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Capsid Polypeptides of Mouse Elberfeld Virus

I. Amino Acid Compositions and Molar Ratios in the Virion

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The four major polypeptide chains (alpha, beta, gamma, delta) constituting the capsid protein of mouse Elberfeld (ME) virus were isolated by preparative electrophoresis on polyacrylamide gels, and the amino acid composition of each chain was determined. In addition, the molecular weights of the smallest chains of ME virus, mengovirus, and poliovirus, which had previously been determined by gel electrophoretic methods, were redetermined by gel filtration chromatography in 6 M guanidine hydrochloride. Each was found to have a molecular weight about 7,300. Using the reevaluated molecular weights and the known amino acid compositions of the chains, the molar ratio of each chain in the ME virion was determined by quantitative analysis of the distribution of radioactivity in the electrophoretically separated chains of virus which had been specifically radiolabeled with leucine or with methionine. Equimolar proportions of all four chains were found in the virion.

The capsid of mouse Elberfeld (ME) virus, a murine picornavirus, is thought to be arranged of 60 symmetrically organized subunits (7, 15). Each subunit appears to be processed during viral morphogenesis by three specific cleavages operating on a single large precursor chain A having a molecular weight of about 100,000 (2, 14, 15). According to this hypothesis, the virion should contain all four chains in equimolar proportions. However, preliminary attempts to measure chain stoichiometry in this virus indicated that mature virions may contain only half the expected number of delta chains (24).

Selective loss of delta chains from the virion might conceivably have theoretical significance. For example, loss from the virion of a specific number of these chains might reflect previously unrecognized details of the viral maturation process, e.g., rules governing encapsulation of the asymmetric ribonucleic acid (RNA) strand by a symmetrical capsid or rearrangement of capsid elements such as translocation of delta chains from the protomer to new sites in the virion (7). Hence we sought to establish whether the low delta chain content of the virion was real and, if so, how many chains were lost. The results presented here indicate that no delta chains are lost from the virion. Rather, the earlier discrepancy is attributable to imperfections in the assumptions required to estimate the stoichiometry of the chains.

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MATERIALS AND METHODS

Preparative isolation of capsid polypeptides: procedure I. Capsid proteins derivatized with mercury orange were electrophoretically separated on sodium dodecyl sulfate (SDS)-containing polyacrylamide gels. Mercury orange (1-[4-chloromercuriphenylazo]-naphthol-2) is a colored mercurial which forms covalent linkages with free thiol groups in proteins (26). The colored bands were eluted from the crushed gel segments with water and precipitated with acidic acetone which cleaves the mercury mercaptide bond and regenerates the underivatized proteins (26). Precipitation at low *p*H also prevents precipitation of SDS by the acetone (20).

In a typical preparation, 1 mg of ^{3}H-leucine-labeled ME virus (50,000 counts/min) in 90 μliters of 0.25 M NaCl, 0.005 M tris(hydroxymethyl)aminomethane (Tris)-acetate, *p*H 7.5, mixed with 30 μliters of 10% SDS, 3 μliters of mercaptoethanol, 70 μliters of 0.1 M sodium phosphate, *p*H 7.2, and 108 mg of urea. The virus was dissociated by incubation for 30 min at 45 C. After adding 25 μliters of 1% mercury orange in tetramethyl urea, the suspension was incubated for 40 min at room temperature. The suspension was applied directly to the gels without removing the excess mercury orange which precipitated from solution.

For electrophoresis, 50-μliter samples were applied to each of six SDS-containing polyacrylamide gels (0.6 by 7 cm). The gels were polymerized in 0.1 M sodium phosphate, *p*H 7.2, containing 9.8% (w/w) acrylamide, 0.2% (w/w) methylene-bisacrylamide, 0.1% SDS, 0.1% (v/v) *N,N,N',N'*-tetramethylethylenediamine, and 0.05% ammonium persulfate. Electrophoresis was for 5 hr at 8 ma per tube using 0.1% SDS in 0.1 M sodium phosphate, *p*H 7.2, as electrode buffer. The alpha, beta, and gamma chains, which

