JOURNAL OF VIROLOGY, Mar. 1970, p. 338-352
Vol. 5, No. 3

Synthesis, Transport, and Morphogenesis of Type 5 Adenovirus Capsid Proteins

L. F. VELICER[1] AND H. S. GINSBERG

Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received for publication 30 October 1969

During the period between 20 and 24 hr after infection of KB cells with type 5 adenovirus, at a time when approximately 85% of the proteins made were virus-specific, viral proteins were synthesized on polyribosomes with an average sedimentation coefficient of 200*S*. The polypeptide chains synthesized during a 1-min period of labeling with ^{14}C-amino acids had an average sedimentation coefficient of 3.4*S* in sucrose gradients containing 1% sodium dodecyl sulfate. Within 1 min after completion, the newly made polypeptide chains were released from polyribosomes, and the majority were transported into the nuclei within 6 min. Meanwhile, the immunological reactivity of the newly synthesized proteins also increased rapidly. During the same 6-min interval after synthesis, the single polypeptide chains assembled into multimeric proteins with average sedimentation coefficients of 6*S*, 9*S*, and 12*S*. The 6*S* and 12*S* proteins were identified immunologically as the fiber and hexon capsid proteins, respectively. The 9*S* protein was trypsin-sensitive and appeared to be the precursor of the penton; it was tentatively identified as the penton base. The penton had a sedimentation coefficient of about 10.5*S* and sedimented with the hexon in sucrose gradients. The concomitant migration of nascent proteins into the nuclei, development of the capsid proteins' immunological reactivity, and morphogenesis of the multimeric capsid proteins suggest that the single polypeptide chains or small complexes were transported into the nuclei where they assembled into mature structural proteins of the virion.

Viral capsid proteins are synthesized in the cytoplasm and rapidly migrate into the nuclei of KB cells infected with type 5 adenovirus (34). Three independent methods demonstrated the cytoplasmic site of synthesis of viral proteins on polyribosomes: (i) autoradiography showed that most, if not all, proteins are synthesized in the cytoplasm and then transported rapidly into the nuclei; (ii) immunological coprecipitation detected newly synthesized viral proteins on 200*S* polyribosomes; and (iii) hybridization of denatured viral deoxyribonucleic acid (DNA) with a species of ribonucleic acid (RNA) from polyribosomes identified viral messenger RNA.

It was predicted that the chains of the capsid proteins of type 5 adenovirus, synthesized on polyribosomes with a mean of 200*S* (hereafter referred to as 200*S* polyribosomes), would have an average molecular weight of approximately 25,000 (34). It was further hypothesized that the newly made polypeptide chains were the precursors of the macromolecular viral proteins. The results of investigations to be summarized in this communication will substantiate the validity of these predictions. It will be shown that the nascent polypeptide chains sediment as small nonrandomly coiled molecules with an average sedimentation coefficient of 3.4*S*, that the newly made proteins are rapidly released from polyribosomes and migrate immediately into the nucleus, that the polypeptide chains are precursors of the multimeric capsid structures, and that the polypeptide chains assemble into the capsid proteins and acquire specific immunological reactivity at the same rapid rate. The experiments to be described were carried out when synthesis of host proteins was reduced to a minimum and 70 to 85% of the proteins produced were viral capsid proteins (1, 34).

MATERIALS AND METHODS

The methods for cell growth, viral propagation and purification, and viral plaque assay were described previously (1, 9, 10).

Preparation of antisera. The method of immunization of rabbits with purified type 5 adenovirus was previously presented (34). Rabbit antisera against

[1] Pennsylvania Plan Scholar. Present address: Department of Microbiology and Public Health, Michigan State University, East Lansing, Mich. 48823.

purified hexon and fiber were prepared by the same injection schedule and with similar amounts of protein. Immunological tests could not detect penton in fiber preparations. However, antisera prepared against fiber reacted with penton because the fiber is an integral component of the intact penton. The sera from rabbits immunized with purified virus, hexon, or fiber will be called anti-Ad 5, anti-hexon, and anti-fiber, respectively. Goat-anti-rabbit serum (serum from goats immunized with rabbit gamma globulin), termed GAR, was obtained as previously described (34).

Complement-fixation assay. The microtiter technique described by Sever was employed (28). Antigen titers are expressed as the highest dilution that completely fixed 2 exact units of guinea pig complement in the presence of 8 units of antibody.

Immunological precipitation of viral proteins: direct precipitation. Viral proteins were selectively precipitated from a cell homogenate with antiserum against purified virus or viral antigen. Even viral proteins labeled during a 1-min pulse could be effectively precipitated because sufficient antigens had accumulated prior to addition of isotope. Samples were prepared for analysis as previously described (1). The fraction of radioactivity not precipitated by antiserum represents a maximal estimate of the percentage of ^{14}C-labeled amino acids incorporated into host proteins. The amount of antiserum needed to obtain maximal precipitation was determined in control experiments. The direct precipitation procedure was also used in some experiments to identify and to quantitate radioactive capsid proteins, when sufficient amounts of protein were present in fractions of sucrose gradients.

Coprecipitation. To detect the small amounts of newly synthesized protein on polyribosomes or in sucrose gradients, the technique of immune coprecipitation was employed. The sera used were from rabbits immunized with purified virus or a viral capsid protein and from a goat immunized with rabbit gamma globulin (34). In the experiments to be described, however, sodium dodecyl sulfate (SDS) was not included because nonspecific trapping of capsid proteins was usually less than 20%. All analyses were done in duplicate.

Purification of type 5 adenovirus antigens. The protein antigens were purified and their purity was determined as previously described (10), except that the crude material was not treated with trypsin and the protein solution was dialyzed against 0.001 M phosphate buffer at *p*H 7.2 before chromatography. Commercially prepared hydroxylapatite powder (Bio-Gel HTP; Bio-Rad Laboratories, Richmond, Calif.) was reswollen in 0.001 M phosphate buffer (*p*H 7.2), and packed in a column (1.4 × 7 cm) according to the manufacturer's directions. The protein was eluted with a linear gradient from 0.001 to 0.35 M sodium phosphate, *p*H 7.2 (175 ml of each). Washed diethylaminoethyl (DEAE) cellulose was used to make columns of 1.1 × 5 cm. Each protein was eluted from DEAE cellulose with a linear gradient from 0 to 0.5 M NaCl in 0.01 M phosphate buffer, *p*H 7.2 (125 ml of each).

Iodination of protein. The method of iodination described by McConahy and Dixon (14) was adapted for use with bovine serum albumin (BSA), hexon, and fiber: 10 mg of BSA and 2.7 mg of purified hexon were iodinated with 200 μc of ^{125}I (4 mc/ml; New England Nuclear Corp., Boston, Mass.), and 1 mg of purified fiber was reacted with 100 μc of the same ^{125}I preparation. After the reaction was stopped with sodium metabisulfite, the samples were extensively dialyzed against several changes (total volume, 8 liters) of 0.01 M phosphate-buffered saline (*p*H 7.6) over a 3-day interval. Portions (2 ml) of the hexon and BSA solutions were chromatographed on Sephadex G-200 to remove any remaining free iodine and to separate single protein molecules from aggregates. The fiber solution was chromatographed on Sephadex G-25 to remove free iodine. Phosphate-buffered 0.15 M NaCl (*p*H 7.6) was used as the eluant. Fractions of 3.0 ml were collected in disposable tubes (13 × 75 mm) and assayed in an auto-gamma spectrometer.

Sucrose gradient analysis of adenovirus capsid proteins. The proteins were sedimented through 4.6-ml linear gradients of 5 to 20% (w/v) sucrose in gradient buffer (0.01 M phosphate buffer at *p*H 7.6, 0.001 M ethylenediaminetetraacetate, 0.15 M NaCl). In some experiments, sodium decyl sulfate (SDeS; 0.1, 0.25, or 1%) or guanidine·HCl (1 M) was included in the gradient. Centrifugation was carried out in a Spinco SW 39 or SW 65 Ti rotor at 20 C. About 35 fractions (0.14 ml) were collected through the bottom of the tube. The specific details of sample preparation and the times and speeds of centrifugation varied in the experiments to be described, and the variations are stated in the figure legends. In general, samples for analyses were prepared in final volumes of 0.42 to 0.5 ml. One-hundredth of the sample (0.04 ml) was spotted on a filter-paper disc for determination of radioactivity, and 0.4 ml was layered on the gradient for analysis. Approximate sedimentation coefficients were calculated by the method of Martin and Ames (17) with the following assumed values: 4.4*S* for BSA (12), 6.5*S* for rabbit gamma globulin (32), 12*S* for hexon, and 6*S* for fiber (10, 22, 23).

Cell fractionation and isolation of polyribosomes. The methods previously described were used to fractionate cells (34). Sedimentation in linear sucrose gradients was employed to separate polyribosomes from cytoplasmic extracts (34).

Radioactive labeling of protein. Proteins were labeled with radioactive amino acids for periods varying from 1 to 60 min (34). When the labeled cells were to be cultured in the absence of isotope (i.e., "chased"), they were washed once in cold isotope-free medium and resuspended to a concentration of 150,000 cells/ml in prewarmed isotope-free medium. To stop the chase, the desired volume of cell suspension was poured on partly frozen phosphate-buffered saline.

