Evidence for the Existence of Protomers in the Assembly of Encephalomyocarditis Virus

S. MCGREGOR,* L. HALL,¹ AND R. R. RUECKERT

Biophysics Laboratory and Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706

Received for publication 26 December 1974

Two capsid precursor subunits, which sediment on glycerol gradients at 13S and 14S, respectively, have been identified in cytoplasmic extracts of encephalomyocarditis virus-infected HeLa cells. The 13S subunit, which was detected after a 10-min pulse label with ³H-labeled amino acids, contained only capsid precursor chain A (mol wt 100,000). When the 10-min pulse label in such cells was chased for 20 min, the A-containing 13S subunit in the cytoplasmic extracts was replaced by a 14S subunit containing equimolar proportions of three chains: ϵ , γ , and α . This (ϵ, γ, α)-containing 14S subunit could be dissociated into 6S subunits with the same polypeptide composition. The sedimentation properties and the polypeptide stoichiometry of these three precursor subunits, when compared with those of the 13S, (β, γ, α)₈, and 5S, (β, γ, α), subunits derived by acid dissociation of purified virions, suggest the following structural assignments: 13S, (A)₈; 14S, (ϵ, γ, α)₈; 6S, (ϵ, γ, α). The molecular weights of the individually isolated capsid chains were determined by gel filtration in 6 M guanidine hydrochloride to be: ϵ , 36,000; α , 32,000; β , 29,500; γ , 26,500; and δ , 7,800. With the exception of the δ -chain, these values are in reasonable agreement with the values previously determined by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels. These data support the hypothesis that picornavirus capsids are assembled from identical protomers according to the following scheme:

where n is the number of immature protomers per virion.

Picornaviruses contain four nonidentical polypeptide chains here designated α , β , γ , and δ (29). All four chains are generated by post-translational cleavage of a single, large (mol wt 100,000) precursor, the A chain (3, 13, 17, 22). The virions also frequently contain traces of a fifth polypeptide, ϵ , which is an uncleaved precursor of β and δ chains (3, 13). The cardioviral (encephalomyocarditis [EMC] virus, mouse Elberfeld [ME] virus, mengovirus) chains A, ϵ , α , β , γ , and δ correspond to the polioviral chains NCVP-1a, VP-0, VP-1, VP-2, VP-3, and VP-4, respectively (13, 32). We have adopted the cardiovirus nomenclature which lends itself more readily to the description of oligomeric structures.

Cytoplasmic extracts of poliovirus-infected HeLa cells contain two capsid-related structures, one sedimenting at 14S and the other at 73S (25-27). Both contain the capsid peptides

ϵ , γ , α and are thought to be intermediates in capsid assembly. In the presence of rough membrane fractions from infected cells, the 14S subunit can be assembled *in vitro* into 73S protein shells which are indistinguishable from the RNA-devoid, empty capsids found in infected cells (24, 25, 27). The kinetics of *in vivo* labeling experiments are also consistent with the postulated precursor role of these two subunits (14, 23).

It has been suggested that the capsid polypeptides are organized into two closely related types of subunits designated "mature protomers" (a protomer is defined as the smallest identical subunit of an oligomeric protein [21]) ($\delta, \beta, \gamma, \alpha$), and "immature protomers," (ϵ, γ, α) (29, 30). These hypothetical subunits are presumably derived from the A chain in the sequence: $A \rightarrow (\epsilon, \gamma, \alpha) \rightarrow (\delta, \beta, \gamma, \alpha)$.

According to the protomer hypothesis, the immature empty capsid, by analogy with the structure of the virion, is comprised of 60 im-

* Present address: Department of Biology, 16-717, Massachusetts Institute of Technology, Cambridge, Mass. 02139.

mature protomers, $(\epsilon, \gamma, \alpha)_{60}$, whereas the sedimentation coefficient of the 14S subunit is about that expected for a pentameric structure, $(\epsilon, \gamma, \alpha)_5$ (29). However, the stoichiometry reported for the polioviral 14S precursor is $\epsilon_3\beta_2\gamma_5\alpha_3$ (26).

A capsid-related antigen, which sediments at 14S, has also been reported in extracts from EMC virus-infected Krebs II ascites cells (15). In this communication we confirm the existence of such a 14S subunit in EMC virus-infected HeLa cells and show that it has the properties of a precursor, not a capsid breakdown product. We also show that this subunit can be dissociated into a smaller subunit with the properties expected of the postulated immature protomer. A second subunit, which appears to be an uncleaved precursor of the 14S subunit, is also described.

MATERIALS AND METHODS

Media. Medium A is Eagle minimal medium in Earle saline supplemented with 0.1 mM nonessential amino acids and 2×10^{-6} M inositol (20). Medium F is Eagle minimal medium in Earle saline lacking calcium and magnesium and supplemented with 0.1 mM glycine and serine and 0.1% Pluronic F68. Medium AH is medium A containing 25 mM *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid buffer, pH 7.4 (3). Medium AL is medium AH lacking amino acids.

Buffers. When Dulbecco phosphate-buffered saline (6) is supplemented with 0.1% bovine serum albumin (BSA), it is designated PBSA. Buffer IV is 1 M sodium chloride in 0.02 M Tris acetate (pH 7.5). TSA is 0.25 M sodium chloride, 0.005 M Tris acetate (pH 7.4) containing 0.01% BSA. Reticulocyte standard buffer (RSB) is 0.01 M sodium chloride, 1.5×10^{-3} M magnesium chloride and 0.01 M Tris-hydrochloride (pH 7.4).

Cells. HeLa cells, designated W-HeLa, were originally obtained through the courtesy of G. C. Mueller, Department of Oncology, University of Wisconsin. They were propagated in suspension in medium F supplemented with 6% bovine serum by shaking 300-ml cultures in siliconized Florence flasks at 37°C. The cultures, which did not require trypsinization, grew with a generation time of 24 h. They were continuously maintained at this growth rate of serial dilution to keep the cell density between 0.5×10^6 to 6.0×10^6 cells per ml.

Virus. EMC virus was originally obtained from C. Fuerst, University of Toronto. It had been adapted by serial passage to growth in Sarcoma 180 ascites tumor cells (12). The virus was then plaque-purified three times in HeLa cells. Stock virus was prepared by growing the plaque isolate (hereafter designated the F2 strain) in HeLa cell suspension cultures. The stock was concentrated 50- to 100-fold by sedimentation. The pelleted virus was resuspended and purified by isopycnic centrifugation in cesium chloride. The virus

suspension was then dialyzed against TSA buffer and stored at -70°C . The infectivity titer of the stock virus was determined by plaque assay on HeLa cell monolayers and was 6.5×10^{11} PFU per ml.