were visible as red bands, were excised with a razor blade. The delta chain which lacks cysteine (see Table 6) was not stained but, since it is well isolated from the other chains, could be recovered from radioactivity-bearing gel segments near the electrophoretic front. Pooled gel segments were crushed by forcing them through a 100-mesh stainless steel screen (Small Parts Inc., Miami, Fla.) fitted in the base of a disposable 2.5-ml syringe, and protein was extracted from the gel particles by soaking overnight at 37 C with about 2.5 ml of 0.1% SDS, 0.1% mercaptoethanol, and 0.01 M sodium phosphate, *p*H 7.2, per batch of six segments. The solvent was removed and a second extraction was carried out for an additional 5 hr. The pooled extracts were mixed with 5 volumes of acetone containing 0.1 M hydrochloric acid, and the protein which precipitated after standing overnight at room temperature was sedimented by low-speed centrifugation and was dissolved in 1 ml of 90% formic acid. This solution was stored frozen at −70 C. About 60 to 70% of the ^{3}H-leucine applied to the gels was recovered in the alpha, beta, and gamma chains; since these three chains carry about 90% of the capsid leucine (see Table 4), this represents 65 to 75% of theoretical recovery. The delta chain which failed to precipitate was not recovered by this procedure; it was isolated by procedure 2.

Procedure 2. Ten milligrams of ME virus containing tracer amounts of ^{3}H-leucine-labeled virus (about 100,000 counts/min) was thermally disrupted by heating for 20 min at 37 C in 5 ml of 0.1 M sodium chloride–0.005 M sodium citrate, *p*H 5.7, and the resulting precipitate (I-protein) which is greatly enriched in delta chains (7) was recovered by low-speed centrifugation. The precipitate was dissolved in 200 μliters of 1% SDS, 6 M urea, and 0.2% mercaptoethanol, and was incubated at 45 C for 20 min. Samples (100 μliters) were applied to 10% polyacrylamide gels (10-mm internal diameter by 8-cm long) containing 0.1% dodecyl sulfate in 0.1 M sodium phosphate, *p*H 7.2, as described above, and were subjected to electrophoresis 5.5 hr at 23 ma/tube (40 v). The delta chain was well separated from other polypeptide chains under these conditions. The gel was crushed by forcing it with a piston from the electrophoresis tube through a 100-mesh stainless-steel wire screen, and fractions representing 5-mm segments of gel were extracted overnight at 37 C each in 1 ml of 0.1 M sodium phosphate, *p*H 7.2, containing 0.1% SDS and 0.1% mercaptoethanol. Fractions containing the delta chain, detected by counting portions for radioactivity, were pooled and the gel particles were removed by filtration. The protein was precipitated with 10% trichloroacetic acid (2 hr at 37 C). The precipitate was recovered by low-speed centrifugation, washed once with ether-ethanol (1:1) and once with ether. The dry precipitate was dissolved in 0.5 ml of 90% formic acid and stored frozen at −70 C. About 65 μg of delta chain, 15% of the theoretical yield based on recovery of ^{3}H-leucine label, was recovered by this procedure.

The low recovery of delta chain was due mainly to incomplete dissociation of the acid precipitate by the solubilization procedure described here. Studies carried out after completion of this work indicate that the precipitate would have been more effectively dissociated by heating for 1 min at 100 C in 1% SDS and 1% mercaptoethanol prior to electrophoresis.

Procedure 3. The alpha and gamma chains of ME virus could also be isolated in pure form by differential precipitation of the reduced carboxymethylated protein. In a typical experiment, 5 mg of ME virus was dissociated and reduced by incubating for 30 min at 37 C in 2.5 ml of 5 M guanidine hydrochloride, 0.037 M mercaptoethanol, 0.5 M Tris neutralized to *p*H 8.6 with HCl. The protein was alkylated by incubation for 10 min at 37 C in the dark with 103 mg of iodoacetamide. After adding 0.1 ml of mercaptoethanol to destroy excess iodoacetamide, the solution was diluted twofold with distilled water, and the protein was extracted into 5.3 ml of redistilled 80% phenol by gently flushing in and out of a pipette at 45 C. After 5 min, the emulsion was chilled in ice water, then centrifuged for 5 min at full speed in an International clinical centrifuge. The upper phenol phase was recovered, the lower phase was reextracted with 1 ml of fresh 80% phenol, and the two phenol phases, containing the protein, were combined.