Autoradiography. The autoradiography procedure was previously described (34).

Protein determinations. The protein in whole cells and samples after cell fractionation was precipitated with cold 5% trichloroacetic acid (1). Protein was measured by the method of Lowry et al. (13) with crystalline BSA as the standard.

Radioactivity assays. After being heated at 90 C for 45 min in 5% trichloroacetic acid, the protein precipitate was solubilized in 1.0 ml of 0.1 N NaOH, and a

0.1-ml sample was spotted on a filter-paper disc (1). In many experiments, fractions from sucrose gradients were collected directly on paper discs. The filter-paper discs were oven-dried, and those not previously heated in trichloroacetic acid were treated sequentially with 5% cold trichloroacetic acid two times, 5% trichloroacetic acid at 90 C for 45 min, 5% cold trichloroacetic acid, and acetone. The dried papers were assayed for ^{125}I in a Packard auto-gamma spectrometer and for ^{14}C in a Packard liquid scintillation spectrometer (1, 9). Between 30 and 40% of the ^{125}I radioactivity was counted in the liquid scintillation spectrometer under conditions used to assay ^{14}C-labeled proteins; corrections were made by use of an ^{125}I standard. It was unnecessary to correct for ^{14}C in the ^{125}I assays because only about 0.02% of the ^{14}C radioactivity was detected when ^{125}I was counted.

RESULTS

Characterization of newly synthesized protein. After a 1-min pulse with ^{14}C-labeled amino acids, approximately 80% of the newly synthesized proteins in the cytoplasm was found on polyribosomes. For the study of nascent proteins, the polyribosomes from a sucrose gradient were sedimented; the polypeptide chains were released by adding 1% SDeS and ribonuclease (225 μg/ml), and incubating the mixture at 37 C for 4 hr. In preliminary experiments, it was found that ribonuclease treatment improved solubilization and recovery of radioactive proteins. In addition, the ribonuclease treatment was included to prevent contamination of the samples with charged soluble RNA (sRNA). Between 93 and 95% of the ^{14}C-labeled amino acids in the 200*S* polyribosome peak was recovered in the pellet, and only 10% of the recovered radioactivity was soluble in hot trichloroacetic acid. The sedimentation characteristics of the polypeptide chains were analyzed in a 5 to 20% sucrose gradient containing 1% SDeS. Most of the radioactivity was found in a single, homogeneous peak with an average sedimentation coefficient of about 3.4*S* (Fig. 1A). Eighty-six per cent of the radioactive proteins was recovered in the fractions of the gradient; the remaining radioactive proteins were in aggregates larger than 12*S* at the bottom of the tube. The high optical density at the top of the gradient reflected the hydrolysis products of RNA effected by the ribonuclease treatment. Recentrifugation in sucrose gradients of two fractions in the leading limb of the peak (5*S* region) and two fractions in the trailing limb (2.2*S* region) yielded two sharp, homogeneous peaks with average sedimentation coefficients of 3.6*S* and 3*S*, respectively. Hence, the apparently homogeneous peak (Fig. 1A) was composed of polypeptide chains of varying sizes, but the range of sizes was small.

The average sedimentation coefficient of nascent proteins obtained from polyribosomes in several determinations was 3.4*S* in the presence of 1% SDeS. Newly synthesized proteins obtained from whole infected cells were of similar size (Fig.

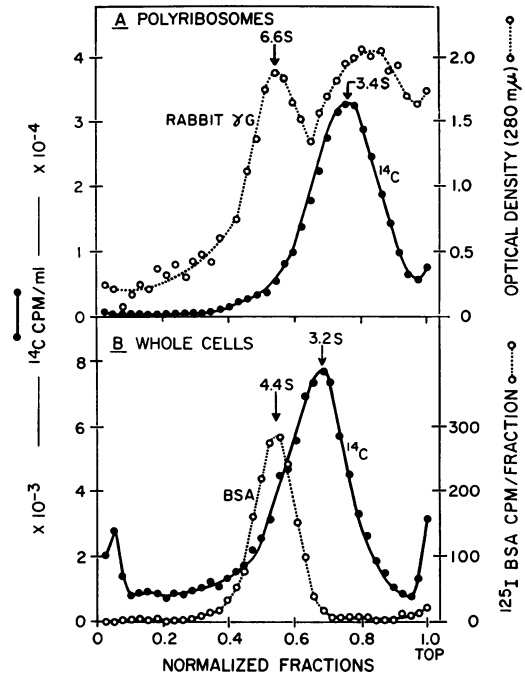

FIG. 1. *Sucrose gradient sedimentation analysis of newly synthesized proteins in cells 20 hr after infection with 200 plaque-forming units of type 5 adenovirus per cell. (A) Polyribosomes were isolated from cells labeled for 1 min with a mixture of ^{14}C-amino acids (final radioactivity, 4 μc/ml) as described in Materials and Methods. The fractions in the 200S peak (34) were pooled and centrifuged for 16 hr at 22,500 rev/min in an SW 25 rotor. The polyribosome pellet was solubilized in 1% sodium decyl sulfate (SDeS) and treated with ribonuclease (225 μg/ml) for 4 hr at 37 C. Rabbit gamma globulin (12 mg) was added, and the mixture was analyzed in a 5 to 20% (w/v) sucrose gradient containing 1.0% SDeS by centrifugation for 18 hr at 30,000 rev/min in a Spinco SW39 rotor at 20 C. (B) Cells were labeled with ^{14}C-amino acids for 1 min as in part A. A pellet containing 5 × 10^6 whole cells was resuspended in 0.05 ml of ribonuclease (1 mg/ml), 0.05 ml deoxyribonuclease (1 mg/ml), 0.02 ml of ^{125}I-labeled bovine serum albumin, and gradient buffer (0.01 M phosphate buffer, pH 7.6, 0.001 M ethylenediaminetetraacetate, 0.15 M NaCl) to a final volume of 0.45 ml. The suspension was sonically treated for 10 sec and incubated for 2 hr at 37 C. SDeS was added (0.05 ml of 10% SDeS) and the sample was incubated at 37 C for 2 more hr. The mixture was layered on a 5 to 20% (w/v) sucrose gradient containing 1% SDeS and centrifuged for 18 hr at 41,000 rev/min in a Spinco SW65 Ti rotor.*

1B). This finding made it possible to isolate and study nascent polypeptide chains without initially obtaining polyribosomes, thereby facilitating subsequent experiments.

Rate of release of nascent protein. The apparent rapid migration of newly made protein into the nuclei (34) implied that polypeptide chains were rapidly released from the polyribosomes. When the rate of release of nascent protein from polyribosomes was studied in pulse-chase experiments (Fig. 2), most of the newly labeled proteins were released from the polyribosomes after a 1-min chase. The radioactive protein remaining on the polyribosomes after a 1-min chase may have resulted from incomplete release of polypeptides or from continued incorporation of labeled amino acids from the cell pool into protein and into charged sRNA which was 10% of the acid-precipitable radioactivity after a 1-min pulse. The latter possibility seems likely because: (i) the amount of hot trichloroacetic acid-insoluble radioactivity increased by 12% during the first 3 min of incubation in the absence of labeled amino acids and then remained constant for 4 hr, and (ii) the size of the cold and hot trichloroacetic acid-soluble isotope pools decreased during the first 3 min of the chase period. The results of the pulse-chase experiments indicate that the polypeptides were synthesized on and released from polyribosomes within the first 3 min of the chase period; most polypeptide chains were released within 1 min after they were completed (Fig. 2).

Rate of protein transport into nuclei. Because the nuclei of infected cells leak viral capsid proteins and virions (34), it was necessary to use autoradiography to determine unambiguously the distribution of radioactive proteins in cells. The results of two experiments conducted to study the rate of protein transport (Fig. 3) clearly indicate that proteins rapidly moved from the cytoplasms into the nuclei of both infected and uninfected cells. The percentage of grains found in the nuclei after a 5 to 6-min chase was 75 to 80% of the number that accumulated during a 1-hr chase. In these experiments, 70 to 75% of the proteins synthesized in the infected cells were virus-specific.