Infection of suspension cultures. HeLa cells were grown in suspension culture in medium F supplemented with 6% bovine serum. The cells were sedimented by low-speed centrifugation, washed once, and resuspended in medium AL at a concentration of 4×10^7 cells per ml. The cell suspension was inoculated with 100 PFU per cell. After an attachment interval of 20 min at room temperature, the suspension was diluted 10-fold with warm medium AL. Actinomycin D was then added to give a final concentration of 5 μg per ml. The infected cell suspension (4×10^6 cells per ml) was incubated at 37°C with gentle agitation.

Isotopic labeling and purification of EMC virus. Isotopically labeled amino acids were added to infected cell suspensions at 3 h postinfection. Incubation was continued at 37°C. At 5 h postinfection, the cells, containing greater than 90% of the total infectivity, were sedimented and washed once in cold medium AL. The virus was released from the cells by freeze-thawing twice. Cell debris was removed by low-speed centrifugation, and the virus was pelleted through a cushion of 30% sucrose in buffer IV. The pellet was resuspended in PBSA by flushing with a needle and syringe and insoluble material was removed by centrifugation at $12,000 \times g$ for 5 min. The virus in the clarified supernatant fluid was further purified by isopycnic centrifugation in cesium chloride, followed by gel filtration on 6% agarose equilibrated with TSA. The purified virus was stored at -70°C .

Glycerol density gradient. Linear gradients of glycerol (wt/wt) contained RSB, 0.1% 2-mercaptoethanol, and 0.1% BSA. Five-milliliter gradients (Spinco SW65 rotor) were generated by the method of Britten and Roberts (1). Twelve-milliliter gradients (Spinco SW41 rotor) were prepared by layering five concentrations of glycerol (30, 25, 20, 15, and 10%) and storing at 6°C for 6 h to allow diffusion. Both types of gradients were centrifuged at 40,000 rpm and had average gravity forces of 114,000 and 196,000 for the SW65 and SW41 rotors, respectively. All gradients were fractionated by bottom puncture. Radioactivity was determined by scintillation spectrometry in scintillation solvent B-10 (20).

Dissociation of virions into 13S and 5S protein subunits. Purified EMC virus in TSA buffer was treated with 0.1% 2-mercaptoethanol at room temperature for 10 to 15 min. The reduced virus was dissociated into 13S subunits by dialysis against 0.1 M sodium chloride in 0.05 M sodium citrate buffer, pH 5.7, at 37°C for 6 h. The 13S subunit was then isolated by sedimentation on a 12-ml, linear, 10 to 30% glycerol gradient at 6°C for 13 h at 40,000 rpm in a Spinco SW41 rotor.

To prepare 5S subunits, reduced virions were first dissociated at pH 5.7 as described above, and the resulting 13S subunits were then dissociated into 5S subunits by adding to the dialyzed preparation sufficient 6 M urea to bring the final concentration to 1.5

M. After 15 min at room temperature the preparation was applied to a 12-ml, linear, glycerol gradient and centrifuged at 6°C for 14 h at 40,000 rpm in a Spinco SW41 rotor.

Polyacrylamide gel electrophoresis. Electrophoresis was by a modification (20) of the procedure of Maizel (16). The gels contained 9.7% acrylamide, 0.3% ethylene diacrylate (vol/vol), 0.1% *N,N,N',N'*-tetramethylethylenediamine, 0.1% sodium dodecyl sulfate (SDS), 0.5 M urea and 0.1 M sodium phosphate at pH 7.2. The electrophoresis buffer was 0.1 M sodium phosphate, pH 7.2, containing 0.1% SDS and 0.01 M mercaptopropionic acid. Samples to be electrophoresed on SDS gels were dissociated at 100°C for 5 min in 1% SDS, 0.5 M urea, and 0.1% 2-mercaptoethanol in 0.01 M sodium phosphate buffer, pH 7.2. Electrophoresis was carried out at 9 mA per tube.

Soft gels, utilized in the purification of high-molecular-weight subunits, contained 5% polyacrylamide (acrylamide-bisacrylamide ratio of 33), 0.5% agarose, 0.1% *N,N,N',N'*-tetramethylethylenediamine, and 0.1 M sodium phosphate buffer (pH 7.2). The gels were polymerized at 37°C and then incubated at room temperature for 1 h before use. The electrophoresis buffer contained 0.1 M sodium phosphate buffer, pH 7.2, and 0.02 M mercaptopropionic acid. Electrophoresis was carried out at 4 mA per tube.

All gels were fractionated with a Gilson automatic gel fractionator (11). Determination of radioactivity in SDS gel fractions by liquid scintillation counting has been described (20).

Determination of polypeptide molecular weights. The apparent molecular weights of individually isolated virus peptides were determined by gel filtration in 6 M guanidine hydrochloride according to the method of Fish et al. (10). The virus peptides, labeled with a ³H-labeled amino acid mixture, were recovered from peak regions of the fractionated SDS-polyacrylamide gels by extraction with 10 gel volumes of distilled water containing 0.1% 2-mercaptoethanol and 0.1% BSA for 12 to 48 h. The proteins in this extract were carboxymethylated by the following procedure.

Solutions containing the isolated virion peptides were mixed with marker proteins (never exceeding 20 mg of total) in a 1-dram vial containing the vacuum-dried residue from 1 ml of 6 M guanidine hydrochloride in 0.5 M Tris-hydrochloride, pH 8.6. After adjusting the volume to 1.0 ml with distilled water, 4 μ l of 2-mercaptoethanol was added and the solution was incubated for 1 h at 45°C to complete reduction of disulfide bonds. Iodoacetamide was then added to a final concentration of 0.15 M and incubation was continued at 45°C for 10 min in the dark. Alkylation was terminated by adding 30 μ l of 2-mercaptoethanol. After cooling, 300 μ g of Blue dextran 2000 (Pharmacia Fine Chemicals, Piscataway, N. J.) and 78 μ g of sodium 2,4-dinitrophenyl-alanine (Mann Research Laboratories, Inc., New York) were added as volume markers for the column. Gel filtration of 200- μ l samples was carried out on a 6% agarose column (75 by 0.9 cm) equilibrated with 6 M guanidine hydrochloride. The marker proteins were spectrophotomet-

rically detected using a micro-flow cell (Instrumentation Specialties Co., Lincoln, Neb.) with a volume of 50 μ l. The flow rate was maintained at 1.5 ml per h.

Determination of acid-insoluble radioactivity. Samples from glycerol gradients were placed onto Whatman 3MM filter disks and dried at 37°C for 4 h. The filter disks were washed three times in 5% trichloroacetic acid at 6°C for 10 min. The trichloroacetic acid was removed by washing twice in 95% ethanol and the disks were dried at 100°C. The filter disks were then counted in scintillation solvent B-10.

