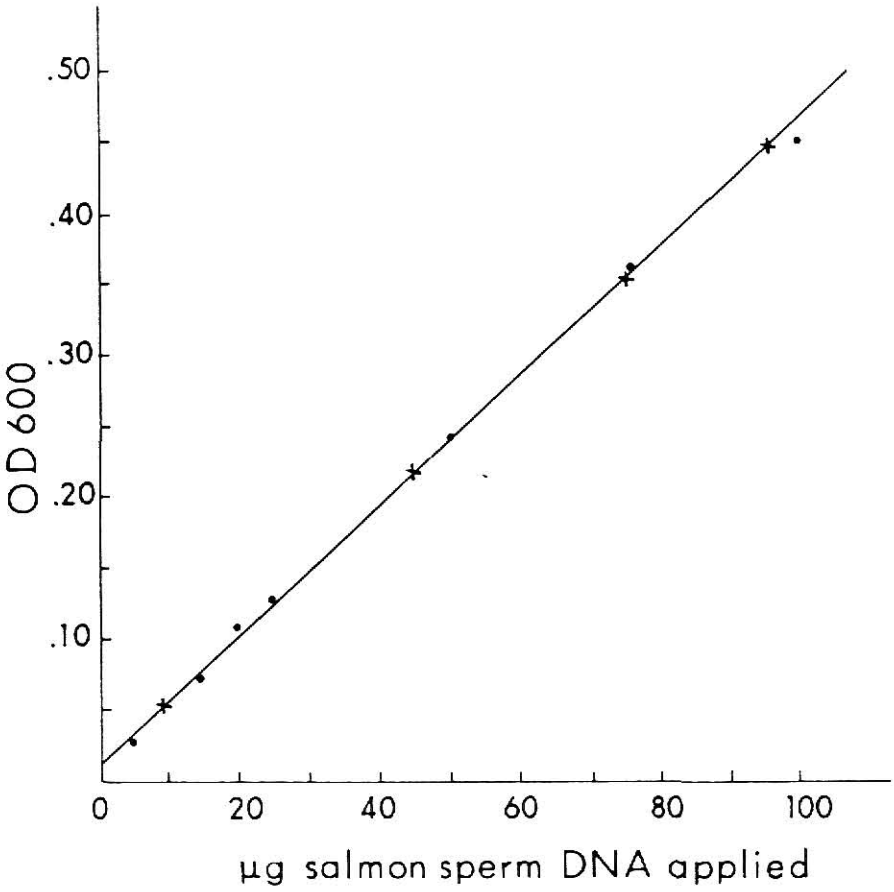

FIGURE 23. Standard curve for the determination of the amount of DNA bound to nitrocellulose filters. Salmon sperm DNA was denatured and applied to filters in the usual manner. The filters were dried overnight and baked for 2 hr at 80°C. DNA was assayed by the method of Burton (1956). The absorbance at 600 nm was determined for each concentration of DNA. Closed circles are experimentally determined values. X's are theoretical points.

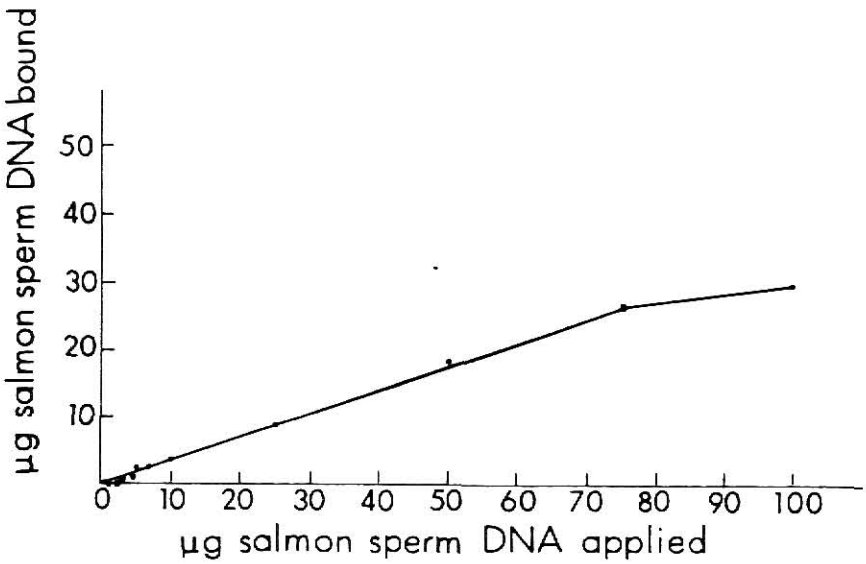

FIGURE 24. Saturation of S&S type B6 filters with salmon sperm DNA. Increasing amounts of denatured salmon sperm DNA were applied to separate filters. The filters were washed and dried as usual. The method of Burton (1956) was used to determine the amount of DNA bound to each filter.

occurred between 75 and 100 μg of salmon sperm DNA. The DNA binding capacity of this lot of filters was unusually high, making it impractical to saturate the filters with the experimental DNA samples. In all experiments, 10 μg of either cellular or viral DNA were applied to the filters.

C. Other Control Experiments

Treatment of filters with preincubation mixture (PM) which is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin, serves to coat the surface of the filters and prevent nonspecific binding of DNA. Control experiments were carried out to determine the efficiency of PM treatment. The effects of PM treatment and the extensive washings on previously bound DNA was determined. The results are presented in Table 13. The PM was found to be 99-100% efficient in preventing the binding of additional adenovirus or cellular DNA. The PM treatment and subsequent washings eluted less than 1% of previously bound adenovirus or cellular DNA.

III. Adsorption and Uptake of Adenovirus Type 3 Particles

The adenovirus incomplete particles apparently do not replicate (Burlingham et al., 1974; Wadell et al., 1973, Prage et al., 1972). This lack of infectivity could be due to blockage of early events in the infectious cycle, such as adsorption or penetration. Adsorption and uncoating of incomplete and complete particles of Ad 3 was studied in KB cells, a permissive host.

Radioactively labeled Ad 3 was purified by a step gradient and an equilibrium density gradient. The equilibrium gradient was dripped,

Table 13

Control Experiments for DNA-DNA Hybridization

DNA Applied	Treatment	^{3}HdR CPM	% of Control
^{3}H Ad 2	Control[a]	7289	
^{3}H Ad 2	PM before DNA[b]	62	0.85
^{3}H KB	Control	10520	
^{3}H KB	PM before DNA	115	0.01
^{3}H Ad 2	Control	8865	
^{3}H Ad 2	PM[c]	8963	101.10
^{3}H KB	Control	6517	
^{3}H KB	PM	6499	99.72

[a]Denatured ^{3}H TdR-labeled DNA was applied to a filter. The filter was washed, dried, and counted.