Immunological maturation. Between 30 and 40% of the polypeptide chains synthesized during a

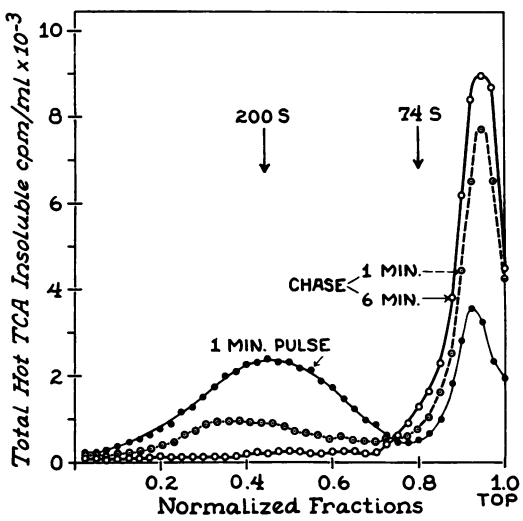

FIG. 2. *Rate of release of nascent proteins from polyribosomes of cells infected with type 5 adenovirus. Cells were labeled for 1 min with ^{14}C-labeled amino acids (1 μc/ml) 20 hr after infection with type 5 adenovirus (200 plaque-forming units/cell); a portion of the culture was selected immediately, and the remainder was cultured in the absence of labeled amino acids as described in Materials and Methods. Cytoplasmic extracts were prepared from 50 × 10^6 cells obtained after a 1-min pulse and from similar numbers of cells after a 1-min pulse followed by 1- and 6-min chases. The cytoplasmic extracts were analyzed in 15 to 30% (w/v) sucrose gradients centrifuged for 90 min at 22,500 rev/min in a Spinco SW25 rotor at 4 C.*

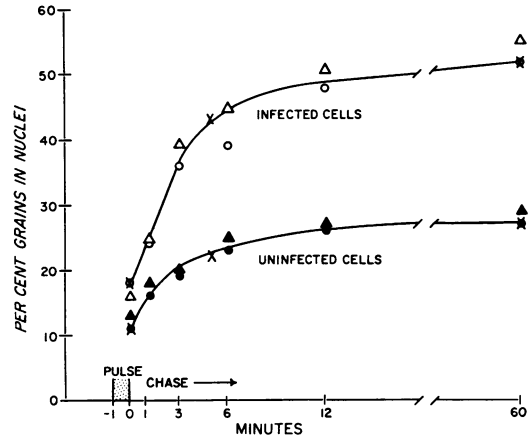

FIG. 3. *Transport of nascent proteins from the cytoplasm to nuclei of uninfected KB cells and cells infected with type 5 adenovirus. Measurements made with autoradiographic analyses. Monolayer cultures were labeled with ^{3}H-valine 26 hr after infection (200 plaque-forming units/cell). Distribution of grains was determined in cells labeled during a 1-min pulse with ^{3}H-valine (1.5 mc/ml: 117 mc/mmole; New England Nuclear Corp., Boston, Mass.) or labeled in the same manner and then chased for various periods of time in the presence of excess unlabeled valine. The results of two separate experiments are plotted (△, ○), along with the average results (×) of three preliminary experiments (34). Uninfected cells were labeled and chased as above. The results are presented in similar manner (▲, ●) except that the preliminary data (×) are the average of two experiments (34).*

1-min isotopic pulse were immunologically reactive at a time when 70 to 85% of the proteins produced by the cell were viral capsid proteins (34). It was anticipated that the immunological reactivity of the newly synthesized polypeptides would increase as all the polypeptide chains were completed and assembled into the multimeric viral capsid structures. The results of a representative pulse-chase experiment to test this prediction indicate that the increase in immunological reactivity occurred rapidly during the first 6 min of the chase period, after which the rate decreased as the maximal reactivity was approached (Fig. 4). The increased immunological precipitation probably reflects the aggregation of the polypeptide chains into viral capsid proteins, forming additional antigenic reactive groups or increasing the association constant between the antigens and antibodies.

Assembly of macromolecular capsid structures. If the 3.4*S* nascent polypeptide chains (Fig. 1) were precursors of the large morphological proteins present in the virion, it might be expected that those polypeptides labeled during a short isotopic pulse would be incorporated into identifiable structures during a subsequent chase period. The macromolecular capsid subunits (hexon, penton, and fiber) have been identified (33, 39) and purified (10, 21–23). The hexon and fiber have also been physically characterized: the hexon and fiber have sedimentation coefficients of 12*S* and 6*S* (10, 22, 23) and molecular weights of 310,000 and 65,000 to 70,000, respectively (16; Ginsberg, Scherz, and Dixon, *unpublished data*). Accordingly, the newly formed hexons and fibers could be identified in sucrose gradients on the basis of (i) their cosedimentation with iodinated purified hexons and fibers and (ii) their immunological precipitation by antisera from rabbits immunized with purified hexons or fibers (38).

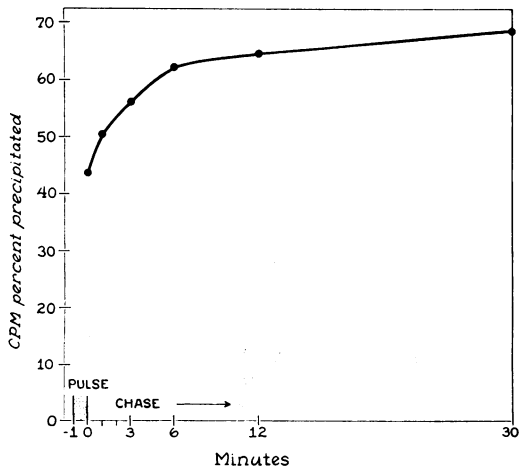

FIG. 4. *Immunological maturation of type 5 adenovirus capsid proteins in infected cells. Cells were labeled for 1 min with ^{14}C-labeled amino acids (final radioactivity, 1 μc/ml) 20 hr after infection (200 plaque-forming units/cell). Part of the cell culture was harvested, and the remainder was subjected to varying chase periods as in Fig. 2. The cells were harvested, sonically treated, and analyzed by direct immune precipitation with antiserum prepared against purified type 5 adenovirus.*

To determine the precursor-product relationship of the 3.4*S* polypeptide chain and the capsid proteins, 24 hr after infection cells were harvested after a 1-min pulse with ^{14}C-labeled amino acids and after subsequent chase periods of 1 to 60 min. Cells were sonically treated and then were incubated with ribonuclease (50 μg) for 2 hr at 37 C. Guanidine·HCl was added to the mixture to make a final concentration of 2 M, and after 2 hr at room temperature the mixture was analyzed in sucrose gradients containing 1 M guanidine·HCl to limit artificial aggregation of the nascent polypeptide chains. This concentration of guanidine does not denature either the hexon or fiber (Ginsberg, Scherz, and Dixon, *unpublished data*). After a 1-min pulse, only approximately 60% of the newly made protein was soluble in the gradient under the conditions employed, and most of the soluble protein sedimented as molecules with average sedimentation coefficients of about 3*S* (Fig. 5). Immediately after radioactive labeling, there was a very small protein peak under the 12*S* marker and a slight shoulder in the 6*S* region of the gradient. The first 6 min of chase were characterized by a rapid increase in the size of the 12*S* peak, development of a distinct 6*S* peak, and a slight decrease in the size of the 3*S* peak. The solubility of the nascent proteins progressively increased during the chase period until more than 80% of the label was recovered in the gradient. There was only a small increase in the size of the 6*S* and 12*S* peaks during the remainder of the hour. After a 1-hr chase, a majority of the protein cosedimented with hexon and fiber markers. However, about 25% of the radioactivity persisted as 3*S* protein molecules.

Since 2 M guanidine·HCl does not denature purified hexon or fiber, the results described suggested that the persistence of polypeptides represented unstable noncapsid proteins or newly assembled capsid macromolecules which were less stable to denaturing agents. The latter appeared possible when it was found that no 6*S* peak appeared in the presence of 1% SDeS; only a small, heterogeneous 12*S* peak formed, and the majority of isotope remained in polypeptide chains of

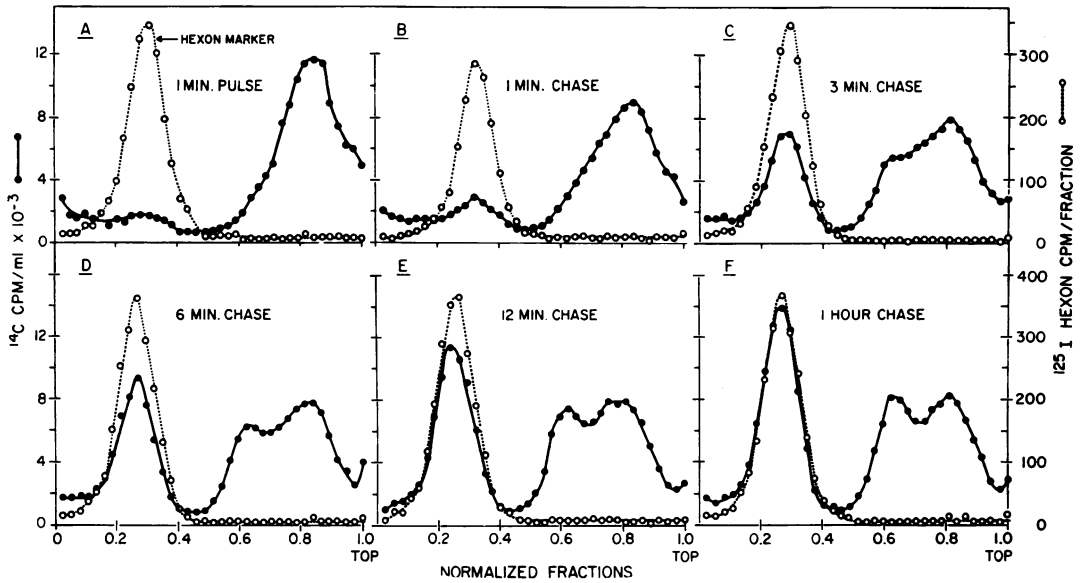

FIG. 5. *Morphogenesis of type 5 adenovirus capsid protein in cells 20 hr after infection (200 plaque-forming units/cell). Pulse-chase procedures were the same as described in Fig. 2 except that the final radioactivity employed was 4 μc/ml. Homogenates of cell pellets containing 5 × 10^6 cells were prepared as in Fig. 1B except that a final concentration of 2.0 M guanidine·HCl was added and an iodinated hexon marker was used. After 4 hr at room temperature, the samples were layered on 5 to 20% (w/v) sucrose gradients containing 1 M guanidine and centrifuged for 18 hr at 27,500 rev/min in a Spinco SW39 rotor at 20 C.*

about 3*S*. The experiments were therefore repeated without dissociating agent in either the cell homogenate or the gradient.