Material. Actinomycin D (Dactinomycin) was obtained from Merck, Sharp and Dohme (West Point, Pa.). The 6% agarose (Bio-Gel A-5M) was from BioRad Laboratories (Richmond, Calif.). The L-[4,5-³H]leucine and L-[U-¹⁴C]leucine were purchased from Schwarz/Mann (Orangeburg, N.Y.); the L-[U-¹⁴C]-amino acid mixture was from New England Nuclear (Boston, Mass.).

RESULTS

Identification of two capsid-related protein precursor subunits. There is evidence that the EMC viral A chain, which is the precursor of all capsid protein, has a half-life of about 7 min (4). Accordingly, we sought evidence for the existence of capsid-related structures within the first 10 min of synthesis. Figure 1a shows the sedimentation profile of a cytoplasmic extract from EMC virus-infected HeLa cells after a 10-min pulse of [³H]leucine. Labeling was carried out at 3.5 h postinfection when virtually all protein synthesis was virus coded. The bulk of the acid-insoluble radioactivity sedimented as a broad band in the 2S to 10S region with a small shoulder cosedimenting with a 13S marker. When the label in such pulsed cells was chased for 20 min, the 13S shoulder was replaced with a prominent sharp peak which sedimented 8% faster than the 13S marker, or at about 14S (Fig. 1b).

The electrophoretic profile of the 13S and 14S peaks on SDS-polyacrylamide gels is shown in Fig. 2. The 13S peak, observed after the 10-min pulse, contained a dominant peak of A chain and a trace of capsid-related B chain (3). It also contained about 12% of the recovered label in E chain, a noncapsid protein (3), and a trace of label in at least one other minor, unidentified peak. The 14S peak, observed after the 20-min chase, consisted predominantly of capsid-related peptides (ϵ, α, γ) and only very small proportions of A and E chains. These results raise the possibility that the E chain is a component of the A-containing 13S peak, in which case the distribution of the A and E chains in the glycerol gradient should coincide.

To test this possibility, the distribution of each chain in gradient 1a was determined by

FIG. 1. Sedimentation profile of cytoplasmic extracts from EMC-infected cells. (a) Pulse labeled 10 min; (b) pulse labeled 10 min and chased 20 min. Infected HeLa cells (8×10^7) were pulse labeled with [^3H]leucine (100 μCi per ml) at 3.5 h postinfection. Labeling was allowed to proceed for 10 min at 37 C. Half of the cells were then Dounce homogenized in cold RSB and the cytoplasmic extract was stored in ice. The other half of the culture was sedimented and resuspended in 10 ml of medium AH and the ^3H label was chased by incubation for an additional 20 min at 37 C. The cells were then homogenized. Equivalent amounts of both cytoplasmic extracts were placed onto 12-ml glycerol gradients (10 to 30%) and centrifuged at 6 C for 13 h at 40,000 rpm in a Spinco SW41 rotor. A [^{14}C]13S marker (not shown) prepared by acid dissociation of the EMC virion (see Materials and Methods) was included in both gradients. The gradients were fractionated into 14-drop (270 μl) fractions by bottom puncture. Sedimentation was from right to left.

analyzing fractions 10 to 20 on SDS-polyacrylamide gels. The total radioactivity in the A and E chains in each gradient fraction was calculated and plotted against fraction number (Fig. 3). The small amount of label in the B chain precluded analysis by this procedure. Also shown in Fig. 3 are the results of a similar analysis of the 14S, ϵ -containing peak from gradient 1b. The ϵ , α , and γ chains in the 14S peak all coincided, supporting the idea that all are elements of a single structure. In contrast, the lack of coincidence in the distribution of the A and E chains in the 13S region of gradient 1a argues against the hypothesis that the E chain is a component of the 13S peak. A more complete analysis (not shown) indicated that the E chain is part of a separate but overlapping component which peaks at about 7S.

These data suggest that the A chain is assem-

bled into an oligomeric structure before being cleaved into the smaller ϵ , α , and γ chains. After cleavage, the sedimentation velocity of the precursor increased from 13S to 14S. In addition, it should be pointed out that while the ϵ -containing peak was very symmetrical the A-containing peak was not, but tended to skew toward the 14S peak (Fig. 3).

Polypeptides in the 2S to 10S region. The monomeric form of the A chain, with a mass of 100,000 daltons, would be expected to sediment at about 6S (29). A search for such a monomeric structure in the 2S to 10S region of the gradient (Fig. 1a) revealed less than 2% of the total radioactivity in this region in A chains. However, after the 20-min chase (Fig. 1b), approximately 10% of the total label in the 2S to 10S region was found in ϵ chains. A trace of label was also found in capsid-related peptides in a narrow band at the top of the gradient. The bulk of the label in the 2S to 10S region was

FIG. 2. Electrophoretic profile of peak fractions from density gradients in Fig. 1. Samples of 200 μl were electrophoresed on 20-cm, SDS-polyacrylamide gels at 9 mA per tube for approximately 18 h. (a) 13S subunit (fraction 17) from gradient a in Fig. 1. (b) 14S subunit (fraction 14) from gradient b in Fig. 1.

FIG. 3. Distribution of virus-coded peptides in the 13S to 14S region of sedimentation gradients of cytoplasmic extracts. Gradient fractions from Fig. 1 (a and b) were analyzed by SDS-polyacrylamide gel electrophoresis. The amount of each virus peptide per fraction was calculated and their distributions were plotted. The values plotted for the A chain and E chain were from (a); the value for the ϵ chain was from (b) (Fig. 1). The distribution of α and γ chains (not shown) coincided with that of the ϵ chain. The maximum values for each peptide were: A, 1,163 counts/min; E, 544 counts/min; and ϵ , 2,576 counts/min. A chain, ●—●; ϵ chain, ○---○; and E chain, □—□.

associated with the noncapsid polypeptides E (mol wt 56,000), H (12,000), and I (11,000). The E chain sedimented at about 7S and the H and I chains at about 5S. The amount of these three noncapsid proteins in the 2S to 10S region was increased at least fivefold when the extracts were treated with 0.5% Nonidet P-40 (NP-40) before centrifugation.

Distribution of A chains in the pellet. In the course of these experiments, 40 to 60% of the total A-chain label in the cytoplasmic extracts was found in the membrane-rich pellet at the bottom of the glycerol gradients. When cytoplasmic extracts of pulse-labeled, infected cells were centrifuged on discontinuous sucrose gradients (5), 30 to 35% of the A chain was found in the rough endoplasmic reticulum band; an additional 5% was found in the smooth endoplasmic reticulum band. In an attempt to detect a monomeric (6S) form of the A chain on membranes, pellets from glycerol gradients were solubilized with 0.5% NP-40 and sedimented on glycerol gradients. A chains were found in the 14S but not in the 6S region. This result suggests that the hypothetical A chain, monomeric subunits are rapidly assembled into the 14S form. Another possibility is that A chains were present, but, because of a tendency to precipitate or attach to surfaces, were not solubilized by NP-40 treatment.