The alpha and beta chains were precipitated from the phenol with 6 volumes of 0.1 M potassium acetate in 95% ethanol. After 1 hr at room temperature, the precipitate (precipitate A) was recovered by low-speed centrifugation. The gamma chain which remains in solution under these conditions was precipitated by adding glacial acid to a final concentration of 3% and by storing overnight at 6 C. The flocculent precipitate, which consisted of electrophoretically pure gamma chain, was recovered by centrifugation, dissolved in 67% acetic acid and dialyzed against 1,000 volumes of distilled water to produce a water clear solution.

To recover alpha chain, precipitate A was dissolved in 8 M urea and dialyzed against 10% acetic acid, whereupon another precipitate formed. This precipitate, which consisted mainly of beta chain contaminated with smaller amounts of alpha chain, was removed by centrifugation. The supernatant fluid contained electrophoretically pure alpha chain.

Amino acid analysis. Formic acid or acetic acid was removed by drying in vacuo. The protein residue was hydrolyzed in 1 ml of redistilled, constantly boiling HCl for 24 or 72 hr at 108 C in sealed, evacuated tubes (18). Acid was removed by rotary evaporation with the tube immersed in a 45 C water bath. The residue was redissolved in *p*H 2.2 sodium citrate buffer and was stored frozen until it was analyzed.

The alpha, beta, and gamma chains, isolated by procedure 1, were each hydrolyzed for 24 and 72 hr. The delta chain, which was isolated by procedure 2, and the alpha and gamma chains, which were also isolated by procedure 3, were hydrolyzed for 24 hr. Duplicate amino acid analyses were carried out on each hydrolysate using a Beckman model 120C amino acid analyzer equipped with a scale-expanding device which increased the sensitivity of the recorder 10-fold. The values for serine and threonine were calculated from the composition of 24-hr hydrolysates assuming losses of 10 and 5%, respectively, during hydrolysis. The tryptophan content of the gamma chain, calcu-

lated from its ultraviolet absorption spectrum, was measured with a Cary model 15 recording spectrophotometer, by the procedure of Goodwin and Morton (11).

Molecular-weight determination of virus polypeptides on agarose gels containing 6 M guanidine hydrochloride. Ovalbumin (molecular weight 43,000), horse heart cytochrome *c* (molecular weight 12,300), and zinc insulin (molecular weight 5,740) were reduced and alkylated with iodoacetamide as described by Fish, Mann, and Tanford (8). ^{3}H-labeled ME virus (about 10^5 counts/min) in 100 μliters of 0.25 M NaCl, 0.005 M Tris-acetate, *p*H 7.5, and 0.01% bovine serum albumin was dissociated by addition to 20 μliters of 1 M Tris-hydrochloride buffer, *p*H 9, 1 μliter of mercaptoethanol, and 100 mg of guanidine hydrochloride, and was reduced by incubating for 2 hr at 37 C. The protein was alkylated by incubating for 10 min at 37 C with 9.6 mg of iodoacetamide. Unreacted iodoacetamide was destroyed by addition of 5 μliters of mercaptoethanol. The sample, about 0.17 ml, was mixed with 0.1 ml of protein standard solution and 25 mg of sucrose and was applied to the top of a 0.9 by 52 cm column of 6% agarose beads (Biogel A-5M, Bio-Rad Laboratories) packed in a K9/60 acrylic column (Pharmacia Fine Chemicals Co.). Fractions (about 0.7 g each) were collected at a flow rate of 1.2 ml/hr which was generated by a pressure head of about 40 cm of solvent. Virus was detected by radioactivity, and the standard markers were detected spectrophotometrically and turbidimetrically (8). Radioactivity was determined (17) by analysis of 100-μliter portions of each fraction for 5% trichloroacetic acid-insoluble radioactivity on Whatman 3MM filter-paper discs in 5 ml of scintillation fluid I. The elution weight (V_e) in grams was determined by weighing the test tubes before and after collection of eluted solvent (8).