Under these conditions, after a 1-min pulse only about 17% of the nascent protein was recovered in the gradient (Fig. 6); the remainder of the labeled protein was in the pellet in aggregates larger than 15*S*. About one-third of the soluble material cosedimented with the hexon marker, a small protein peak with a sedimentation coefficient of about 9*S* was noted, and the remainder was present in a broad peak of 2 to 6*S*. During the first 6 min of the chase, the 12*S*, 9*S*, and 6*S* protein peaks became more prominent and increased in size rapidly while the 3*S* material progressively disappeared. The distinct 9*S* peak which appeared during the short chase periods seemed to be lost after a 1-hr chase. The 12*S* and 6*S* peaks increased slightly from 6 to 60 min during the chase. With the increasing size of the peaks, more of the newly synthesized protein became soluble, so that at the end of the chase 65 to 70% of the radioactivity was recovered in the gradient and was found to be in two apparently homogenous peaks having sedimentation coefficients of 12*S* and 6*S*. The radioactive protein which sedimented faster than 12*S* did not form peaks between 12*S* and 33*S* in gradients centrifuged for short periods. With sonic treatment of the pelleted material, about 10 to 15% was resolubilized as two peaks of 2 to 6*S* and 12*S*.

Identification of the hexon and fiber. The 12*S* and 6*S* proteins were similar in size to the hexon and fiber, respectively: the 12*S* protein cosedimented precisely with an iodinated hexon marker (Fig. 5 and 6), and the 6*S* peak cosedimented exactly with iodinated fiber.

Immune precipitation was employed for further identification of the hexon and fiber. The 12*S* and 6*S* peaks were obtained from experiments in which a 1- or 10-min pulse was followed by a 1-hr chase; the results were identical with both labeling methods. The average results of two experiments are summarized in Table 1. Sera from rabbits immunized with purified type 5 virus (anti-Ad 5) coprecipitated almost all of the radioactive protein in the 12*S* peak. In this instance, when most of the radioactive protein was precipitated, the correction for nonspecific trapping may result in an underestimate of the amount of antigen precipitated by specific antibody. A large amount of the labeled protein was precipitated with antibodies directed solely against hexon, but the amount was 17% less than that which reacted with anti-Ad 5 serum, suggesting the presence of a viral capsid protein other than hexon in the apparently homogeneous peak. The finding that anti-fiber (which also reacts with the fiber component of the pen-

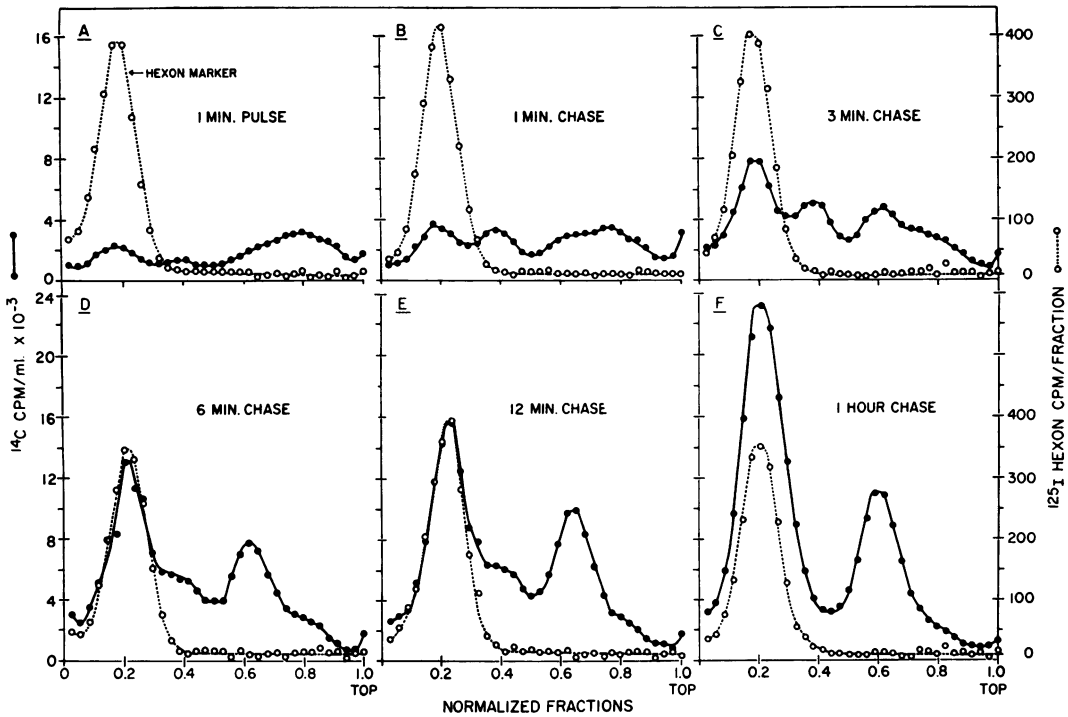

FIG. 6. *Morphogenesis of type 5 adenovirus capsid protein. Analyses were carried out in the absence of dissociating agents. Cell pellets (5 × 10⁶ cells) were obtained, prepared, and analyzed as in Fig. 5 except that the incubation with nucleases proceeded for 4 hr at 37 C, and guanidine was eliminated from the samples and gradients.*

ton) coprecipitated about 25% of the radioactivity from the 12*S* region strengthened this interpretation. The data obtained with direct precipitation of the 12*S* material agree with the coprecipitation results. The amounts of radioactive protein precipitated by anti-hexon and anti-fiber were 84 and 14%, respectively. These results indicate that the 12*S* peak was predominantly hexon, but contained a significant amount of at least one other capsid protein. Because of its reactivity with the anti-fiber antiserum, these data suggested that the other protein was aggregated fiber, or penton.

The 6*S* peak was shown to consist mainly of fiber: anti-hexon was unreactive with the fiber peak and about two-thirds of the radioactive protein was precipitated by anti-fiber. Anti-Ad 5 and anti-fiber coprecipitated the same amount of labeled material, suggesting that the remaining unreactive proteins were host proteins or noncapsid proteins coded for by the virus. The fact that host proteins might be present in the 6*S* region was demonstrated by analysis of uninfected cells after a 1-min pulse and a 1-hr chase: radioactivity was found in a broad band between 3 and 8*S*. The presence of host proteins or noncapsid viral proteins was further suggested by the effect of trypsin on the immunological reactivity of the fiber peak. Trypsin does not hydrolyze purified fiber (33); nevertheless, trypsin rendered about 10% of the radioactive 6*S* proteins acid-soluble. Moreover, after trypsin treatment, anti-fiber serum precipitated 10% more of the radioactive protein than it did prior to enzyme hydrolysis, suggesting that nonviral or noncapsid proteins had been removed.

Identification of the penton in cell extracts. The appearance of a newly synthesized protein with a sedimentation coefficient of about 9*S*, and its disappearance within 1 hr, suggested that it was precursor material. The apparent merging of the 9*S* material with the 12*S* peak (Fig. 6) suggested that the 9*S* protein might be an intermediate macromolecule in the assembly of the hexon. However, other data implied that so-called hexon 12*S* peak contained more than one protein and that the additional protein might be the penton: (i) trypsin hydrolyzed a small proportion of the protein, although hexon is not hydrolyzed by trypsin (10) whereas the penton base is (33); (ii) the protein in the peak was not immunologically homogeneous, as it reacted more with antibodies to purified virions than to hexon antibodies (Table 1); and (iii) the large protein peak which contained hexon, observed after a 1-hr chase (Fig. 6F), was not symmetrical, but rather it was skewed on the trailing edge.

TABLE 1. *Immunological identification of 12S and 6S proteins*

Protein	Serum[a]	Per cent radioactivity in precipitate	
		Coprecipitation[b]	Direct precipitation[c]
12S	Anti-Ad 5 virions	89[d]	—[e]
	Anti-hexon	72	84
	Anti-fiber	25	14
6S	Anti-Ad 5 virions	67	—
	Anti-hexon	1	0
	Anti-fiber	67	68

[a] The antisera were from rabbits immunized with purified type 5 adenovirus, hexon, or fiber.

[b] Duplicate 0.3-ml samples of each pooled peak of radioactivity were analyzed with each antiserum. A 0.1-ml amount of diluted antiserum (10 μliters/0.1 ml) or normal rabbit serum (NRS; 15 μliters/0.1 ml) was added to each sample, and the mixture was incubated at 37 C for 2 hr. A 0.1-ml amount of diluted goat-anti-rabbit serum (GAR) was added, and the reaction mixture was incubated an additional 2 hr at 37 C. The precipitate was sedimented at 1,000 × *g* for 10 min, washed three times in cold 0.15 M NaCl, and solubilized in 0.5 ml of 0.1 N NaOH. Portions of the dissolved precipitates (0.4 ml) and of the supernatant fractions (0.35 ml) were spotted on filter paper discs, dried, and assayed for radioactivity.