Electrophoretic purification of capsid precursors on nondissociating polyacrylamide gels. When material from the 14S region of a density gradient (Fig. 1b) was electrophoresed on nondissociating (no SDS) polyacrylamide gels at pH 7.2, two components were observed, a large peak I and a slower peak II (Fig. 4). Peak II was typically less abundant than that shown in Fig. 4. To examine the possibility that these peaks might correspond to the A-containing, 13S precursor and the cleaved, 14S precursor subunits, each component was eluted from the gel and its polypeptide composition was determined by electrophoresis on SDS-polyacrylamide gels.

The electrophoretic profile of peak I (Fig. 5) revealed three major polypeptides; these comigrated with the ϵ , α , and γ chains of the EMC virion. Sedimentation analysis of peak I on glycerol gradients further showed a single peak sedimenting at 14S (not shown); hence the majority of the applied 14S material survived electrophoresis and elution without degradation. The total lack of E chains in this profile further supports our earlier conclusion that the E chain is not an integral element of the 14S subunit. In addition, the absence of β chains indicates that the 14S subunit is a precursor of the virion and not a degradative product.

A similar electrophoretic analysis of peak II revealed 93% of the recovered radioactivity in ϵ , α , and γ chains (not shown); these chains were present in the same relative proportions as in peak I. Traces of label were also found in capsid precursor chains A (0.7%) and B (0.7%) and in a

FIG. 4. Electrophoretic profile of the intact 14S precursor subunit on a 5% polyacrylamide gel (no SDS). The 14S subunit was isolated from cytoplasmic extracts of [^3H]leucine-labeled EMC-infected cells as described in Fig. 1b. After dialysis against RSB, the subunit was further purified by sedimentation in a second glycerol density gradient. The 14S peak fractions were pooled and electrophoresed on a 5% polyacrylamide gel containing 0.1 M sodium phosphate, pH 7.2, and 0.02 M mercaptopropionate but lacking SDS (see Materials and Methods). Electrophoresis was carried out at 4 mA per tube for 32 h. The gel was fractionated and the protein subunit was eluted into distilled water containing 0.1% 2-mercaptoethanol. Fractions were incubated for at least 12 h at 6 C before samples were removed for determination of radioactivity.

noncapsid chain G (4%). Hence, peak II cannot contain more than a trace amount of subunits composed of A chain. It consists instead mainly of peak I-related material, possibly in the form of an aggregate, a conformer or even a dissociation product. Because of its smaller size, the mobility of such a dissociation product would normally be expected to exceed that of peak I. However, similar electrophoretic studies of the virion-derived 13S, (β, γ, α)_s, and 5S, (β, γ, α)_s subunits (see below) yielded the opposite result; i.e., the 5S subunit migrated slower than the 13S. Such a result could be explained by a

decrease in the isoelectric point of the protein accompanying the dissociation of the 13S subunit.

Attempts to electrophoretically purify the A-containing 13S peak from pulse-labeled, cytoplasmic extracts were frustrated by the small amount of radioactivity available and by serious losses encountered during storage and manipulation of this material.

Dissociation of the 14S capsid precursor with urea. Figure 6 shows the sedimentation profile of the 14S subunit after a 30-min treatment with 1.5 M urea at 35 C. Approximately 55% of the 14S subunits was dissociated by this treatment into material which sedimented just ahead of a 5S marker; this smaller component was assigned a nominal sedimentation value of 6S. An additional 30% of the radioactivity sedimented as undissociated 14S subunits. The remaining 15% of the material was adsorbed to the centrifuge tube. To minimize the loss of material due to adsorption, the 6S region was collected into siliconized glass vials. When examined by electrophoresis on SDS-polyacrylamide gels, the 6S material exhibited a polypeptide composition very similar to that of the parent, 14S subunit (Fig. 7; see also Table 4, below).

13S and 5S subunits from pH-dissociated EMC virions. ME virus (7) and mengovirus (18), which are serologically related to EMC virus, can be dissociated through a 13S-14S intermediate, (β, γ, α)_s, into protomeric 5S subunits, (β, γ, α). In this section we describe the properties of the analogous subunits derived from pH-dissociated EMC virions (Fig. 8). The sedimentation profile of the dissociation product of [^{14}C]leucine-labeled EMC virions (Fig. 8a) was indistinguishable from that of the (β, γ, α)_s subunits from ME virus (data not shown). We assume therefore that the 13S-14S subunits derived from EMC virus correspond in structure to those from ME virus.

The (β, γ, α)_s subunit of ME virus has previously been assigned a sedimentation value of 14S (7); on this basis, the (ϵ, γ, α)-containing precursor subunit of EMC virus was assigned a value of 15S (29). The sedimentation coefficient of the mengovirus (β, γ, α)_s subunit, determined by analytical ultracentrifugation, has been reported to be 13.4S (19). In this paper, we have attempted to simplify the nomenclature by adopting a nominal value of 13S for the (β, γ, α)_s subunit of ME virus, EMC virus, and mengovirus. This leads to a calculated value of 14S for the EMC virus capsid precursor (Fig. 1b), a value which has been extensively used in

FIG. 5. SDS-polyacrylamide gel pattern of the purified 14S precursor subunit. The subunit eluted from fraction 14 to 15 of Fig. 4 was coelectrophoresed with [14 C]EMC virus on a 20-cm polyacrylamide gel at 9 mA per tube for approximately 15 h. Arrows indicate the positions of the EMC virion polypeptides.

FIG. 6. Sedimentation profile of the 14S subunit after treatment with urea. The purified 14S subunit from Fig. 4 was heated at 35 C for 30 min after the addition of 6 M urea to give a final concentration of 1.5 M urea. It was sedimented on a 12-ml linear 15 to 35% glycerol gradient at 6 C for 14 h at 40,000 rpm in a Spinco SW41 rotor. A [14 C]5S subunit was included as a sedimentation marker (not shown). A parallel gradient contained a [14 C]13S marker prepared from EMC virus (not shown).

FIG. 7. SDS-polyacrylamide gel pattern of the 6S peak from Fig. 6. Electrophoresed on a 22-cm polyacrylamide gel at 9 mA per tube for approximately 18 h.

describing the poliovirus capsid precursor, which presumably has a similar structure.