Radioactivity and measurements. Polyacrylamide gels were fractionated on an automatic unit (10), hydrolyzed with alkali, and counted in scintillation solvent tT21 (19) as described by Butterworth et al. (2). Radioactivity was measured at appropriate channel settings, on the Packard Tri-Carb model 3375 liquid scintillation counter at 6 C. Scintillation solvent I contained 0.5% 2,5-diphenyloxazole and 0.01% 1,4-bis-2-(4-methyl-5-phenoxyazoyl)-benzene in toluene.

Materials. Materials were obtained from the following sources: L-leucine-*4,5-*^{3}H (58.1 Ci/mmole), L-^{14}C-leucine (316 mCi/mmole) and L-^{35}S-cysteine (28.5 mCi/mmole) were from Schwarz BioResearch Inc.; L-methionine-*methyl-*^{14}C (60 mCi/mmole) was from Amersham-Searle Corp.; cytochrome *c* was from Sigma Chemical Co.; ovalbumin was from Schwarz/Mann; insulin was from United States Reference Standards, New York; and lysozyme was from Worthington Biochemical Co. Ethylene diacrylate was purchased from the Borden Chemical Co., Philadelphia, Pa. and mercury orange from Dajac Laboratories, Borden, Inc., Philadelphia, Pa. Tetramethyl urea was obtained from Matheson, Coleman, and Bell, East Rutherford, N.J.

Abbreviations. Abbreviations for amino acids are those recommended by the IUPAC-IUB Combined Commission on Biochemical Nomenclature (13).

RESULTS

Amino acid composition. The amino acid composition of each of the four major electrophoretic components isolated from ME virus is summarized in Table 1. As a control on the procedures used for isolation and analysis of the viral polypeptide chains, a protein of known amino acid composition, lysozyme, was isolated by the same preparative gel electrophoresis as that used to isolate the alpha, beta, and gamma chains. Except for methionine and glutamate, the experimental values obtained with lysozyme agree within 9% or better with the expected values (Table 1, columns 1 and 2). The slightly low value for methionine is tentatively attributed to oxidative losses which occur if the acid hydrolysis tube is imperfectly evacuated (4, 18). The significance of the high (about 16%) glutamate value is unclear.

Each viral component differed significantly in amino acid composition from each of the others (Table 1). The largest differences were in proline, methionine, isoleucine, tyrosine, phenylalanine, histidine, and arginine. The delta chain, which contained only traces of histidine, was unusually rich in (aspartate plus aspargine) residues and in serine residues but was relatively poor in threonine, lysine, and arginine residues.

Except for cysteine, which was too scarce to be detected by the amino acid analyzer (but see Table 3), methionine and histidine were the least frequent amino acids. Minimum molecular weights calculated from the content of these amino acids in each chain were: alpha, 11,400 (Met); beta, 7,400 (Met); gamma, 9,300 (His) or 5,000 (Met); and delta, 10,400 (Met). The molecular weights of the major polypeptides in ME virus, calculated from their electrophoretic mobilities on dodecyl sulfate-containing polyacrylamide gels, have been reported (6) to be: alpha, 33,000; beta, 30,500; gamma, 25,800, and delta, 10,000. From these values and the minimum molecular-weight values cited above, one calculates methionine contents of 2.9 residues per alpha chain, 4.1 per beta chain, 5.2 per gamma chain, and 1.0 per delta chain.