[c] The pooled fractions of each peak of radioactivity obtained by analysis of 15 × 10^6 cells were diluted and divided into six samples containing 0.4 ml each. Duplicate samples were then mixed with 0.1 ml of diluted NRS, anti-hexon serum, or anti-fiber serum (10 μliters in 0.1 ml). After spotting 0.1 ml of the reaction mixture on a filter paper disc, the remainder was incubated for 4 hr at 37 C, followed by centrifugation for 30 min at 1,800 × *g*. For assay, 0.1 ml of each supernatant fluid was spotted on a filter paper disc.

[d] Per cent radioactivity precipitated = counts per minute in precipitate/(counts per minute in precipitate plus counts per minute in supernatant fluid) × 100. The percentage of counts per minute precipitated by the NRS-GAR precipitate was subtracted from the percentage precipitated by the antiserum-GAR precipitate to correct for nonspecific trapping. The correction was 10% for the 12S protein and 7% for the 6S protein.

[e] Per cent radioactivity precipitated = 100% − counts per minute per 0.1 ml of supernatant fraction/counts per minute per 0.1 ml of reaction mixture × 100. The radioactivity precipitated in the presence of NRS was negligible and a correction was not necessary.

If the large 12S peak contained penton, it seemed possible that it could be separated from the hexon because chromatography on DEAE cellulose separates fiber, penton, and hexon (7, 38). The leading half of a typical 12S peak cochromatographed with iodinated hexon as a single peak (Fig. 7A). (The iodinated hexon marker had been stored at 4 C for about 6 months and showed partial breakdown, accounting for the leading shoulder. Fresh preparations of iodinated hexon did not show this characteristic.) The trailing half of the 12S peak eluted from DEAE cellulose in three distinct peaks (Fig. 7B): peak I eluted at the same NaCl concentration as fiber and the separated protein sedimented with a sedimentation coefficient of 6S; peak III cochromatographed with iodinated hexon; and peak II showed the elution characteristics of penton under the conditions employed (7, 22, 23, 38). When the protein of peak II was studied immunologically, it was shown to be unreactive with anti-hexon serum, but it was precipitated by antibodies directed against the fiber (Table 2). These results are consistent with the hypothesis that the peak II protein is the penton because the fiber is an integral component of the intact structure (33, 39). These results also agree with the earlier observation that anti-fiber serum could precipitate a portion of the protein contained in the 12S peak (Table 1).

Upon centrifugation of the peak II protein in a sucrose gradient, most of the protein sedimented as a distinct peak with a sedimentation coefficient of 10.6S, but there was a small peak in the position at which fiber usually sediments (Fig. 8A). The fiber in the gradient may have resulted from spontaneous disruption of some of the penton during storage. Reaction of the protein of peak II with trypsin provided strong evidence that the protein was present in the penton: (i) the penton peak disappeared almost completely and (ii) the isotope from the 10.6S penton peak was recovered quantitatively in protein having the sedimentation characteristics of the fiber, and in cold acid-soluble material in the fractions at the top of the gradient (Fig. 8B).

It had been reported that the reaction of penton with 2 M guanidine·HCl resulted in dissociation of the complex structure into free base and fiber (18, 19). Therefore, to identify the peak II protein further, the reaction was carried out and the products were identified on sucrose gradients containing 1 M guanidine·HCl. A protein having the sedimentation coefficient of fiber (i.e., 6S) was liberated (Fig. 9A), a reaction similar to that of the protein with trypsin (Fig. 8B), but a macromolecular structure having the characteristics of penton base was not detectable. About 50% of the radioactive material was found in the pellet or adherent to the walls of the tube. Despite the inability to identify free penton base, the separation of fiber from the original protein adds further evidence indicating that the protein in peak II

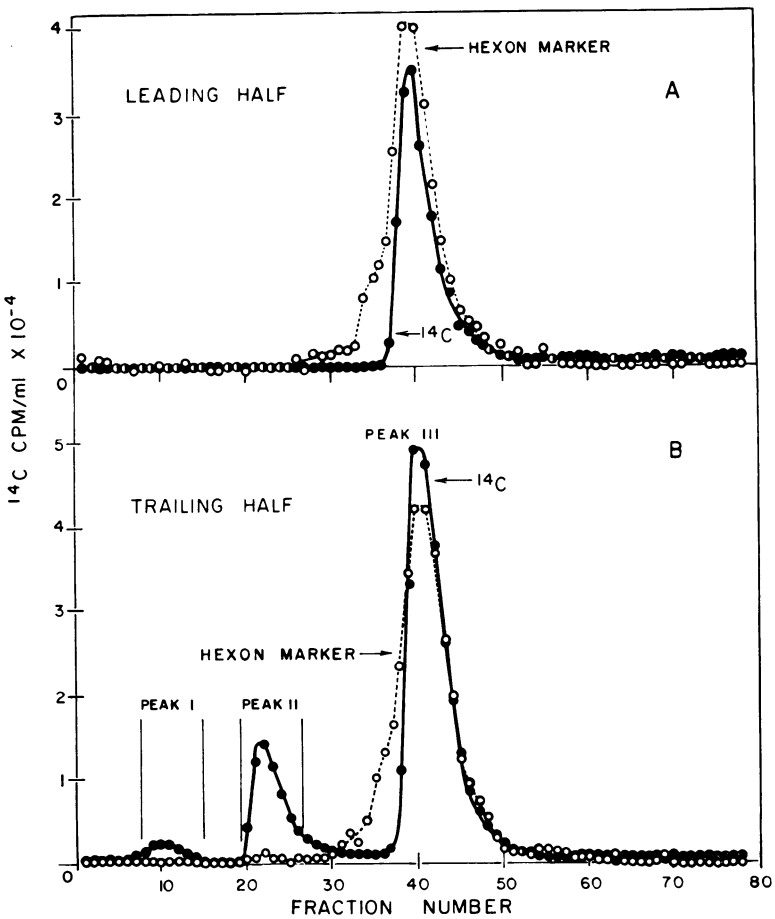

FIG. 7. *Chromatographic analysis of the protein in the 12S peak from a sucrose gradient. Cells (15 × 10^6) were labeled with ^{14}C-amino acids (10 μc/ml) for 1 min and incubated in the absence of isotope for 1 hr as in Fig. 2. The cells were prepared and analyzed as in Fig. 6. The fractions of the leading and trailing halves of the typical 12S peak were combined to form two pools; each was chromatographed on DEAE cellulose columns. The protein was eluted in a linear gradient between 0.01 M phosphate buffer (125 ml, pH 7.2) and 0.5 M NaCl in 0.01 M phosphate buffer (125 ml, pH 7.2). Iodinated hexon (approximately 10,000 counts/min) was included as a marker. (A) Chromatography of the leading half (pool I, fractions 3–7) of the 12S peak. (B) Chromatography of the trailing half (pool II, fractions 8–13) of the 12S peak. Fractions 8–14 (peak I) and 20–26 (peak II) were pooled for further analyses.*

TABLE 2. *Immunological identification of possible penton protein*[a]

Serum	Per cent radioactivity coprecipitated[b]
Anti-hexon	2
Anti-fiber	77

[a] Peak II from chromatographic separation on DEAE cellulose (Fig. 7B).

[b] The analysis and calculations were carried out as in Table 1. The correction for nonspecific trapping was 22%.

was intact penton. The average sedimentation coefficient (three determinations) of the peak II protein, considered to be penton, was 10.5*S*.

The results of the experiments described in Fig. 8 suggested that the penton was somewhat unstable, and that the penton base or its component polypeptide chains stick to the sides of the tube. Analyses of the same preparation employed in the experiments summarized in Fig. 8 after 16 days of storage at 4 C strengthened the above conclusion. The 6*S* fiber peak was larger than the 10.5*S* penton peak, suggesting spontaneous disruption of the penton during storage; however, a structure

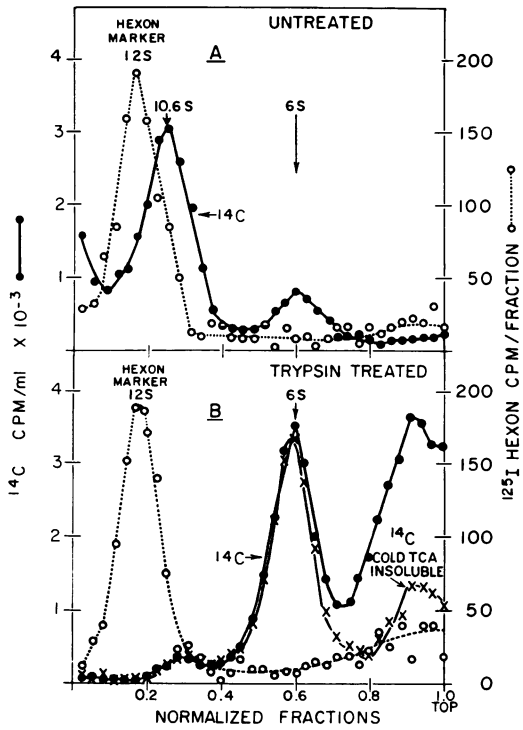

FIG. 8. *Sucrose gradient sedimentation analysis of peak II from DEAE cellulose chromatography (Fig. 7B). The pooled fractions (20 ml) were concentrated to 2.5 ml by pressure dialysis, and 0.35-ml samples were analyzed on sucrose gradients with an iodinated hexon marker as in Fig. 6. (A) Untreated control. (B) Trypsin-treated sample (100 μg/ml; 1 hr at 37 C) followed by inactivation with an equal amount of soybean trypsin inhibitor. A sample from each fraction of the gradient was reacted with 5% cold trichloroacetic acid, and the radioactivity of acid-precipitable material was also assayed.*