The 13S subunit was completely dissociated into smaller 5S subunits by treatment with 1.5 M urea at room temperature (Fig. 8b). Its stability differed significantly in this respect from that of the 14S precursor subunit which was only partially dissociated even when exposed to an equivalent urea concentration for a longer time at a higher temperature (35 C rather than 21 C).

SDS-polyacrylamide gel analysis of the 13S and 5S subunits demonstrated that both subunits were composed of α , β , and γ chains (Fig.

FIG. 8. Sedimentation profile of the 13S (a) and 5S (b) subunits derived from the EMC virion by acid dissociation. The 13S subunit was prepared by dialyzing purified, thiol-reduced, [^{14}C]leucine-labeled EMC virus against 0.1 M sodium chloride, 0.05 M sodium citrate at pH 5.7 (see Materials and Methods). The dissociated virus was sedimented on a 12-ml, linear, 10 to 30% glycerol gradient at 6 C for 12 h at 40,000 rpm in a Spinco SW41 rotor. The 5S subunit was also prepared from the purified virion. After acid dissociation of the virus at pH 5.7 in 0.1 M sodium chloride, it was further treated with 1.5 M urea for 15 min at room

9). The distribution of [^3H]leucine in the profiles of the two subunits was very similar (see Table 3).

Stoichiometry of polypeptides in the EMC virion. The number of polypeptide chains of species i (n_i), and molecular weight (m_i), in a capsid of known mass (M) can be calculated using equation (1) if the relative distribution of radioactivity (r_i) and the relative specific activity (a_i) are known:

$$n_i = (a_i)(r_i)(M)/(m_i) \quad (1)$$

Purified EMC virions, labeled with a mixture of 15 radioactive amino acids, were disrupted with SDS and electrophoresed on SDS-polyacrylamide gels. The distribution of radioactivity in each polypeptide peak was determined (Table 1, column 2). Assuming that all five chains were labeled to a constant specific activity by the radioactive amino acid mixture, the average chain stoichiometry was calculated using peptide molecular weights determined by two different methods: (i) electrophoresis on SDS-polyacrylamide gels, and (ii) gel filtration chromatography in 6 M guanidine hydrochloride.

The stoichiometry obtained using the electrophoretically determined molecular weights was $\epsilon_2\alpha_{60}\beta_{57}\gamma_{69}\delta_{45}$ (Table 1), compared to a formula of $\epsilon_2\alpha_{60}\beta_{58}\gamma_{60}\delta_{58}$ predicted for a 60-protomer capsid containing two immature protomers. Agreement with the theoretical stoichiometry fell well within the 6 to 10% reliability limit attributed to the electrophoretic method (8, 33), except for the two smallest chains, γ (15% high) and δ (25% low). The apparent molecular weight of the γ chain of ME virus, which is electrophoretically indistinguishable from that of EMC virus, is known to be sensitive to small changes in electrophoretic conditions, varying from 23,000 to 26,500 (8, 29). An apparently low delta chain content of the virion has also been observed with ME virus and was traced to an anomalous molecular weight value (30, 31). Such anomalies are now known to occur commonly in SDS-gel electrophoresis with polypeptides smaller than 15,000 daltons (8, 28).

To examine this issue with EMC virus, the molecular weight of each polypeptide was determined by gel filtration chromatography in 6 M guanidine hydrochloride using isolated, [^3H]leucine-labeled chains extracted from SDS-polyacrylamide gels (Table 1). The stoichiome-

temperature. The dissociated virus was then sedimented on a 12-ml, linear, 15 to 35% glycerol gradient at 6 C for 14 h at 40,000 rpm in an SW41 rotor. A [^3H]14S marker was included in both gradients (not shown). Sedimentation is from right to left.

FIG. 9. SDS-polyacrylamide gel pattern of the 13S and 5S subunits derived from the virion. The peak fractions from Fig. 8 (a and b) were electrophoresed on separate 30-cm polyacrylamide gels at 9 mA per tube for approximately 20 h. [^{14}C]EMC virus was coelectrophoresed with each subunit (not shown). Arrows indicate the positions of the EMC virion polypeptides. [^3H]13S subunit (-----); [^3H]5S subunit (—).

TABLE 1. Molar proportions of polypeptide chains in EMC virions

Peptide	Recovered radioactivity (%) ^a	SDS-gel electrophoresis		6 M GUHCL gel filtration ^b	
		Apparent mol wt ^c	No. of chains per virion ^d	Apparent mol wt	No. of chains per virion ^d
ε	1.1 ± 0.1	40,000	1.6	36,500 ± 200	1.7
α	35.0 ± 0.1	34,000	59.7	32,000 ± 100	63.4
β	29.3 ± 0.2	30,000	56.6	29,500 ± 200	57.6
γ	27.5 ± 0.3	23,000	69.3	26,500 ± 150	60.2
δ	7.0 ± 0.1	9,000	45.1	7,800 ± 150	52.1

^a The average values (± average error) of three determinations utilizing purified EMC virus labeled with a mixture of 15 ^{14}C -labeled amino acids. The standard error of counting was 3% for ε chain.

^b The apparent molecular weights were determined by gel filtration chromatography of isolated peptides in 6 M guanidine hydrochloride (GUHCL). The average errors given indicate the precision of two or three separate determinations.

^c From Butterworth et al. (3).

^d The number of chains per virion was calculated using equation (1) (see text).

try calculated with these molecular weight values was $\epsilon_2\alpha_{63}\beta_{58}\gamma_{60}\delta_{52}$. The molecular weight value required to obtain a value of 58 delta chains per virion from the radioactivity distribution data is 7,000 rather than the value of 7,800 actually found. These values can be compared with similarly determined values for the delta chain: EMC virus, 6,800 (2); ME virus and mengovirus, 7,200; and poliovirus, 7,600 (31). Using the average molecular weight value

of all these determinations, 7,300, yielded a calculated delta chain content of 56 per virion.

There is good agreement between the chain stoichiometry calculated by these methods and that expected for a 60-protomer virion. This is possibly fortuitous considering the 7 to 10% reliability limit attributed to the gel filtration method of determining molecular weights, and the uncertainty of the error involved in assuming the polypeptides are labeled to a constant

specific activity by the amino acid mixture (10). In addition, there is also good agreement on the mass of the capsid protomer calculated by different methods: 100,000 daltons for the A chain (3); 97,000 and 96,000 daltons for the immature ($\epsilon + \gamma + \alpha$) and mature ($\delta + \beta + \gamma + \alpha$) protomers using the mass values determined by SDS-gel electrophoresis; and 95,000 and 96,000 daltons for the immature and mature protomers using the mass values determined by gel filtration chromatography.