[b]A washed filter was treated with PM. It was then washed, dried, and challenged with denatured ^{3}H TdR-labeled DNA.

[c]Denatured ^{3}H TdR-labeled DNA was applied to a filter. The filter was washed, dried, and PM treated. It was then washed 6 times with 20 ml of 0.01 M Tris pH 8.1, dried, and counted.

collecting 0.2 ml fractions and optical densities at 260 and 280 nm were determined. The fractions containing the complete virus (V) and the fractions containing the incomplete particles (II) were pooled and dialyzed against PBS. The particles were adsorbed to confluent monolayers of KB cells at a physical multiplicity of 5,000. Adsorption was carried out at 4°C and was facilitated by gently rocking the cell monolayers every 15 min. After two hours, the inoculum was removed, retained, and residual particles removed by washing the cell monolayers three times in PBS. The inoculated cells were incubated in MEM suspension culture medium containing 10% calf serum for 0, 1, or 6 hours. A portion of the inoculum removed from the monolayer was counted. The remainder was added to fresh monolayers for another cycle of adsorption.

The radioactive counts lost from the inoculum after each cycle of adsorption are presented in Table 14. The data suggest differences in adsorption between incomplete and complete particles. The incomplete particles are more readily adsorbed on subsequent exposures. A greater percentage of complete particles are adsorbed during the first exposure to cells. However much of this data is based on estimates. The percentage of complete particles adsorbed on subsequent exposures seems to drop slightly. This data might reflect the structural differences between the incomplete and complete particles. A number of investigators have shown that the polypeptide compositions of complete and incomplete particles are not identical (Ishibashi and Maizel, 1974; Rosenwirth et al., 1974; Wadell et al., 1973; Prage et al., 1972). Perhaps structural changes must occur in incomplete particles before they can be adsorbed to cells, for instance, degradation or activation of

Table 14

Differential Adsorption of Complete and Incomplete Particles of Ad 3

	Percentage of counts lost from inoculum upon successive adsorption number			
	1	2	3	4
Incomplete Particles (II) Labeled with				
^{3}H TdR	17.2	72.2	71.4	58.3
^{3}H TdR	73.3	80.9	92.4	98.3
^{32}P	53.1[a]	75.9	90.6	95.4
^{32}P*	39.7	81.3	85.0	90.7
Complete Particles (V) Labeled with				
^{3}H TdR	94.8	61.2	84.5	31.5
^{3}H TdR	70.9[a]	76.7	90.2	95.3
^{32}P	89.1[a]	74.5	79.6	90.3
^{32}P*	55.7[a]	89.7	96.6	98.7

*^{32}P labeled Ad 3 particles which had been frozen for about 6 weeks.

[a]Estimated percentage.

protein subunits or factors. Another possibility would be that an inhibitor of adsorption would have to be removed from the incomplete particle preparation. These changes would occur during the first adsorption cycle allowing the particles to be readily adsorbed during subsequent exposure to cells.

To determine the differences in uncoating, at 0, 1, and 6 hours after adsorption the medium was removed from the infected cells, 2 ml of TE were added, and the cells were frozen and thawed three times. The lysates were divided into three aliquots: 0.5 ml was acid precipitated and counted, 0.5% was digested twice with DNase then acid precipitated and counted, DNA was extracted from the remaining sample and was hybridized to Ad 3 DNA and to KB DNA. The results are presented in Table 15.

These results must be interpreted cautiously since the cell lysates were stored at 4°C for several weeks before these analyses were performed. An adenovirus particle is defined as uncoated when its nucleic acid becomes susceptible to DNase degradation. The data in Table 15 suggest that uncoating occurs for both complete and incomplete particles, however the process seems to occur much more efficiently and rapidly with complete particles. Most of the complete particles were uncoated in 1 hr. This is in agreement with previously published studies on Ad 2 (Sussenbach, 1967) and Ad 5 (Lawrence and Ginsberg, 1967). Uncoating occurs more slowly with adsorbed incomplete particles. This could be due to structural differences between the two types of particles. Another possibility is that the incomplete particles were adsorbed to the cell but a large percentage did not penetrate and therefore were not uncoated.

Table 15

Uncoating of Incomplete Particles and Infectious Virions

Sample	Harvest time after adsorption #1	Acid ppt cpm	DNase resistant ppt cpm	% DNase resistand	Total cpm in DNA	CPM hybridized to Ad 3 DNA	% Input hybridized to Ad 3 DNA	CPM hybridized to KB DNA	% Input hybridized to KB DNA	Specific[a] hybridization ratio
Incomplete Particles (II)	0 hr	303	173	57.17	340	70	41.18	16	9.41	4.38
	1 hr	315	107	34.07	288	61	42.36	13	9.03	4.69
	6 hr	101	27	27.23	-	-	-	-	-	-
Complete Particles (V)	0 hr	945	121	12.85	1092	121	22.16	11	2.02	11.00
	1 hr	670	32	4.85	980	341	69.59	8	1.63	42.62
	6 hr	1021	45.5	4.46	1520	16	2.10	0	0	16.00

[a]% hybridizing to Ad 3 DNA/% hybridizing to KB DNA.

The labeled DNA extracted from the cell lysates hybridized more readily to Ad 3 DNA than to KB DNA. However the DNA from incomplete particles hybridized about two times better to KB DNA than did the DNA from the complete virions. This result agrees with the in vitro hybridization data presented in Table 12. This suggests that if the incomplete particles contain some cellular DNA or viral DNA sequences which are complementary to host cell sequences, these particles are at least adsorbed by the same mechanism as particles containing viral DNA.

BIBLIOGRAPHY

Ada, G. L., and B. T. Perry. 1958. Influenza virus nucleic acid: Relationship between biological characteristics of the virus particle and properties of the nucleic acid. J. Gen. Microbiol. 14:623-633.

Anderson, C. W., P. R. Baum, and R. F. Gesteland. 1973. Processing of adenovirus 2-induced proteins. J. Virol. 12:241-252.

Baum, S. G., and R. I. Fox. 1974. Human adenovirus infection in monkey cells. An example of host restriction at a step late in replication. Cold Spring Harbor Symp. Quant. Biol. 39:567-573.