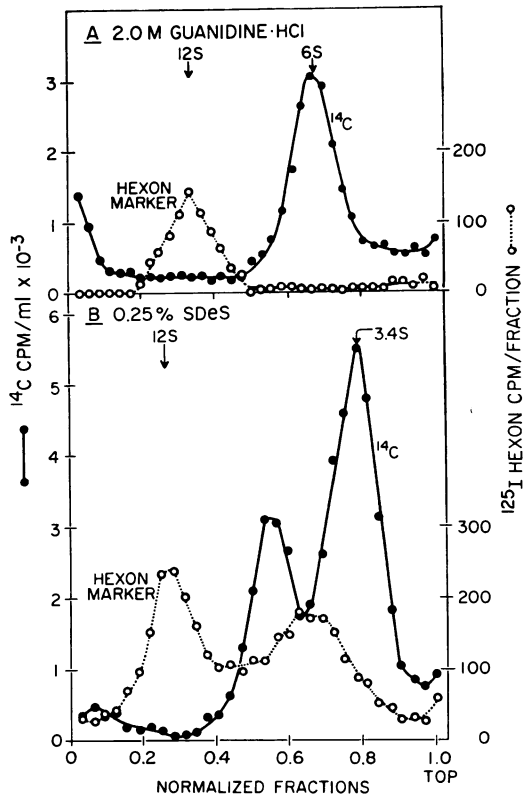

FIG. 9. *Effect of guanidine·HCl and sodium decyl sulfate* (SDeS) *on penton. Penton obtained from DEAE cellulose chromatography (peak II, Fig. 7) was prepared and analyzed as in Fig. 8. (A) The penton preparation was treated with guanidine·HCl (final concentration, 2.0 M) for 1 hr and analyzed on a gradient containing 1 M guanidine·HCl. SDeS (0.25% final concentration) was added to the penton preparation, and the sample was analyzed in a sucrose gradient containing 0.25% SDeS.*

which could be identified as the penton base was not found as a distinct peak in the gradient.

Additional evidence on the structure of the penton was obtained when the peak II protein was mixed with 0.25% SDeS and analyzed in sucrose gradients containing the same concentration of the detergent. Penton was completely disrupted, yielding a large 6*S* fiber peak (as in Fig. 8 and 9A) and another homogeneous peak with an average sedimentation coefficient of 3.4*S* (Fig. 9B; the hexon marker was either partially disrupted by SDeS or incompletely enmeshed in detergent micelles). In contrast to the experiments in which 2 M guanidine·HCl was used to disrupt the penton, 97% of the radioactivity was recovered in the gradient. These data imply that the penton base is assembled from polypeptide chains having a sedimentation coefficient of about 3.4*S*, that the polypeptide chains remain soluble in the presence of detergent, and that after reaction with guanidine·HCl the denatured components of the penton base aggregate and are not soluble in the 1 M guanidine·HCl-sucrose mixture.

Characterization of the 9S protein. The disappearance of the 9*S* peak of newly synthesized proteins and the apparent merging of the protein with material in the hexon peak (Fig. 6) suggested that the 9*S* protein was a precursor of a larger macromolecule. Since the intact penton with a sedimentation coefficient of 10.5*S* was submerged in the peak having its maximum at 12*S*, and since penton base had not been identified as a separate structure, it appeared possible that the 9*S* protein was penton base. But it was also possible that the 9*S* protein consisted of partially assembled poly-

peptide chains destined to form hexons. A 9S peak was not present in extracts of uninfected cells obtained after a 1-min pulse and a 3-min chase, which clearly demonstrated that the transient 9S structure was not a normal cell component.

Trypsin readily hydrolyzes the base unit of the penton (33). Therefore, the newly synthesized 9S protein should be trypsin-sensitive if it is the penton base. Extracts of infected cells obtained after a 1-min pulse followed by a 3-min chase were treated with trypsin and centrifuged in sucrose gradients. Trypsin completely hydrolyzed the protein in the 9S region of the gradient, whereas the proteins sedimenting as hexons and fibers were trypsin-resistant (Fig. 10).

To identify the 9S protein further, the appropriate fractions from several gradients were pooled, concentrated, and recentrifuged in sucrose gradients. A large 9S peak was again obtained, as well as small 6S and 10.5 to 12S peaks which came from the contaminating fiber and hexon-penton peaks, respectively, of the original gradients.

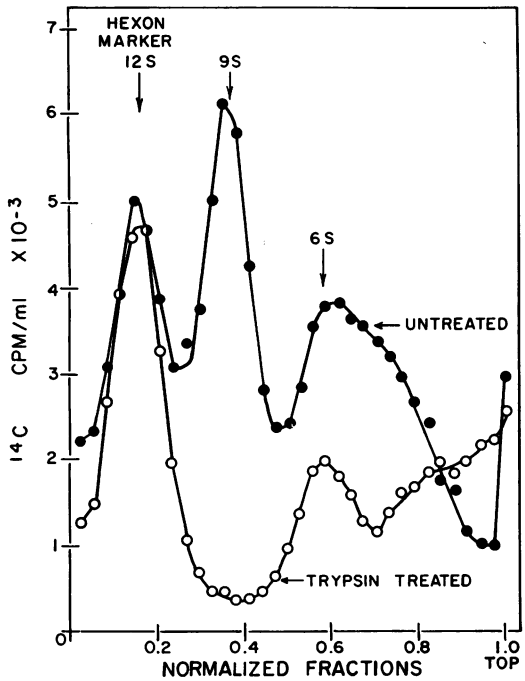

FIG. 10. *Effect of trypsin on the 9S protein: 5 × 10⁶ cells were labeled with ¹⁴C-amino acids (10 μc/ml) for 1 min and chased for 3 min as in Fig. 2. Pellets of 2.5 × 10⁶ cells were prepared and analyzed as in Fig. 6. One preparation received trypsin (100 μg/ml) during the last hour of incubation at 37 C after which an equal amount of soybean trypsin inhibitor was added. Samples were sedimented through 5 to 20% (w/v) sucrose gradients.*

Examination of the immunological properties of the three protein peaks (from Fig. 10) indicates that the material in the 9S region did not react with hexon antibodies (Table 3), and anti-fiber serum only precipitated 26% of the protein. Most of the protein that reacted with fiber antibodies was not trypsin-sensitive and probably was derived from the overlapping lighter fiber peak and the heavier penton peak. The contaminating penton could have provided the small amount of trypsin-sensitive protein. The 9S material did react with antisera against virions, and trypsin markedly reduced this reactivity. The 6S protein peak appeared to contain only fiber, and the data suggested that the 10.5 to 12S peak consisted of approximately equal proportions of hexon and penton.

Rate of capsid protein morphogenesis. The data presented in Fig. 5 and 6 suggest that the majority of the capsid proteins are formed within 6 min after synthesis of the polypeptide chains. It should be noted that after a 1-min pulse only a

TABLE 3. *Immunological identification of radioactive proteins obtained from the 9S regions of sucrose gradients*

Protein peak[a]	Serum	Percentage of counts/min in precipitate[b]
Untreated 9S	Anti-hexon	2
	Anti-fiber	26
	Anti-Ad 5	68
Trypsin-treated 9S[c]	Anti-hexon	1
	Anti-fiber	19
	Anti-Ad 5	20
Untreated 6S	Anti-hexon	1
	Anti-fiber	76
Untreated 10.5 to 12S	Anti-hexon	55
	Anti-fiber	55

[a] Cells (22.5 × 10⁶) were labeled with ¹⁴C-amino acids (20 μc/ml) for 3 min and incubated in the absence of isotope for 1 min as in Fig. 2. Three equal-sized cell pellets were prepared and analyzed on sucrose gradients as in Fig. 6. The 9S regions were pooled, concentrated, and reanalyzed to yield distinct but not completely separate 6S, 9S, and 10.5 to 12S peaks.

[b] The coprecipitation analyses, calculations, and correction for nonspecific trapping were carried out as in Table 1.

[c] Incubation with trypsin (100 μg/ml) for 1 hr at 37 C followed by addition of an equal amount of soybean trypsin inhibitor.

small proportion of the nascent polypeptide chains are soluble in the absence of any dissociating agent such as guanidine·HCl or detergent (Fig. 6A). It seemed reasonable to assume that the rate at which the newly synthesized proteins become soluble (i.e., appeared in the gradient in the absence of guanidine·HCl) accurately reflected the rate at which the capsid proteins were formed. On the basis of this assumption, the data summarized in Fig. 6 were employed to determine the rate of morphogenesis of the capsid proteins. The resulting curve (Fig. 11) demonstrates that a rapid assembly of the capsid proteins occurs during the first 6 min of the experimental period, and that about 85% of the proteins made are assembled within 12 min. The fiber, penton base, and hexon appear to form at approximately the same rates at 20 hr after infection. Because the whole penton could not be identified as a separate peak, the rate of assembly of the penton could not be estimated.