The nonintegral amounts of ϵ chain (1.6 and 1.7) observed in these experiments do not necessarily reflect a 15 to 20% error in a true stoichiometry value of 2. A nonintegral value could be attributed to a heterogeneous population of virions containing 0, 1, 2, 3 or more immature protomers (7). For example, a value of 1.6 might represent a mixture of virions, 40% containing one immature protomer and 60% containing two. In this case, the average stoichiometry would appear to be $\epsilon_{1.6} \alpha_{60} \beta_{58.4} \gamma_{60} \delta_{58.4}$.

Distribution of leucine residues in capsid polypeptides. [^3H]leucine is incorporated into EMC viral protein without significant conversion to other amino acids (D. Omilianowski, personal communication). Therefore, the distribution of label in the electrophoretic profiles of each of the capsid-precursor and capsid-product subunits could be used to measure their chain stoichiometry if the leucine content of each capsid chain were known. These values can be computed from the radioactivity profile of purified ^3H -labeled leucine virions by assuming the polypeptide stoichiometry predicted by the 60-protomer model.

The capsid protein of EMC virus is reported

to contain 8.3 mol% leucine (9). Assuming a mass of 111 daltons per average amino acid residue (29), the leucine content of EMC virus protein is calculated to be $(0.083/111 =) 7.5 \times 10^{-4}$ mol/g of protein. Hence, a protomer with a mass of 96,000 daltons would contain about $(7.5 \times 10^{-4} \times 96,000 =) 72$ leucine residues and a 60-protomer capsid about $(60 \times 72 =) 4,300$ leucine residues.

The leucine content of each chain can now be calculated for a capsid of known stoichiometry (Table 2). For example, the number of leucine residues in a virion complement of α chains is $(0.330 \times 4320 =) 1,430$, or $(1,430/60 =) 24$ leucine residues per α chain. The results of similar calculations for each chain in the virion are summarized in Table 2 for a 60-protomer capsid containing (n) immature protomers, where $n = 2$ and $n = 3$. The calculated leucine content of α , β , γ , and δ chains is not affected by the variation in (n). However, in the case of the ϵ chain, the number of leucine residues is 45 if $n = 2$, but 30 if $n = 3$. The latter value agrees with the leucine content of β plus δ chains, which are the cleavage products of the ϵ chain. This suggests that $n = 3$, i.e., the average virion in this preparation contained three ϵ chains.

The alternative interpretation, that $n = 2$, would require the loss of 15 leucine residues during the cleavage of the ϵ chain. This hypothesis requires that the mass of the ϵ chain exceed that of its cleavage products ($\beta + \delta$) by at least 1,700 daltons (the mass of 15 leucine residues). A discrepancy of this magnitude might conceivably go undetected since the estimated uncertainty of the molecular weight determination is on the order of 10%, or $\pm 3,600$ daltons, for the epsilon chain. However, such a sequence would

TABLE 2. *Distribution of leucine residues in the polypeptides of EMC virus*

Peptide	Recovered radioactivity (%) ^a	Two ϵ chains per virion		Three ϵ chains per virion	
		No. of chains per virion ^b	No. of leucine residues per chain ^c	No. of chains per virion ^b	No. of leucine residues per chain ^c
ϵ	2.12 ± 0.04	2	45.5	3	30.0
α	33.01 ± 0.23	60	23.8	60	23.8
β	30.72 ± 0.37	58	22.9	57	23.3
γ	25.18 ± 0.18	60	18.2	60	18.2
δ	8.98 ± 0.12	58	6.7	57	6.8

^a The average values ($\pm$ average error) of two determinations utilizing [^3H]leucine-labeled EMC virus. The chains were separated on SDS-polyacrylamide gels.

^b Assuming 60 protomers per virion, including (n) immature protomers (ϵ, γ, α) and $(60-n)$ mature protomers ($\delta, \beta, \gamma, \alpha$).

^c The number of leucine residues per chain was calculated by dividing the product of the total leucine residues in the virion and the fraction of [^3H]leucine in a particular peptide by the number of chains per virion. It was assumed that the virion contained 4,320 leucine residues.

have to be very leucine rich (>50%) in order to have such a small mass. Further evidence against this hypothesis is discussed below.

Peptide stoichiometry in capsid-related subunits. Electrophoretic profiles of [³H]leucine-labeled 13S, (β, γ, α)₈, and 5S, (β, γ, α) subunits (Fig. 9), derived by acid dissociation of EMC virions, provided very reproducible distributions of radioactivity. These are summarized in Table 3 (columns 3 and 5, respectively). These data were used to calculate the molar stoichiometry of the polypeptide chains assuming that the α chain contained 24 leucine residues; the β chain, 23; and the γ chain, 18 (Table 2, column 6). The chain stoichiometry of the 13S and 5S subunits fell within a few percent of the theoretical equimolar ratios (Table 3, columns 4 and 6). These results strongly reinforced our confidence in the values determined for the leucine content of each

virion chain and in the ability of this method to determine chain stoichiometry in capsid subunits with considerable precision.

It should be noted here that the calculated chain stoichiometry depends on the distribution of leucine in the subunit and in the virion, but not on the gross leucine content or mass of the average amino acid residue in the capsid protein. Thus an error in the value of 0.083 mol% used for the leucine content of the virion capsid or in the value of 111 for the mass of the average amino acid residue would change the apparent number of leucine residues per chain but not the relative chain stoichiometry.

The chain stoichiometry of the 14S precursor subunit and the 6S subunit was determined assuming the ϵ chain contained 30 leucine residues (Table 2, column 6). The ratio of all three chains in the 14S subunit fell within 2% of the equimolar values predicted by the protomer

TABLE 3. Distribution of [³H]leucine in the 13S and 5S subunits derived from the virion

Peptide	Fraction of leucine residues ^a	13S subunit		5S subunit	
		Recovered radioactivity (%) ^b	Molar ratio ^c	Recovered radioactivity (%) ^d	Molar ratio ^c
α	24/65	36.3 ± 0.7	0.98 ± 0.02	36.3 ± 0.8	0.98 ± 0.02
β	23/65	36.1 ± 0.5	1.02 ± 0.02	35.9 ± 0.8	1.01 ± 0.02
γ	18/65	27.6 ± 1.0	1.00 ± 0.04	27.8 ± 0.3	1.00 ± 0.01

^a From Table 2, last column, assuming a (β, γ, α) protomer contains 65 leucine residues.

^b The average values (± average error) of four determinations utilizing the purified subunit labeled with [³H]leucine. The 13S subunit was prepared by dissociating the purified virion as described in Fig. 8. The chains were separated on SDS-polyacrylamide gels (see Fig. 9).

^c The relative molar amount of each chain (n) was calculated from the relation $n = r/m$, where (r) is the fraction of radioactivity and (m) is the fraction of leucine residues in the chain (column 2). For example, the number of α chains was calculated to be $0.363 \div 24/65 = 0.98$.