Bablanian, R., and W. C. Russell. 1974. Adenovirus polypeptide synthesis in the presence of non-replicating polio virus. J. Gen. Virol. 24:261-279.

Bellett, A. J. D., and H. B. Younghusband. 1972. Replication of the DNA of chick embryo lethal orphan virus. J. Mol. Biol. 72:691-709.

Bellett, A. J. D., and P. D. Cooper. 1959. Some properties of the transmissible interferring component of vesicular stomatitis virus preparations. J. Gen. Microbiol. 21:498-509.

Blackstein, M. E., C. P. Stanners, and A. J. Farmilo. 1969. Heterogeneity of polyoma virus DNA: Isolation and characterization of non-infectious small supercoiled molecules. J. Mol. Biol. 42:301-313.

Bourgaux, P., L. Delbecchi, and D. Bourgaux-Ramoisy. 1976. Initiation of adenovirus type 2 DNA replication. Virology 72;89-98.

Breedis, C., L. Berwick, and T. Anderson. 1962. Fractionation of Shope papilloma virus in cesium chloride density gradients. Virology 17:84-94.

Brockman, W., T. Lee, and D. Nathans. 1973. The evoluation of new species of viral DNA during serial passage of simian virus 40 at high multiplicity. Virology 54:384-397.

Brown, D. T., and B. T. Burlingham. 1973. Penetration of host cell membranes by adenovirus 2. J. Virol. 12:386-396.

Brown, D. T., M. Westphal, B. T. Burlingham, U. Winterhoff, and W. Doerfler. 1975. Structure and composition of the adenovirus type 2 core. J. Virol. 16:366-387.

Burlingham, B. T., D. T. Brown, and W. Doerfler. 1974. Incomplete particles of adenovirus. I. Characteristics of the DNA associated with incomplete adenovirions of types 2 and 12. Virology 60:419-430.

Burlingham, B. T., W. Doerfler, U. Pettersson, and L. Philipson. 1971. Adenovirus endonuclease: Association with the penton of adenovirus type 2. J. Mol. Biol. 60:45-64.

Burnett, J. P., and J. A. Harrington. 1968. Infectivity associated with simian adenovirus type SA7 DNA. Nature (Lond.) 220:1245-1246.

Burns, W. H., and P. H. Black. 1969. Induction experiments with adenovirus and polyoma virus transformed cell lines. Int. J. Cancer 4:204-211.

Burton, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62:315-322.

Chardonnet, Y., and S. Dales. 1970a. Early events in the interaction of adenoviruses with HeLa cells. I. Penetration of type 5 and intracellular release of the DNA genome. Virology 40:462-477.

Chardonnet, Y., and S. Dales. 1970b. Early events in the interaction of adenoviruses with HeLa cells. II. Comparative observations on the penetration of types 1, 5, 7, and 12. Virology 40:478-485.

Chardonnet, Y., and S. Dales. 1972. Early events in the interaction of adenoviruses with HeLa cells. III. Relationship between an ATPase activity in nuclear envelopes and transfer of core material: a hypothesis. Virology 48:342-359.

Craig, E. A., M. McGrogan, C. Mulder, and H. J. Raskas. 1975. Identification of early adenovirus type 2 RNA species transcribed from the left hand end of the genome. J. Virol. 16:905-912.

Craig, E. A., and H. J. Raskas. 1974. Two classes of cytoplasmic viral RNA synthesized early in productive infection with adenovirus 2. J. Virol. 14:751-757.

Dales, S. 1962. An electron microscope study of the early association between two mammalian viruses and their hosts. J. Cell Biol. 13:303-322.

D ıell, J. E., L. Philipson, R. Wall, and M. Adesnik. 1971. Polyadenylic acid sequences: Role in conversion of nuclear RNA into messenger RNA. Science 174:507-510.

Denhardt, D. T. 1966. A membrane-filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. 23:641-646.

Doerfler, W. 1968. The fate of the DNA of adenovirus type 12 in baby hamster kidney cells. Proc. Nat. Acad. Sci., U.S.A. 60: 636-643.

Doerfler, W. 1970. Integration of the DNA of adenovirus type 12 into the DNA of baby hamster kidney cells. J. Virol. 6:652-666.

Doerfler, W., and A. K. Kleinschmidt. 1970. Denaturation pattern of the DNA of adenovirus type 2 as determined by electron microscopy. J. Mol. Biol. 50:579-593.

Duesberg, P. H. 1968. The RNA's of influenza virus. Proc. Nat. Acad. Sci., U.S.A. 59:930-937.

Edvardsson, B., E. Everitt, H. Jörnvall, L. Prage, and L. Philipson. 1976. Intermediates in adenovirus assembly. J. Virol. 19:533-547.

Eliceiri, G. L. 1973. Ribosomal RNA synthesis after infection with adenovirus type 2. Virology 56:604-607.

Ellens, D. J., J. S. Sussenbach, and H. S. Jansz. 1974. Studies on the mechanism of replication of adenovirus DNA. III. Electron microscopy of replicating DNA. Virology 61:427-442.

Everitt, E., and L. Philipson. 1974. Structural proteins of adenoviruses. XI. Purification of three low molecular weight proteins of adenovirus type 2 and their synthesis during productive infection. Virology 62:253-269.

Everitt, E., B. Sundquist, U. Pettersson, and L. Philipson. 1973. Structural proteins of adenoviruses. X. Isolation and topography of low molecular weight antigens from the virion of adenovirus type 2. Virology 52:130-147.

Everitt, E., B. Sundquist, and L. Philipson. 1971. Mechanism of arginine requirement for adenovirus synthesis. I. Synthesis of structural proteins. J. Virol. 8:742-753.

Flanagan, J. F., and H. S. Ginsberg. 1962. Synthesis of virus-specific polymers in adenovirus-infected cells: Effect of 5-fluorodeoxyuridine. J. Exp. Med. 116:141-157.

Garon, C. F., K. W. Berry, J. C. Hierholzer, and J. A. Rose. 1973. Mapping of base sequence heterologies between genomes from different adenovirus serotypes. Virology 54:414-426.

Garon, C. F., K. Berry, and J. Rose. 1972. A unique form of terminal redundancy in adenovirus DNA molecules. Proc. Nat. Acad. Sci., U.S.A. 69:2391-2395.

Gilead, Z., and H. S. Ginsberg. 1968a. Characterization of the tumor-like (T) antigen induced by type 12 adenovirus. I. Purification of the antigen from infected KB cells and a hamster cell line. J. Virol. 2:7-14.