Molecular weight of the capsid chains. Molecular weights based upon the gel electrophoretic method depend upon the empirical observation that the electrophoretic mobility of reduced and denatured proteins in the presence of dodecyl sulfate is inversely proportional to the logarithm of their molecular weights (28). Apparent molecular weights obtained by this method generally fall within 10% of theory for proteins larger than about 10,000 to 15,000 (6, 29). However, the

TABLE 1. *Amino acid compositions of the polypeptide chains of mouse Elberfeld virus*[a]

Amino acid residue	Lysozyme (4 determinations)		Alpha (6 determinations)		Beta (4 determinations)		Gamma (6 determinations)		Delta (2 determinations)	
	Moles per 100 moles of amino acid		Moles per 100 moles of amino acid	μMoles per milligram	Moles per 100 moles of amino acid	μMoles per milligram	Moles per 100 moles of amino acid	μMoles per milligram	Moles per 100 moles of amino acid	μMoles per milligram
	Found	Theory								
	(1)[b]	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Asx	16.2	16.3	9.15	0.861	10.1	0.925	7.51	0.719	19.6	1.87
Thr	5.83	5.43	7.68	0.722	9.66	0.886	10.06	0.963	4.19	0.399
Ser	8.03	7.75	9.85	0.927	8.06	0.739	10.08	0.965	16.6	1.58
Glx	4.52	3.88	9.73	0.915	10.1	0.925	7.07	0.677	8.45	0.804
Pro	1.61	1.55	7.04	0.662	5.07	0.465	8.30	0.795	4.33	0.412
Gly	9.70	9.30	10.7	1.01	8.86	0.813	9.23	0.884	9.04	0.861
Ala	9.50	9.30	6.03	0.567	7.72	0.708	8.84	0.847	7.55	0.719
Val	4.66	4.65	6.86	0.645	6.94	0.637	5.49	0.526	2.94	0.280
Met	1.22	1.55	0.92	0.087	1.46	0.134	2.10	0.201	.987	0.094
Ile	4.26	4.65	2.94	0.277	3.52	0.323	5.22	0.500	3.68	0.350
Leu	6.53	6.20	6.94	0.653	7.79	0.715	6.70	0.642	8.67	0.825
Tyr	2.10	2.33	2.77	0.261	2.93	0.269	4.21	0.403	3.12	0.297
Phe	2.38	2.33	5.63	0.530	3.92	0.360	4.38	0.419	4.24	0.404
Lys	4.53	4.65	4.71	0.443	3.48	0.319	4.01	0.384	2.16	0.206
His	0.83	0.78	1.75	0.165	2.88	0.264	1.12	0.107	.336	0.032
Arg	8.75	8.53	3.66	0.344	4.83	0.443	2.89	0.277	1.99	0.189
Trp		4.65					1.59	0.152		

[a] Lysozyme was isolated by preparative gel electrophoresis as described in Materials and Methods (method I). The theoretical amino acid composition was taken from Canfield (3). The weight of protein analyzed was calculated from the sum of the weights of the recovered amino acid residues (after correcting for amino acid losses as described in Materials and Methods). The alpha, beta, and delta chains were assumed to contain 2 moles % tryptophan. The cysteine content of each chain was assumed to be 1.59, 0.72, 1.19, and 0 mole percent for the alpha, beta, gamma, and delta chains, respectively (see Table 4). Glutamine and asparagine are converted during acid hydrolysis of the protein to glutamic and aspartic acids respectively; hence Asx signifies Asp plus Asn and Glx signifies Glu plus Gln.

[b] Numbers in parentheses indicate column numbers.

reliability of the method for smaller proteins has been less well documented and has in fact been questioned on the grounds that the relatively constant size-to-length ratio of reduced denatured polypeptide–SDS complexes becomes erratic for polypeptide chains smaller than about 15,000, i.e., the Stoke's radii of these complexes no longer vary in a predictable manner with molecular weight at sizes below this limit (9, 21). Fortunately the Stoke's radii of reduced polypeptides do vary predictably down to a size of about 1,000 daltons in aqueous solutions of 6 M guanidine hydrochloride. Thus there are important theoretical grounds for believing that the size of small polypeptides is more reliably measured by gel filtration chromatography in 6 M guanidine hydrochloride than by gel electrophoresis in SDS solution. The gel filtration procedure is reported to measure the size of such small polypeptides with an accuracy of 7 to 10% (5, 8).