^d The average values (± average error) of five determinations utilizing the purified subunit labeled with [³H]leucine. The 5S subunit was prepared by dissociating the 13S subunit as described in Fig. 8. The peptides were separated on SDS-polyacrylamide gels (see Fig. 9).

TABLE 4. Distribution of [³H]leucine in the 14S precursor and its 6S subunit

Peptide	Fraction of leucine residues ^a	14S subunit		6S subunit	
		Recovered radioactivity (%) ^b	Molar ratio ^c	Recovered radioactivity (%) ^d	Molar ratio ^c
ϵ	30/72	42.4 ± 0.6	1.02 ± 0.02	44.1 ± 1.0	1.06 ± 0.02
α	24/72	32.6 ± 0.6	0.98 ± 0.02	33.1 ± 0.5	0.99 ± 0.01
γ	18/72	25.0 ± 0.3	1.00 ± 0.01	22.8 ± 0.6	0.91 ± 0.03

^a From Table 2.

^b The average values (± average error) of three determinations utilizing the purified subunit labeled with [³H]leucine. The chains were separated on SDS-polyacrylamide gels (see Fig. 5).

^c Described in footnote c, Table 3. For example, the number of ϵ -chains was calculated to be $0.424 \div 30/72 = 1.02$.

^d The average value (± average error) of four determinations utilizing the purified subunit labeled with [³H]leucine. The chains were separated on SDS-polyacrylamide gels (see Fig. 7).

hypothesis (Table 4, column 4).

However, the peptide ratio of the 6S subunit, which had been generated by dissociating the 14S subunit with urea, deviated significantly from the equimolar values expected for an immature protomer (Table 4, column 6). Normalizing to the ϵ chain yielded a ratio of 1.00:0.93:0.86 for the ϵ , α , and γ chains, respectively. This discrepancy could be accounted for by partial degradation of about 15% of the immature protomers. Such degradation would not be surprising in view of the relatively harsh urea treatment required to dissociate the 14S precursor into the 6S subunit (see above).

DISCUSSION

The results reported here reveal a satisfying internal consistency between the chain ratios predicted by the protomer hypothesis and those actually found for the virion and its four related subunits. Moreover, the finding of exact (i.e., within a few percent) equimolar stoichiometry in the 14S precursor subunit, using the leucine compositions calculated for each chain in the virion, justifies a previously unstated, but fundamental, assumption. That is, the 14S precursor subunit loses little, if any, mass (leucine

residues) during or after the maturation cleavage to form the virion.

In fact, leucine might have been lost during maturation in at least two different ways. One, suggested by the result in Table 2, is by elimination of a small, undetected, leucine-rich fragment from the ϵ chain when it is cleaved to form the β and δ chains. Another way is by loss of chains, e.g., delta chains, from the capsid during maturation or purification of the virions.

In either case, the apparent leucine content of the ϵ chain, calculated by summing the number of leucine residues in the β and δ chains, would have been low. This would have resulted in a high estimate for the relative number of ϵ chains in the immature protomer. The fact that this did not occur allows us to make two conclusions: (i) the leucine content of the β and δ chains is a good measure of the leucine content of the ϵ chain; and (ii) the purified EMC virion preparation did contain the assumed full complement of each chain. The latter is reinforced by a similar conclusion drawn previously in studies with ME virus (31).

Our current view of the early stages of capsid assembly, and the structural relationship of the subunits involved, are summarized in Fig. 10.

FIG. 10. Model for the assembly and dissociation of the EMC virion. The capsid precursor protein, A, is assembled into 13S subunits, which are thought to be pentameric oligomers. Cleavage of each A chain at two specific processing sites then follows. The 8% increase in sedimentation velocity attending this processing step suggests a conformational change preceding assembly of 14S subunits into the 60-protomer capsid. The 14S precursor could be dissociated with 1.5 M urea into a 6S subunit containing approximately equimolar proportions of ϵ , γ , and α chains and fits the description of an immature protomer. EMC virions can be dissociated into 13S and 5S subunits indistinguishable in sedimentation velocity and chain stoichiometry from those of ME virus. Hence, the dissociation pathway appears to follow that proposed previously for the serologically related ME virus (7).

Also shown are the subunits which are derived by dissociation of the virion. The assignment of a pentameric structure to the 13S and 14S precursor subunits is still tentative and is based on measurements of sedimentation position. These indicate that the 14S precursor sediments 8% faster (Fig. 1b) than the $(\beta, \gamma, \alpha)_5$ subunit, which has a known mass of about 425,000 daltons (7). The molecular weights of particles of similar shape and density are related to their sedimentation velocities by the relationship $s_1/s_2 = (M_1/M_2)^{2/3}$ or, in rearranged form, $M_2 = M_1(s_1/s_2)^{-3/2}$. Assuming the gradient is approximately isokinetic for the two particles, the mass of the 14S precursor subunit is calculated to be 480,000 daltons. This is exactly the mass expected for a pentamer of 96,000-dalton protomers.

However, a similar calculation yields a mass of only 425,000 daltons for the 13S, A-containing precursor. Since cleavage processing is unlikely to increase the mass or density of the 13S subunit, the 8% increase in the sedimentation velocity accompanying its maturation evidently reflects a decrease in the hydrodynamic radius. This in turn implies a substantial conformational change. In view of the surface changes implied by marked differences between the antigenic determinants of the 14S, 73S, and virion of poliovirus (reviewed in reference 29), the occurrence of such conformational changes is not unprecedented. Indeed, it now seems likely that substantial rearrangements of surface structure may accompany each step in picornavirus assembly.

A monomeric form of the capsid precursor, polypeptide A, which is known to be a primary gene product in infected cells, would be expected to sediment at 5S to 6S (30). However, a search for such a form, or for its cleaved derivative, $(\epsilon, \gamma, \alpha)$, was negative. It is possible that the monomeric A chain, which is thought to be synthesized on the rough endoplasmic reticulum, is assembled on the membrane before its release in the soluble 13S form or has a rate of assembly which is rapid enough to preclude appreciable accumulation of the monomeric form. Absence of the $(\epsilon, \gamma, \alpha)$ monomer suggests that polymerization of the A chains may be prerequisite for chain cleavage.

It has been suggested that each picornavirus protomer requires two different types of inter-subunit bonding regions to accomplish capsid assembly (30). Ordering of a two-step bonding sequence might be controlled by a delay in formation of the stereospecific, complementary surface configuration required for the second binding step until the product of the first step is

complete. We suggest that the conformational shift accompanying the cleavage of the 13S subunit may represent just such an unmasking of a second bonding site, and that the double cleavage of the A chain may be required to allow sufficient configurational change to accomplish this unmasking.