Gilead, Z., and H. S. Ginsberg. 1968b. Characterization of the tumor-like (T) antigen induced by type 12 adenovirus. II. Physical and chemical properties. J. Virol. 2:15-20.

Gilead, Z., and H. S. Ginsberg. 1965. Characterization of a tumor-like antigen in type 12 and type 18 adenovirus-infected cells. J. Bact. 90:120-125.

Ginsberg, H. S., L. J. Bello, and A. J. Levine. 1967. Control of biosynthesis of host macromolecules in cells infected with adenovirus. In: The Molecular Biology of Viruses (J. S. Colter and W. Paranchych, eds.), pp. 547-572. New York. Academic Press.

Ginsberg, H. S., and M. K. Dixon. 1959. Deoxyribonucleic acid (DNA) and protein alterations in HeLa cells infected with type 4 adenovirus. J. Exp. Med. 109: 407-422.

Ginsberg, H. S., H. G. Pereira, R. C. Valentine, and W. C. Wilcox. 1966. A proposed terminology for the adenovirus antigens and virion morphological subunits. Virology 28:782-783.

Grahm, F. L., P. J. Abrahams, S. O. Warnaar, C. Mulder, F. A. J. De Vries, W. Fiers, and A. J. Van der Eb. 1974. Studies on in vitro transformation with viral DNA and DNA fragments. Cold Spring Harbor Symp. Quant. Biol. 39:637-650.

Grahm, F. L., and A. J. Van der Eb. 1973a. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology 52:456-467.

Grahm, F. L., and A. J. Van der Eb. 1973b. Transformation of rat cells by DNA of human adenovirus 5. Virology 54:536-539.

Green, M. 1962a. Studies on the biosynthesis of viral DNA. Cold Spring Harbor Symp. Quant. Biol. 27:219-235.

Green, M. 1962b. Biochemical studies on adenovirus multiplication. III. Requirement for DNA synthesis. Virology 18:601-613.

Green, M., J. T. Parsons, M. Pina, K. Fujinaga, H. Caffier, and I. Landgraf-Leurs. 1970. Transcription of adenovirus genes in productively infected and in transformed cells. Cold Spring Harbor Symp. Quant. Biol. 35:803-818.

Green, M., and M. Pina. 1963. Similarity of DNAs isolated from tumor-induced viruses of human and animal origin. Proc. Natl. Acad. Sci., U.S.A. 50:44-46.

Green, M., and M. Pina. 1964. Biochemical studies on adenovirus multiplication. VI. Properties of highly purified tumorigenic human adenoviruses and their DNA's. Proc. Natl. Acad. Sci., U.S.A. 51:1251-1259.

Green, M., M. Pina, R. C. Kimes, P. C. Wensink, L. A. MacHattie, and C. A. Thomas, Jr. 1967. Adenovirus DNA I. Molecular weight and conformation. Proc. Natl. Acad. Sci., U.S.A. 57:1302-1309.

Green, M., and G. E. Daesch. 1961. Biochemical studies on adenovirus *multiplication*. II. Kinetics of nucleic acid and protein synthesis in suspension cultures. Virology 13:169-176.

Halperen, S., H. Eggers, and I. Tamm. 1964. Complete and coreless hemagglutinating particles produced in ECHO 12 virus-infected cells. Virology 23:81-89.

Hilleman, M. R., and J. H. Werner. 1954. Recovery of new agent from patients with acute respiratory illness. Proc. Soc. Exptl. Biol. Med. 85:183-188.

Horne, R. W., S. Brenner, A. P. Waterson, and P. Wildy. 1959. The icosahedral form of an adenovirus. J. Mol. Biol. 1:84-86.

Horwitz, M. 1971. Intermediates in the synthesis of type 2 adenovirus deoxyribunucleic acid. J. Virol. 8:675-683.

Horwitz, M. S., C. Brayton, and S. G. Baum. 1973. Synthesis of type 2 adenovirus DNA in the presence of cycloheximide. J. Virol. 11:544-551.

Horwitz, M. S., M. D. Scharff, and J. V. Maizel, Jr. 1969. Synthesis and assembly of adenovirus 2. I. Polypeptide synthesis, assembly of capsomers and morphogenesis of the virion. Virology 39:682-694.

Huang, A. S., and D. Baltimore. 1970. Defective viral particles and viral disease processes. Nature (London) 226:325-327.

Huang, A., J. Greenawalt, and R. Wagner. 1966. Defective T particles of vesicular stomatitis virus. I. Preparation, morphology, and some basic properties. Virology 30:161-172.

Huang, A., and R. Wagner. 1966. Defective T particles of vesicular stomatitis virus. II. Biologic role in homologous interference. Virology 30:173-181.

Huebner, R. J. 1967. Adenovirus-directed tumor and T *antigens*. *In*: Perspect. Virol. (M. Pollard, ed.). Vol. V, pp. 147-166. New York, Academic Press.

Huebner, R. J., W. P. Rowe, and W. T. Lane. 1962. Oncogenic effects in hamsters of human adenovirus type 12 and 18. Proc. Natl. Acad. Sci., U.S.A. 48:2051-2058.

Huebner, R. J., W. P. Rowe, H. C. Turner, and W. T. Lane. 1963. Specific adenovirus complement-fixing antigens in virus-free hamster and rat tumors. Proc. Natl. Acad. Sci., U.S.A. 50:379-389.

Ifft, J. B., D. N. Voet, and J. Vinograd. 1961. The determination of density distributions in density gradients in binary solutions at *equilibrium in the ultracentrifuge*. J. Phys. Chem. 65:1138-1145.

Igarashi, K., Y. Niiyama, K. Tsukamoto, T. Kurokawa, and Y. Sugino. 1975. Biochemical studies on bovine adenovirus type 3. II. Incomplete virus. J. Virol. 16:634-641.

Ishibashi, M., and J. V. Maizel, Jr. 1974a. The polypeptides of adenovirus. V. Young virions, structural intermediate, between top components and aged virions. Virology 57:409-424.

Ishibashi, M., and J. V. Maizel, Jr. 1974b. The polypeptides of adenovirus. VI. Early and late glycopeptides. Virology 58:345-361.

Kingsbury, D. W., and A. Portner. 1970. On the genesis of incomplete sendai virions. Virology 42:872-879.

Kingsbury, D. W., A. Portner, and R. W. Darlington. 1970. Properties of incomplete sendai virions and subgenetic viral RNAs. Virology 42:857-871.