The chromatographic elution profile of the capsid protein of disrupted, reduced, alkylated ME virus in 6 M guanidine hydrochloride on a column of 6% agarose revealed three radioactive peaks (Fig. 1A): peak I, a small peak which elutes with blue dextran in the void volume; peak II, a large broad peak assumed to contain unresolved alpha, beta, and gamma chains; and peak III, assumed to represent the delta component. Two other picornaviruses, mengovirus and type 1 poliovirus (Mahoney strain), were also examined and yielded profiles very similar to that of ME virus (Table 2).

The nature of the material eluting in peak I, which contained only 3 to 4% of the radioactivity (Table 2) was not further identified because of the limited amount of label it contained; it probably represents aggregated protein possibly still associated with the viral RNA. In this regard, peak III from ME virus contained only 6.0% of the recovered label which is significantly less than the 9.3% of leucine label recovered in the delta

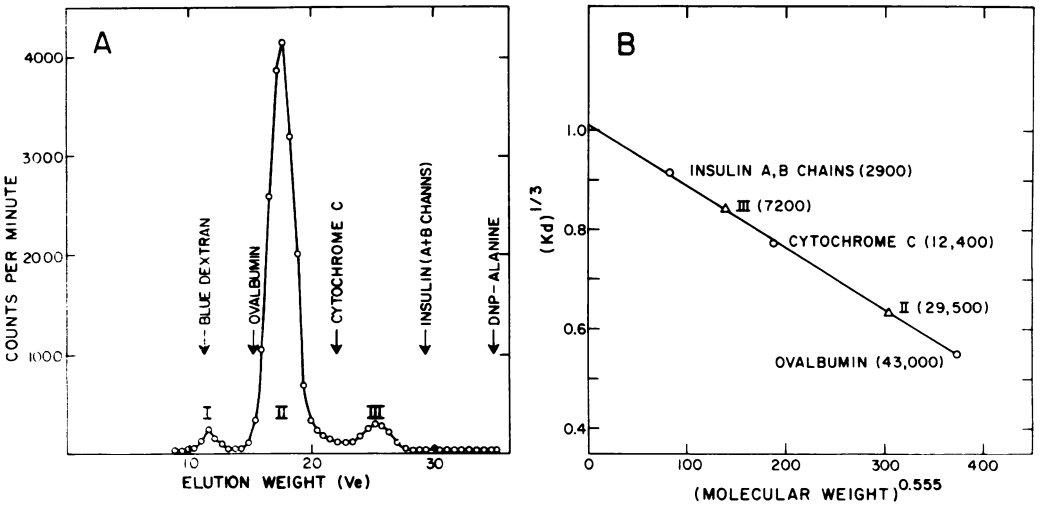

FIG. 1. *Gel filtration profile of reduced and alkylated mouse Elberfeld (ME) virus protein on an agarose column containing 6 M guanidine hydrochloride. Reduced alkylated ^{3}H-leucine labeled ME-virus (10^5 counts/min) was co-chromatographed with the indicated reduced alkylated proteins, blue dextran and DNP-alanine. These markers were used to calibrate the column as described in Materials and Methods. A, Elution profile of ME-viral protein. B, Plot of partition coefficient $(K_d)^{1/3}$ versus (molecular weight)$^{0.555}$. According to Fish et al. (8) this plot best approximates a linear function between partition coefficient and molecular weight of a protein in 6 M guanidine hydrochloride.*

TABLE 2. *Apparent molecular weights of the capsid protein from several picornaviruses by gel filtration chromatography in 6 M guanidine hydrochloride*[a]

Virus	Peak I	Peak II			Peak III		
	Counts/min recovered (%)	Counts/min recovered (%)	K_d[b]	Apparent mol wt	Counts/min recovered (%)	K_d	Apparent mol wt
	(1)[c]	(2)	(3)	(4)	(5)	(6)	(7)
ME virus	2.7	91.4	0.258	29,500	6.0	0.593	7,200
Mengovirus	3.7	88.0	0.262	29,000	8.4	0.592	7,200
Poliovirus	4.1	92.8	0.268	28,500	3.1	0.582	7,600

[a] Recoveries were based on total label recovered in peaks I, II and III. Apparent molecular weights were determined from plots similar to that shown in Fig. 1B.