DISCUSSION

Biosynthesis of type 5 adenovirus proteins is accomplished on cytoplasmic polyribosomes having an average sedimentation coefficient of 200S (34). From these data, it was possible to estimate the size of the polypeptide chains synthesized

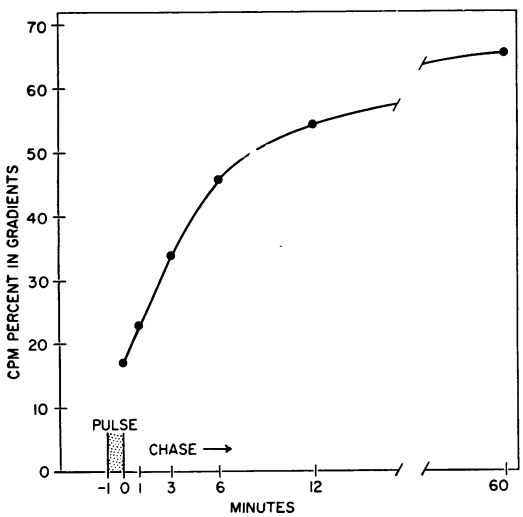

FIG. 11. *Rate of morphogenesis of type 5 adenovirus capsid proteins. The rate of morphogenesis was estimated by calculating the percentage of radioactivity recovered in the fractions of each gradient in Fig. 6, with the assumption that the solubility of the proteins reflects the assembly of the hexons, penton bases, and fibers.*

because a relationship appears to exist between the number of amino acid residues in a polypeptide chain and the number of ribosomes in the polyribosome complex, assuming uniform spacing (25, 36). For example, the light chain of γG globulin, which has a molecular weight of 20,000 to 25,000 daltons, is synthesized on polyribosomes of 180 to 190S (29, 40), and the chains of hemoglobin (16,000 daltons) are made on polyribosomes of 170S (35). It was therefore predicted that the polypeptide chains of the capsid proteins of type 5 adenovirus synthesized on polyribosomes of about 200S would have an average molecular weight of approximately 25,000 (34).

Examination of the actual size of the polypeptides which make up the hexon and fiber confirmed the prediction of the polypeptide chain size for at least two of the viral proteins. The major capsid protein of adenoviruses, the hexon, is composed of 12 polypeptide chains, each having a molecular weight of 25,400. The hexon subunits have a sedimentation coefficient of about 3S in 5 M guanidine·HCl. Preliminary data indicate that the fiber is composed of three polypeptide chains, each having a molecular weight of about 22,000 and a sedimentation coefficient of approximately 3S (Ginsberg, Scherz, and Dixon, *in preparation*). The results of experiments reported in this paper indicate that the newly assembled penton base in 0.25% SDeS yields subunits whose sedimentation coefficient also is about 3S. Additional confirmation of the prediction comes from the finding that the polypeptide chains obtained from polyribosomes or whole cells after a 1-min pulse have an average sedimentation coefficient of 3.4S (Fig. 1), which is similar to the sedimentation characteristics of the polypeptide chains obtained from purified capsid proteins. It should be noted that, even when the sizes of nascent proteins were measured after a 2- or 3-min pulse of ^{14}C-labeled amino acids, the only proteins detectable had sedimentation coefficients of 3.4S, 6S, 9S, and 12S, corresponding to nascent polypeptide chains, fibers, penton bases, and hexons, respectively (*unpublished data*). Proteins which were intermediate in size between 3.4S and 6S were not found. These data imply that the average size of the completed polypeptide chain is 3.4S, and that the proteins are synthesized within 1 min. Hence, the sedimentation properties of the nascent polypeptide chains are consistent with proteins of about 25,000 daltons whose hydrodynamic characteristics are those of nonrandom coils. These data are consistent with the conclusion that the polypeptide chains of the hexon, fiber, and pen-

ton base are translated from monocistronic messages.

Since the polypeptide chains of the capsid proteins are of similar sizes, they would be expected to sediment in a homogeneous peak. However, after a 1-min pulse, it was assumed that some radioactive amino acids would also be present in incomplete polypeptide chains of various lengths. It is not clear why incomplete chains were not detected. The following possibilities could explain the experimental findings. (i) The γG light chain, which has a molecular weight of 20,000 to 25,000, is synthesized in 30 to 45 sec (29). Therefore, the adenovirus polypeptide chains having comparable molecular weights were probably also completed in less than 1 min, but 30 sec or more might have been necessary for the chains to be released from the polyribosome complex. These circumstances would result in a relatively small proportion of the chains being incomplete under the conditions employed. (ii) The pulse was not immediately stopped at 0 to 4 C, so that partially synthesized chains were completed but new chain synthesis could not be initiated and release of completed chains did not occur. (iii) The incomplete chains were insoluble in 1 to 2 M guanidine·HCl, and in 1% SDeS some of the incomplete chains associated with large detergent micelles and sedimented with the completed viral polypeptide chains.

The data presented are not consistent with the results of Maizel and colleagues (15, 16), who reported that the hexon is composed of only three polypeptide chains each having a molecular weight of 120,000, that the penton base contains eight polypeptide chains each with a molecular weight of 70,000, and that the fiber consists of eight polypeptide chains of 62,000 daltons. These data are based on the assumption that 1 to 2% SDS and 1% 2-mercaptoethanol completely disrupt the capsid proteins to yield single polypeptide chains. If this assumption is incorrect, their subsequent finding of some smaller polypeptide chains on polyribosomes (37) might represent detection of the actual subunits rather than incomplete chains. The finding that the capsid polypeptide chains are probably synthesized in less than 1 min on polyribosomes of 200*S* is consistent with the synthesis of molecules of the order of 25,000 daltons (4, 29) rather than ones three to five times that size.

The argument that the capsid proteins are all constructed from polypeptide chains of about 3.4*S* is strengthened by the demonstration that proteins of that size are precursors of the multimeric morphological units of the capsid. The evidence is clear that after synthesis of the 3.4*S* polypeptide chains the appropriate subunits assemble with like proteins to form hexons, fibers, and a protein having a sedimentation of coefficient of about 9*S*. The data indicate that this 9*S* structure is the penton base, although immunological evidence as well as chemical and physical characterization of purified penton base are required to confirm this conclusion. If one accepts the assumption that the 9*S* protein is the penton base, then its disappearance, and apparent merging with the hexon peak, results from its combination with free fiber to form the intact penton. The identification of the penton as a protein with a sedimentation coefficient of 10.5*S* is consistent with reports that the pentons of types 3 and 4 adenoviruses sediment in sucrose gradients in a peak on the light side of the hexon (18, 19). (A personal communication of unpublished work from U. Pettersson and S. Höglund, The Wallenberg Laboratory, University of Uppsala, indicates that the purified penton of type 2 adenovirus has a sedimentation coefficient of 10.5*S* and a molecular weight of about 400,000.) Calculation of the sedimentation coefficients from the published data (18, 19) yielded a value of 10.6 for the penton.

The data summarized above pertain only to the capsid proteins. The internal proteins appear to consist of three to five polypeptide chains (8, 6, 24, 27) and make up about 20% of the viral proteins. They have not been identified as multimeric structures, and the methods employed in this investigation could not distinguish precursors of the internal proteins if they were present in the pool of polypeptide chains during the periods studied. The immunological data (1, 34), however, set an upper limit of 15 to 30% for the quantity of internal proteins which could be synthesized at 20 to 24 hr after infection if production of host proteins were completely stopped.

Maturation of the capsid proteins has been measured by two independent techniques, immunological reactivity and morphogenesis, but it was not possible to test directly whether these maturation processes were cytoplasmic or nuclear events. When the nascent polypeptide chains were removed from polyribosomes, about 30% was precipitated by antibody (34). It could not be determined, however, whether single chains reacted poorly with antibody or whether, after removal from the polyribosomes, 30% of the chains spontaneously folded into their tertiary structures and aggregated to attain immunological reactivity. Nevertheless, the major immunological maturation required 6 to 12 min, and occurred at a rate similar to the rate of transport of the nascent polypeptide chains into the nucleus (Fig. 12). It is striking that the rate at

which morphological development of the capsid proteins occurred was also similar (Fig. 12). These correlative data suggest that the polypeptide chains or small aggregates are rapidly transported into the nucleus, in which location immunological maturation and morphogenesis immediately begin.

The proteins of pseudorabies virus (2, 5) and herpes simplex virus (20, 30, 31), members of another family of nuclear DNA viruses, are also synthesized in the cytoplasm and then migrate into the nuclei of infected cells. Although the results obtained with herpesviruses resemble the data presented for the production of adenovirus proteins, some distinct differences should be noted: (i) the newly synthesized proteins of pseudorabies virus were not immunologically reactive and only became precipitable after transfer to the nuclei (5), and (ii) the nascent proteins of herpesviruses appeared to be transported relatively slowly into the infected nuclei (2, 5, 20, 30). In contrast, approximately 30% of the nascent adenovirus proteins are immunologically reactive within 1 min (34), most of the proteins are rapidly transported into the nuclei within 5 to 6 min, and the majority of the newly synthesized proteins become immunologically mature and morphologically complete structures within 5 to 6 min.