Klemperer, H. G., and H. G. Pereira. 1959. Study of adenovirus antigens fractionated by chromatography on DEAE-cellulose. Virology 9:536-545.

Kline, L. K., S. M. Weissman, and D. Soll. 1972. Investigation of adenovirus-directed 4S RNA. Virology 48:291-296.

Kusano, T., and J. Yamane. 1967. Transformation in vitro of the embryonal hamster brain cells by adenovirus type 12. Tohoku. J. Exp. Med. 92:141-150.

Landgraf-Leurs, M., and M. Green. 1971. Adenovirus DNA. III. Separation of the complementary strands of adenovirus types 2, 7, and 12 DNA molecules. J. Mol. Biol. 60:185-202.

Larson, V. M., A. J. Girardi, M. R. Hilleman, and R. E. Zwickey. 1965. Studies on oncogenicity of adenovirus type 7 viruses in hamsters. Proc. Soc. Exptl. Biol. Med. 118:15-24.

Lavelle, G., C. Patch, G. Khoury, and J. Rose. 1975. Isolation and partial characterization of single stranded adenoviral DNA produced during synthesis of adenovirus type 2 DNA. J. Virol. 16:775-782.

Lawrence, W. C., and H. S. Ginsberg. 1967. Intracellular uncoating of type 5 adenovirus deoxyribonucleic acid. J. Virol. 1:851-867.

Levine, A. J., and H. S. Ginsberg. 1967. Mechanism by which fiber antigen inhibits multiplication of type 5 adenovirus. J. Virol. 1:747-757.

Lewis, A. M., Jr., A. S. Rabson, and A. S. Levine. 1974. Studies on nondefective adenovirus 2-simian virus 40 hybrid viruses. X. Transformation of hamster kidney cells by adenovirus 2 and the nondefective hybrid viruses. J. Virol. 14:1290-1301.

Lief, F. S., and W. Henle. 1956. Studies on the soluble antigen of influenza virus. III. The decreased incorporation of S antigen into elementary bodies of increasing incompleteness. Virology 2:782-797.

Lindberg, U., and B. Sundquist. 1974. Isolation of messenger ribonucleoproteins from mammalian cells. J. Mol. Biol. 86:451-468.

Lindberg, U., T. Persson, and L. Philipson. 1972. Isolation and characterization of adenovirus messenger RNA in productive infection. J. Virol. 10:909-919.

Lonberg-Holm, K., and L. Philipson. 1969. Early events of virus-cell interaction in an adenovirus system. J. Virol. 4:323-338.

Lowry, O. H., N. J. Rosebrough, A. F. Farr, and R. J. Randall. 1951. Protein measurements with the folin-phenol reagent. J. Biol. Chem. 193:265-275.

Maizel, J. V., Jr. 1971. Polyacrylamide gel electrophoresis of viral proteins. In: Methods in Virology, Vol. V. (K. Maramorosch and H. Koprowski, eds.), pp. 179-246. New York. Academic Press.

Maizel, J. V., Jr., B. A. Phillips, and D. F. Summers. 1967. Composition of artificially produced and naturally occurring empty capsids of poliovirus type 1. Virology 32:692-699.

Maizel, J. V., Jr., D. O. White, and M. D. Scharff. 1968a. The polypeptides of adenovirus. I. Evidence of multiple protein components in the virion and a comparison of types 2, 7, and 12. Virology 36:115-125.

Maizel, J. V., Jr., D. O. White, and M. D. Scharff. 1968b. The polypeptides of adenovirus. II. Soluble proteins, cores, top components and the structure of the virion. Virology 36:126-136.

Mak, S. 1969. Transcription and replication of viral deoxyribonucleic acid in cells coinfected with adenovirus types 2 and 12. J. Virol. 4:651-656.

Mak, S. 1971. Defective virions in human adenovirus type 12. J. Virol. 7:426-433.

Martinez-Palomo, A., and C. Brailovsky. 1968. Surface layer in tumor cells transformed by adeno-12 and SV 40 viruses. Virology 34:379-382.

Martinez-Palomo, A., and N. Granboulan. 1967. Electron microscopy of adenovirus 12 replication. II. High resolution autoradiography of infected KB cells labeled with tritiated thymidine. J. Virol. 1:1010-1018.

Martinez-Palomo, A., J. Le Buis, and W. Bernard. 1967. Electron microscopy of adenovirus 12 replication. I. Fine structural changes in the nucleus of infected KB cells. J. Virol. 1:817-829.

McAllister, R. M., C. R. Goodheart, V. Q. Mirabal, and R. J. Huebner. 1966. Human adenoviruses: Tumor production in hamsters by types 12 and 18 grown from single plaques. Proc. Soc. Exptl. Biol. Med. 122:455-458.

McAllister, R. M., and I. MacPherson. 1968. Transformation of a hamster cell line by adenovirus type 12. J. Gen. Virol. 2:99-106.

McAllister, R. M., M. O. Nicolson, A. M. Lewis, Jr., I. MacPherson, and R. J. Huebner. 1969a. Transformation of rat embryo cells by adenovirus type 1. J. Gen. Virol. 4:29-36.

McAllister, R. M., M. O. Nicolson, G. Reed, J. Kern, R. V. Gilden, and R. J. Huebner. 1969b. Transformation of rodent cells by adenovirus 19 and other group D adenoviruses. J. Nat. Cancer Inst. 43:917-923.

Mayne, N., J. P. Burnett, and L. K. Butler. 1971. Tumour induction by simian adenovirus SA 7 DNA fragments. Nature (New Biol.) 232:182-183.

Mims, C. A. 1956. Rift valley fever virus in mice. IV. Incomplete virus; its production and properties. Brit. J. Exp. Pathol. 37:129-143.

Morgan, C., H. S. Rosenkranz, and B. Mednis. 1969. Structure and development of viruses as observed in the electron microscope. X. Entry and uncoating of adenoviruses. J. Virol. 4:777-796.

Morgan, C., C. Howe, H. M. Rose, and D. H. Moore. 1956. Structure and development of viruses observed in the electron microscope. IV. Viruses of the RI-APC group. J. Biophys. Biochem. Cytol. 2:351-360.

Nicolson, M. O., and R. M. McAllister. 1972. Infectivity of human adenovirus 1 DNA. Virology 48:14-21.

Niiyama, Y., K. Igarashi, K. Tsukamoto, T. Kurokawa, and Y. Sugino. 1975. Biochemical studies on bovine adenovirus type 3. I. Purification and properties. J. Virol. 16:621-633.