ACKNOWLEDGMENTS

This research was supported by grant VC 26C from the American Cancer Society. R.R.R. holds a Faculty Research award (PRA-106) from the American Cancer Society.

LITERATURE CITED

1. Britten, R. J., and R. B. Roberts. 1960. High resolution density gradient sedimentation analysis. *Science* **131**:32-33.
2. Burness, A. T. H., S. M. Fox, and I. V. Pardoe. 1974. The polypeptide composition of the encephalomyocarditis virus particle. *J. Gen. Virol.* **23**:225-236.
3. Butterworth, B. E., L. Hall, C. M. Stoltzfus, and R. R. Rueckert. 1971. Virus-specific proteins synthesized in encephalomyocarditis virus infected HeLa cells. *Proc. Natl. Acad. Sci. U.S.A.* **68**:3083-3087.
4. Butterworth, B. E., and R. R. Rueckert. 1972. Kinetics of synthesis and cleavage of encephalomyocarditis virus specific proteins. *Virology* **50**:535-549.
5. Caliguiri, L. A., and I. Tamm. 1970. The role of cytoplasmic membranes in poliovirus biosynthesis. *Virology* **42**:100-111.
6. Dulbecco, R., and M. Vogt. 1954. Plaque formation and isolation of pure lines with poliomyelitis viruses. *J. Exp. Med.* **99**:167-182.
7. Dunker, A. K., and R. R. Rueckert. 1971. Fragments generated by pH dissociation of ME virus and their relation to the structure of the virion. *J. Mol. Biol.* **58**:217-235.
8. Dunker, A. K., and R. R. Rueckert. 1969. Observations on molecular weight determinations on polyacrylamide gel. *J. Biol. Chem.* **244**:5074-5080.
9. Faulkner, P., E. M. Martin, S. Sved, R. C. Valentine, and T. S. Work. 1961. Studies on protein and nucleic acid metabolism in virus-infected mammalian cells. 2. The isolation, crystallization and chemical characterization of mouse encephalomyocarditis virus. *Biochem. J.* **80**:597-605.
10. Fish, W. W., K. G. Mann, and C. Tanford. 1969. The estimation of polypeptide chain molecular weights by gel filtration in 6 M guanidine hydrochloride. *J. Biol. Chem.* **244**:4989-4994.
11. Gilson, W., R. Gilson, and R. R. Rueckert. 1972. An automatic high-precision acrylamide gel fractionator. *Anal. Biochem.* **47**:321-328.
12. Hall, L., and R. R. Rueckert. 1971. Infection of mouse fibroblasts by cardioviruses: premature uncoating and its prevention by elevated pH and magnesium chloride. *Virology* **43**:152-165.
13. Jacobson, M. F., J. Asso, and D. Baltimore. 1970. Further evidence on the formation of poliovirus proteins. *J. Mol. Biol.* **49**:657-669.
14. Jacobson, M. F., and D. Baltimore. 1968. Morphogenesis of poliovirus. I. Association of the viral RNA with coat protein. *J. Mol. Biol.* **33**:369-378.
15. Kerr, I. M., E. M. Martin, M. G. Hamilton, and T. S. Work. 1965. Studies on protein and nucleic acid metabolism in virus-infected mammalian cells. The formation of a virus-specific antigen in Krebs II ascites-tumour cells infected with encephalomyocarditis virus. *Biochem. J.* **94**:337-344.
16. Maizel, J. V., Jr. 1966. Acrylamide-gel electrophorograms

- by mechanical fractionation: radioactive adenovirus proteins. *Science* **151**:988-990.
17. Lucas-Lenard, J. 1974. Cleavage of mengovirus polypeptides in vivo. *J. Virol.* **14**:261-269.
18. Mak, T. W., J. S. Colter, and D. G. Scraba. 1974. Structure of the mengo virion. II. Physicochemical and electron microscopic analysis of degraded virus. *Virology* **57**:543-553.
19. Mak, T. W., D. J. O'Callaghan, C. M. Kay, and J. S. Colter. 1971. Studies of the protein subunit of pH inactivated Mingo virus variants. I. Physicochemical properties. *Virology* **43**:579-587.
20. Medappa, K. C., C. McLean, and R. R. Rueckert. 1971. On the structure of rhinovirus 1A. *Virology* **44**:259-270.
21. Monod, J., J. Wyman, and J. Changeux. 1965. On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* **12**:88-118.
22. Paucha, E., J. Seehafer, and J. S. Colter. 1974. Synthesis of viral-specific polypeptides in mengovirus infected L cells: evidence for asymmetric translation of the viral genome. *Virology* **61**:315-326.
23. Penman, S., Y. Becker, and J. E. Darnell. 1964. A cytoplasmic structure involved in the synthesis and assembly of poliovirus components. *J. Mol. Biol.* **8**:541-555.
24. Perlin, M., and B. A. Phillips. 1973. *In vitro* assembly of polioviruses. III. Assembly of 14S particles into empty capsids by poliovirus-infected HeLa cell membranes. *Virology* **53**:107-114.
25. Phillips, B. A. 1969. *In vitro* assembly of polioviruses. I. Kinetics of the assembly of empty capsids and the role of extracts from infected cells. *Virology* **39**:811-821.
26. Phillips, B. A., and R. Fennell. 1973. Polypeptide composition of poliovirions, naturally occurring empty capsids, and 14S precursor particles. *J. Virol.* **12**:291-299.
27. Phillips, B. A., D. F. Summers, and J. V. Maizel, Jr. 1968. *In vitro* assembly of poliovirus-related particles. *Virology* **35**:216-226.
28. Reynolds, J. A., and C. Tanford. 1970. The gross conformation of protein dodecyl sulfate complexes. *J. Biol. Chem.* **245**:5161-5165.
29. Rueckert, R. R. 1971. Picornaviral architecture, p. 255-306. In K. Maramorosch and E. Kurstak (ed.), *Comparative virology*. Academic Press Inc., New York.
30. Rueckert, R. R., A. K. Dunker, and C. M. Stoltzfus. 1969. The structure of mouse-Elberfeld virus: a model. *Proc. Natl. Acad. Sci. U.S.A.* **62**:912-919.
31. Stoltzfus, C. M., and R. R. Rueckert. 1972. Capsid polypeptides of mouse Elberfeld virus. I. Amino acid compositions and molar ratios in the virion. *J. Virol.* **10**:347-355.
32. Summers, D. F., J. V. Maizel, Jr., and J. E. Darnell, Jr. 1965. Evidence for virus-specific noncapsid proteins in poliovirus infected HeLa cells. *Proc. Natl. Acad. Sci. U.S.A.* **54**:505-513.
33. Weber, K., and M. Osborne. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **215**:4406-4412.