The rapid movement of nascent proteins from cytoplasm into nucleus is not unique for adenoviruses, however. Proteins of uninfected KB cells, protamine of trout testes cells (11), and probably histones of mammalian cells (3, 6, 26) also rapidly migrate into the nucleus from their cytoplasmic site of synthesis. Hence, adenoviruses and herpesviruses (and probably other nuclear DNA viruses as well) utilize the established cell machineries for protein synthesis and transport of large molecules to serve their needs for making new virions.

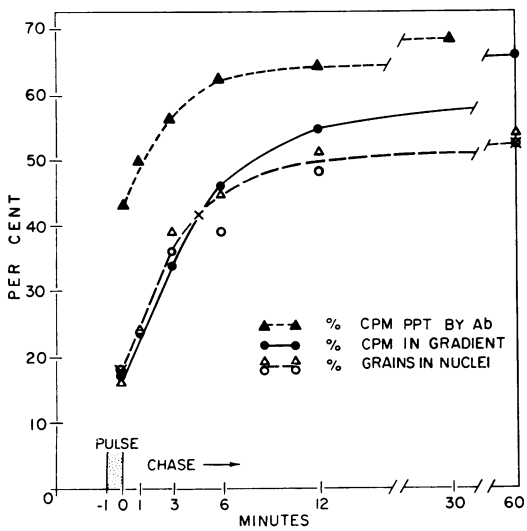

FIG. 12. *Comparison of rates of transport, immunological maturation, and morphogenesis of type 5 adenovirus capsid proteins. The results from Fig. 3, 4, and 11 are presented on the same graph for comparative purposes.*

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grants AI-0362 and AI-05731, and Training Grant AI-203, from the National Institute of Allergy and Infectious Diseases. It was also under the sponsorship of the Commission on Acute Respiratory Diseases of the Armed Forces Epidemiological Board, and was supported by the U.S. Army Medical Research & Development Command, Department of the Army, under research contract DA-49-193-MD-2131.

LITERATURE CITED

1. Bello, L. J., and H. S. Ginsberg. 1969. Relationship between deoxyribonucleic acid-like ribonucleic acid synthesis and inhibition of host protein synthesis in type 5 adenovirus-infected KB cells. J. Virol. 3:106–113.
2. Ben-Porat, T., H. Shimono, and A. S. Kaplan. 1969. Synthesis of proteins in cells infected with herpesvirus. II. Flow of structural viral proteins from cytoplasm to nucleus. Virology 37:56–61.
3. Borun, T. W., M. D. Scharff, and E. Robbins. 1967. Rapidly labeled polyribosome-associated RNA having the properties of histone messenger. Proc. Nat. Acad. Sci. U.S.A. 58:1977–1983.
4. Dintzis, H. M. 1961. Assembly of the peptide chains of hemoglobin. Proc. Nat. Acad. Sci. U.S.A. 47:247–261.
5. Fujiwara, S., and A. S. Kaplan. 1967. Site of protein synthesis in cells infected with pseudorabies virus. Virology 32:60–68.
6. Gallwitz, D., and G. C. Mueller. 1969. Histone synthesis *in vitro* by cytoplasmic microsomes from HeLa cells. Science (Washington) 163:1351–1353.
7. Klemperer, H. G., and H. G. Pereira. 1959. Study of adenovirus antigens fractionated by chromatography on DEAE-cellulose. Virology 9:536–545.
8. Laver, W. G., H. G. Pereira, W. C. Russell, and R. C. Valentine. 1968. Isolation of an internal component from adenovirus type 5. J. Mol. Biol. 37:379–386.
9. Lawrence, W. C., and H. S. Ginsberg. 1967. Intracellular uncoating of type 5 adenovirus deoxyribonucleic acid. J. Virol. 1:851–867.
10. Levine, A. J., and H. S. Ginsberg. 1967. Mechanism by which fiber antigen inhibits multiplication of type 5 adenovirus. J. Virol. 1:747–757.
11. Ling, V., J. R. Trevithick, and H. D. Gordon. 1969. The biosynthesis of protamine in trout testis. I. Intracellular site of synthesis. Can. J. Biochem. 47:51–60.
12. Loeb, G. I., and H. A. Scheraga. 1956. Hydrodynamic and thermodynamic properties of bovine serum albumin at low pH. J. Phys. Chem. 60:1633–1644.
13. Lowry, O. H., N. J. Rosebrough. A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
14. McConahey, P. J., and F. J. Dixon. 1966. A method of trace iodination of proteins for immunologic studies. Int. Arch. Allergy Appl. Immunol. 29:185–189.
15. Maizel, J. V., Jr., D. O. White, and M. D. Scharff. 1968. The polypeptides of adenovirus. I. Evidence for multiple protein components in the virion and a comparison of types 2, 7A and 12. Virology 36:115–125.

16. Maizel, J. V., Jr., D. O. White, and M. D. Scharff. 1968. The polypeptides of adenovirus. II. Soluble proteins, cores, top components and the structure of the virion. Virology 36:126–136.
17. Martin, R. G., and B. N. Ames. 1961. A method for determining the sedimentation of behavior of enzymes: Application to protein mixtures. J. Biol. Chem. 236:1372–1379.
18. Norrby, E., and P. Skarret. 1967. The relationship between soluble antigens and the virion of adenovirus type 3. III. Immunological identification of fiber antigen and isolated vertex capsomer antigen. Virology 32:489–502.
19. Norrby, E., and G. Wadell. 1967. Soluble components of adenovirus type 4. Virology 31:592–600.
20. Olshevsky, U., J. Levitt, and Y. Becker. 1967. Studies on the synthesis of herpes simplex virions. Virology 33:323–334.
21. Pereira, H. G., R. C. Valentine, and W. C. Russell. 1968. The crystallization of an adenovirus protein (the hexon). Nature (London) 219:946–947.
22. Pettersson, U., L. Philipson, and S. Höglund. 1967. Structural proteins of adenoviruses. I. Purification and characterization of adenovirus type 2 hexon antigens. Virology 33:575–590.
23. Pettersson, U., L. Philipson, and S. Höglund. 1968. Structural proteins of adenoviruses. II. Purification and characterization of the adenovirus type 2 fiber antigens. Virology 33:204–215.
24. Prage, L., U. Pettersson, and L. Philipson. 1968. Internal basic proteins in adenovirus. Virology 36:508–511.
25. Rich, A., J. R. Warner, and H. M. Goodman. 1963. The structure and function of polyribosomes. Cold Spring Harbor Symp. Quant. Biol. 28:269–285.
26. Robbins, E., and T. W. Borun. 1967. The cytoplasmic synthesis of histones in HeLa cells and its temporal relationship to DNA replication. Proc. Nat. Acad. Sci. U.S.A. 57:409–416.
27. Russell, W. C., W. G. Laver, and P. J. Sanderson. 1968. Internal components of adenovirus. Nature (London) 219: 1127–1130.
28. Sever, J. L. 1962. Application of a microtechnique to viral serological investigations. J. Immunol. 88:320–329.
29. Shapiro, A. L., M. D. Scharff, J. V. Maizel, and J. W. Uhr 1968. Polyribosomal synthesis and assembly of the H and L chains of gamma globulin. Proc. Nat. Acad. Sci. U.S.A. 56:216–221.
30. Spear, P. G., and B. Roizman. 1968. Proteins specified by herpes simplex virus. I. Time of synthesis, transfer into nuclei, and properties of proteins made in productively infected cells. Virology 36:545–555.
31. Sydiskis, R. J., and B. Roizman. 1966. Polysomes and protein synthesis in cells infected with a DNA virus. Science (Washington) 153:76–78.
32. Utsumi, S., and F. Karush. 1967. The subunits of purified rabbit antibody. Biochemistry 3:1329–1338.
33. Valentine, R. C., and H. G. Pereira. 1965. Antigens and structure of the adenoviruses. J. Mol. Biol. 13:13–20.
34. Velicer, L. F., and H. S. Ginsberg. 1968. Cytoplasmic synthesis of type 5 adenovirus capsid proteins. Proc. Nat. Acad. Sci. U.S.A. 64:1264–1271.
35. Warner, J. R., P. M. Knopf, and A. Rich. 1963. A multiple ribosomal structure in protein synthesis. Proc. Nat. Acad. Sci. U.S.A. 49:122–129.
36. Warner, J. R., A. Rich, and C. E. Hall. 1962. Electron microscopic studies of ribosomal clusters synthesizing hemoglobin. Science (Washington) 138:1399–1403.
37. White, D. O., M. D. Scharff, and J. V. Maizel, Jr. 1969. The polypeptides of adenovirus. III. Synthesis in infected cells. Virology 38:395–406.
38. Wilcox, W. C., and H. S. Ginsberg. 1961. Purification and immunological characterization of types 4 and 5 adenovirus soluble antigens. Proc. Nat. Acad. Sci. U.S.A. 47: 512–526.
39. Wilcox, W. C., H. S. Ginsberg, and T. F. Anderson. 1963. Structure of type 5 adenovirus. II. Fine structure of virus subunits. Morphological relationships of structural subunits to virus specific soluble antigens from infected cells. J. Exp. Med. 118:307–314.
40. Williamson, A. R., and B. A. Askonas. 1967. Biosynthesis of immunoglobulins: the separate classes of polyribosomes synthesizing heavy and light chains. J. Mol. Biol. 23:201–214.