Norrby, E. 1966. The relationship between the soluble antigens and the virion of adenovirus type 3. I. Morphological characteristics. Virology 28:326-248.

Öberg, B., J. Saborio, T. Persson, E. Everitt, and L. Philipson. 1975. Identification of the in vitro translation products of adenovirus mRNA by immunoprecipitation. J. Virol. 15:199-207.

Ohe, K. 1972. Virus-coded origin of a low molecular weight RNA from KB cells infected with adenovirus 2. Virology 47:726-733.

Okubo, C. K., and H. J. Raskas. 1972. A reconstituted system for in vitro synthesis of adenovirus 2 proteins. Virology 47:487-490.

Packman, S., and W. S. Sly. 1968. Constitutive DNA replication by C_{17}, a regulatory mutant related to virulence. Virology 34:778-789.

Parsons, J. T., and M. Green. 1971. Biochemical studies on adenovirus multiplication. XVIII. Resolution of early virus specific RNA species in adeno 2-infected and transformed cells. Virology 45:154 162.

Patch, C. T., A. M. Lewis, and A. S. Levine. 1972. Evidence for a transcription control region of SV 40 in the adenovirus 2-SV 40 hybrid Ad 2 $^{+}ND_1$. Proc. Natl. Acad. Sci., U.S.A. 69:3375-3379.

Pearson, G. D., and P. C. Hanawalt. 1971. Isolation of DNA replication complexes from uninfected and adenovirus infected HeLa cells. J. Mol. Biol. 62:65-80.

Perlman, S., M. Hirsch, and S. Penman. 1972. Utilization of messenger in adenovirus-2-infected cells at normal and elevated temperatures. Nature (New Biol.) 238:143-144.

Pettersson, U. 1973. Some unusual properties of replicating adenovirus type 2 DNA. J. Mol. Biol. 81:521-527.

Pettersson, U., and L. Philipson. 1974. Synthesis of complementary RNA sequences during productive adenovirus infection. Proc. Natl. Acad. Sci., U.S.A. 71:4887-4891.

Pettersson, U., L. Philipson, and S. Höglund. 1967. Structural proteins of adenoviruses. I. Purification and characterization of adenovirus type 2 hexon antigen. Virology 33:575-590.

Pettersson, U., L. Philipson, and S. Höglund. 1968. Structural proteins of adenoviruses. II. Purification and characterization of adenovirus type 2 fiber antigen. Virology 35:204-215.

Pettersson, U., and J. Sambrook. 1973. Amount of viral DNA in the genome of cells transformed by adenovirus type 2. J. Mol. Biol. 73:125-130.

Pettersson, U., J. Sambrook, H. Delius, and C. Tibbetts. 1974. In vitro transcription of adenovirus 2 DNA by Escherichia coli RNA polymerase. Virology 59:153-167.

Philipson, L. 1960. Separation on DEAE cellulose of components associated with adenovirus reproduction. Virology 10:459-465.

Philipson, L. 1967. Attachment and eclipse of adenovirus. J. Virol. 1:868-875.

Philipson, L., U. Lindberg, T. Persson, and B. Vennstrom. 1973. Tran scription and processing of adenovirus RNA in productive infection. In: Advanc. in the Biosciences. Vol. II (G. Raspe, ed.), pp. 167-183. Pergamon Press. Vieweg.

Philipson, L., R. Wall, G. Glickman, and J. E. Darnell. 1971. Addition of polyadenylate sequences to virus-specific RNA during adenovirus replication. Proc. Natl. Acad. Sci., U.S.A. 68:2806-2809.

Philipson, L., K. Lonberg-Holm, and U. Pettersson. 1968. Virus receptor interaction in an adenovirus system. J. Virol. 2:1064-1075.

Philipson, L., U. Pettersson, U. Lindberg, C. Tibbits, B. Vennström, and T. Persson. 1974. RNA synthesis and processing in adenovirus infected cells. Cold Spring Harbor Symp. Quant. Biol. 39:447-456.

Pina, M., and M. Green. 1965. Biochemical studies on adenovirus multiplication. IX. Chemical and base composition analysis of 28 human adenoviruses. Proc. Natl. Acad. Sci., U.S.A. 54:547-551.

Pina, M., and M. Green. 1969. Biochemical studies on adenovirus multiplication. XIV. Macromolecule and enzyme synthesis in cells replicating oncogenic and nononcogenic human adenovirus. Virology 38:573-586.

Pope, J. H., and W. P. Rowe. 1964. Immunofluorescent studies of adenovirus 12 tumors and of cells transformed or infected by adenoviruses. J. Exp. Med. 120:577-588.

Prage, L., S. Höglund, and L. Philipson. 1972. Structural proteins of adenoviruses. VIII. Characterization of incomplete particles of adenovirus type 3. Virology 49:745-757.

Raska, K., D. Frohwirth, and R. W. Schlesinger. 1970. Transfer ribonucleic acid in KB cells infected with adenovirus type 2. J. Virol. 5:464-469.

Raska, K., L. Prage, and R. W. Schlesinger. 1972. The effects of arginine starvation on macromolecular synthesis in infection with type 2 adenovirus. II. Synthesis of virus-specific RNA and DNA. Virology 48:472-484.

Raskas, H. J., D. C. Thomas, and M. Green. 1970. Biochemical studies on adenovirus multiplication. XVII. Ribosome synthesis in uninfected and infected KB cells. Virology 40:893-902.

Reed, L. J., and H. Muench. 1938. Simple method of estimating 50 percent endpoints. Amer. J. Hyg. 27:493-497.

Roberts, R. J., J. R. Arrand, and W. Keller. 1974. The length of the terminal repetition in adenoviruses 2 DNA. Proc. Natl. Acad. Sci., U.S.A. 71:3829-3833.

Robinson, A. J., H. B. Younghusband, and A. J. D. Bellett. 1973. A circular DNA-protein complex from adenoviruses. Virology 56:54-69.

Rosen, L. 1960. A hemagglutination-inhibition technique for typing viruses. Am. J. Hgg. 71:120-128.

Rosenwirth, B., S. Tjia, M. Westphal, and W. Doerfler. 1974. Incomplete particles of adenovirus. II. Kinetics of formation and polypeptide composition of adenovirus type 2. Virology 60:431-437.

Rott, R., and C. Scholtissek. 1963. Investigations about the formation of incomplete forms of fowl plague virus. J. Gen. Microbiol. 33:303-312.

Rowe, W. P., R. J. Huebner, L. K. Gillmore, R. H. Parrott, and T. G. Ward. 1953. Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc. Soc. Exptl. Biol. Med. 84:570-573.

Russell, W. C., K. Hayashi, P. J. Sanderson, and H. G. Pereira. 1967. Adenovirus antigens - A study of their properties and sequential development in infection. J. Gen. Virol. 1:495-507.

Russell, W. C., and B. Knight. 1967. Evidence for a new antigen within the adenovirus capsid. J. Gen. Virol. 1:523-528.

Russell, W. C., J. J. Skehel, R. Machado, and H. G. Pereira. 1972. Phosphorylated polypeptides in adenovirus-infected cells. Virology 50:931-934.

Russell, W. C., and J. J. Skehel. 1972. The polypeptides of adenovirus-infected cells. J. Gen. Virol. 15:45-47.

Schaller, J. P., and D. S. Yohn. 1974. Transformation potentials of the noninfectious (defective) component in pools of adenoviruses type 12 and simian adenovirus 7. J. Virol. 14:392-401.

Sharp, P. A., U. Pettersson, and J. Sambrook. 1974. Viral DNA in transformed cells. I. A study of the sequences of adenovirus DNA in a line of transformed rat cells using specific fragments of the viral genome. J. Mol. Biol. 86:709-726.

Schilling, R., B. Weingärtner, and E. L. Winnacker. 1975. Adenovirus type 2 DNA replication. II. Termini of DNA replication. J. Virol. 16:767-774.

Shimojo, H., H. Yamamoto, and C. Abe. 1967. Differentiation of adenovirus 12 antigens in cultured cells. Virology 31:748-752.

Shiroki, K., H. Shimojo, and K. Yamaguchi. 1974. The viral DNA replication complex of adenovirus 12. Virology 60:192-199.

Sjögren, H. O., J. Minowada, and J. Ankerst. 1967. Specific transplantation antigens of mouse sarcoma induced by adenovirus type 12. J. Exp. Med. 125:689-701.

Slifkin, M., L. Merkow, and N. P. Rapoza. 1968. Tumor induction by simian adenovirus 30 and establishment of tumor cell lines. Cancer Res. 28:1173-1179.

Smith, K. O. 1965a. Studies on adenovirus-12. I. Quantitative correlations between some physical, antigenic, and infectious properties. J. Immunol. 94:976-989.

Smith, K. O. 1965b. Cyclic structure of adenovirus DNA. Science 148:100-102.

Smith, R. E., H. J. Zweerink, and W. Joklik. 1969. Polypeptide components of virions, top components, and cores of reovirus type 3. Virology 39:791-810.

Strohl, W. A., A. S. Rabson, and H. Rouse. 1967. Adenovirus tumorigenesis: Role of the viral genome in determining tumor morphology. Science 156:1631-1633.

Sundquist, B., E. Everitt, L. Philipson, and S. Höglund. 1973. Assembly of adenoviruses. J. Virol. 11:449-459.

Sussenbach, J. S. 1967. Early events in the infection process of adenovirus type 5 in HeLa cells. Virology 33:567-574.

Sussenbach, J. S., D. J. Ellens, and H. S. Janz. 1973. Studies on the mechanism of replication of adenovirus DNA. II. The nature of single-stranded DNA in replicative intermediates. J. Virol. 12:1131-1138.

Sussenbach, J. S., and P. C. Van der Vliet. 1973. Studies on the mechanism of replication of adenovirus DNA. I. The effect of hydroxyurea. Virology 54:299-303.

Sussenbach, J. S., P. C. Van der Vliet, D. J. Ellens, and H. S. Janz. 1972. Linear intermediates in the replication of adenovirus DNA. Nature (New Biol.) 239:47-49.

Tibbetts, C., U. Pettersson, K. Johansson, and L. Philipson. 1974. Relationship of mRNA from productively infected cells to the complementary strands of adenovirus type 2 DNA. J. Virol. 13:370-377.

Tockstein, G., H. Polasa, M. Pina, and M. Green. 1968. A simple purification procedure for adenovirus type 12 T and tumor antigens and some of their properties. Virology 36:377-386.

Tolun, A., and U. Pettersson. 1975. Termination sites for adenovirus type 2 DNA replication. J. Virol. 16:759-766.

Trentin, J. J., and E. Bryan. 1964. Immunization of hamsters and histogenic mice against transplantation of tumors induced by human adenovirus type 12. Proc. Amer. Assoc. Cancer Res. 5:64.

Trentin, J. J., G. L. Van Hoosier, Jr., and L. Samper. 1968. The oncogenicity of human adenoviruses in hamsters. Proc. Soc. Exptl. Biol. Med. 127:683-689.

Tseui, D., K. Fujinaga, and M. Green. 1972. The mechanism of viral carcinogenesis by DNA mammalian viruses: RNA transcripts containing viral and highly reiterated cellular base sequences in adenovirus-transformed cells. Proc. Natl. Acad. Sci., U.S.A. 69:427-430.

Uchida, S., and S. Watanabe. 1969. Transformation of mouse 3T3 cells by T antigen-forming defective SV 40 virions (T particles). Virology 39:721-728.

Uchida, S., and S. Watanabe. 1968. Tumorigenicity of the antigen-forming defective virions in simian virus 40. Virology 35:166-169.

Uchida, S., S. Watanabe, and M. Kato. 1966. Incomplete growth of simian virus 40 in African green monkey kidney culture induced by serial undiluted passages. Virology 28:135-141.

Valentine, R. C., and H. G. Pereira. 1965. Antigens and structure of the adenovirus. J. Mol. Biol. 13:13-20.

Van der Eb, A. J. 1973. Intermediates in type 5 adenovirus DNA replication. Virology 51:11-23.

Van der Vliet, P. C., and A. J. Levine. 1973. DNA-binding proteins specific for cells infected by adenovirus. Nature (New Biol.) 246:170-174.

Van der Vliet, P. C., and J. S. Sussenbach. 1972. The mechanism of adenovirus-DNA-synthesis in isolated nuclei. Europ. J. Biochem. 30:548-592.

Velicer, L., and H. Ginsberg. 1970. Synthesis, transport, and morphogenesis of type 5 adenovirus capsid proteins. J. Virol. 5:338-352.

Vinograd, J., and J. E. Hearst. 1962. Equilibrium sedimentation of macromolecules and viruses in a density gradient. Progr. Chem. Org. Nat. Prod. 20:372-421.

Vlak, J. M., Th. H. Rozign, and F. Spies. 1975. Localization of adenovirus DNA replication in KB cells. Virology 65:535-545.

Vlak, J. M., Th. H. Rozign, and F. Spies. 1976. Replication of adenovirus type 5 DNA in KB cells: Localization and fate of parental DNA during replication. Virology 72:99-109.

Von Magnus, P. 1954. Incomplete forms of influenza virus. Adv. Virus Res. 2:59-79.

Wadell, G., M. L. Hammarskjöld, and T. Varsanyi. 1973. Incomplete virus particles of adenovirus type 16. J. Gen. Virol. 20:287-303.

Wadell, G., and M. Hammarskjöld. 1970. Multiplicity dependent infectivity of incomplete particles. J. Gen. Microbiol. 60:3xii.

Wall, R., J. Weber, Z. Gage, and J. E. Darnell. 1973. Production of viral mRNA in adenovirus-transformed cells by post-translational processing of heterogeneous nuclear RNA containing viral and cell sequences. J. Virol. 11:953-960.

Wall, R., L. Philipson, and J. E. Darnell. 1972. Processing of adenovirus specific nuclear RNA during virus replication. Virology 50:27-34.

Walter, G., and J. V. Maizel, Jr. 1974. The polypeptides of adenovirus. IV. Detection of early and late virus-induced polypeptides and their distribution in subcellular fractions. Virology 57:402-408.

Warocquier, R., J. Samaille, and M. Green. 1969. Biochemical studies on adenovirus multiplication. XVI. Transcription of the adenovirus genome during abortive infection at elevated temperatures. J. Virol. 4:423-428.

Weber, J. 1974. Absence of adenovirus-specific repressor in adenovirus tumour cells. J. Gen. Virol. 22:259-264.

Weinman, R., H. J. Raskas, and R. G. Roeder. 1974. Role of DNA-dependent RNA polymerases II and III in transcription of the adenovirus genome late in productive infection. Proc. Natl. Acad. Sci., U.S.A. 71:3426-3430.

White, D. O., M. D. Scharff, and J. V. Maizel, Jr. 1969. The polypeptides of adenoviruses. III. Synthesis in infected cells. Virology 38:395-406.

Wilcox, W. C., and H. S. Ginsberg. 1963a. Protein synthesis in type 5 adenovirus infected cells. Effect of p-fluorophenylalanine on synthesis of protein nucleic acids and infectious virus. Virology 20:269-280.

Wilcox, W. C., and H. S. Ginsberg. 1963b. Structure of type 5 adenovirus. I. Antigenic relationship of virus structural proteins to virus specific soluble antigens from infected cells. J. Exp. Med. 118:295-306.

Wilcox, W. C., H. S. Ginsberg, and T. F. Anderson. 1963. Structure of type 5 adenovirus. II. Fine structure of virus subunits. Morphological relationship of structural subunits to virus-specific soluble antigens from infected cells. J. Expt. Med. 118:307-314.

Winnacker, E. 1975. Adenovirus type 2 DNA replication. I. Evidence for discontinuous DNA synthesis. J. Virol. 15:744-758.

Winters, W. D., and W. C. Russell. 1971. Studies on assembly of adenovirus in vitro. J. Gen, Virol. 10:181-194.

Wolfson, J., and D. Dressler. 1972. Adenovirus 2-DNA contains an inverted terminal repetition. Proc. Natl. Acad. Sci., U.S.A. 69:3054-3057.

Van der Eb, A. J., L. W. Kesteren, and E. F. J. Van Bruggen. 1969. Structural properties of adenovirus DNAs. Biochim. Biophys. Acta 182:530-541.

Yabe, Y., L. Samper, G. Taylor, and J. J. Trenbon. 1963. Cancer induction in hamsters by human type 12 - adenovirus. Effect of route of injection. Proc. Soc. Exptl. Biol. Med. 113:221-224.

Yohn, D. S. 1973. Sex-related resistance in hamsters to adenovirus oncogenesis. Prog. Exp. Tumor Res. 18:138-165.

Yoshiike, K. 1968. Studies on DNA from low density particles of SV 40. Virology 34:390-401.

PHYSICAL, CHEMICAL, AND BIOLOGICAL PROPERTIES OF THE INCOMPLETE PARTICLES OF HUMAN ADENOVIRUS TYPE 3

by

BETTY JEAN ROSE

B.A., Kansas State College, 1973

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Division of Biology

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1977

ABSTRACT

When KB cells are infected with adenovirus type 3 and the lysates of the infected cells are subjected to equilibrium density sedimentation in CsCl, several populations of particles are observed as light scattering bands by transillumination. These populations of virus particles have distinct buoyant densities. Analyses of these populations of particles in the ultracentrifuge indicate that the buoyant densities are 1.2787, 1.2913, 1.2945, 1.3030, 1.3139, 1.3315, and 1.3595 g/cm^3. The bands of particles are designated I, IIa, IIb, III, IV, V, and VI respectively. The virions are band V. The remaining bands are incomplete particles. The virions of adenovirus type 3 are less dense than the virions of adenovirus type 2.

Optical density determinations at 260 and 280 nm of CsCl equilibrium gradients indicates the presence of additional populations of incomplete particles which were not detected in the analytical ultracentrifuge. These bands are designated as IIIa and Va.

Buoyant densities and OD_{260}/OD_{280} ratios indicate that all of the incomplete particle populations contain nucleic acid. DNA is extractable from incomplete particles (II) and virions (V). The DNA from incomplete particles hybridizes better with adenovirion DNA than with KB cell DNA. Incomplete particle DNA hybridizes better with KB cell DNA than does adenovirus type 3 virion DNA suggesting either that host cell DNA may be present or that incomplete particle DNA contains sequences which are complementary to KB cell DNA.

The incomplete particle populations are produced in constant proportions with repeated passages except for band Va which increases with passage number.

The DNA of incomplete particles and virions is present within the capsids. A portion of both types of particles is resistant to DNase digestion. However, more of the nucleic acid in incomplete particles than in virions is susceptible to digestion by this enzyme, and the digestion of the DNA in incomplete particles occurs more slowly. These observations suggest differences in packaging and/or structure of incomplete particles and virions. The specific radioactivity is lower for incomplete particles than it is for virions. This might indicate that parental viral DNA is incorporated into the incomplete particles.

The biological role(s) of the incomplete particles remains unspecified. These particles may be degradation products of the viral replication process. They may have a role in viral assembly serving as precursors to infectious virions. Incomplete particles may serve to determine transforming ability and oncogenic potential of a virus population.