[b] K_d, Partition coefficient of polypeptide.

[c] Numbers in parentheses indicate column numbers.

chain by quantitative gel electrophoresis (see Table 4). This suggests that some delta chain may have been lost during the guanidine chromatographic procedure, possibly in peak I.

According to Fish et al. (8), a linear relationship between molecular weight (MW) of a polypeptide and its partition coefficient (K_d) on a 6 M guanidine hydrochloride column is best approximated by plotting $MW^{0.555}$ versus $(K_d)^{1/3}$. The data calculated from the chromatographic profiles of these three viruses are summarized in Table 2. The apparent molecular weight of peak II, calculated from plots similar to that shown in Fig. 1B, was about the same for all three viruses and averaged 29,000 (Table 2, column 4). The size of peak III was also about the same for all three viruses and averaged about 7,300 in molecular weight (Table 2, column 7). Since the virus samples were run separately on the same column, the small differences in apparent molecular weights between homologous peaks of ME virus, mengovirus, and poliovirus are probably not significant.

The apparent molecular weight of peak II on guanidine hydrochloride columns is in excellent agreement with the average molecular weight

calculated for the three ME viral chains (alpha, beta, gamma) from the electrophoretically determined values ([33,000 + 30,500 + 25,800]/3 = 29,800; reference 6) and for the three homologous polioviral chains ([35,000 + 28,000 + 24,000]/3 = 29,000; reference 16).

On the other hand, the size of peak III (7,300) differs significantly from the electrophoretically determined molecular weight values of 10,000 reported for the delta chain of ME virus (7) and of 5,000 to 6,000 reported for the VP4 chain of poliovirus (16). For reasons discussed at the introduction of this section, we suggest that the smallest capsid chains of ME virus and of poliovirus are similar, if not identical, in size and that the distinctly different electrophoretic mobilities of the smallest chain in these two viruses (see e.g. Fig. 1, panel C of reference 25) reflect differences in chemical composition rather than differences in size. In selecting a value of 7,300, we reject the minimum molecular weight value of 10,400 based on methionine content of the delta chain as unreliable because of the possibility that some of the methionine may have been destroyed during the acid hydrolysis step and because only two amino acid analyses, these on very small samples, were carried out.

We currently find, however, no reason to question the electrophoretically determined molecular weight values assigned earlier to the alpha, beta, and gamma chains of ME virus. Indeed, gel chromatographic studies on the individually isolated capsid chains of EMC virus, which is immunologically and electrophoretically similar to ME virus (see Fig. 1A of reference 25), are in excellent agreement with the electrophoretically determined assignments (S. McGregor, *personal communication*).

Molar proportion of polypeptides in the virion. Earlier attempts to measure the chain stoichiometry of ME virus (24) were carried out on the electrophoretically resolved chains of purified virus which had been labeled by growing infected cells in the presence of a radioactive amino acid mixture. This determination required the assumption that the amount of radioactivity in each peak is directly proportional to the mass of protein in that peak. Availability of the amino acid composition and size of each chain provides an alternative and theoretically sounder method for determining their molar proportions in the virion. In this procedure, the virus is grown in the presence of a single essential amino acid such as leucine which is specifically incorporated into viral protein without significant conversion to other amino acids in the virion (D. Omilianowski, *personal communication*). Given its leucine content, the relative proportion of each chain in the virion is readily determined from quantitative measurements on the distribution of leucine-specific label in each electrophoretic peak. The number of amino acid residues in each of the four chains was calculated as described in Table 3.

The distribution of leucine in the electrophoretically resolved chains of purified ^{14}C-leucine-labeled ME virus was as follows: alpha, 31.5%; beta, 32.1%; gamma, 26.1%; delta, 9.3%, and epsilon 1.2% (Table 4, column 1). Very similar results were obtained with ^{3}H-leucine-labeled virus (Table 4, column 2). The chain ratios computed from these data were, within experimental error, equimolar (Table 4, column 6). Assuming

TABLE 3. *Calculated amino acid residues per chain in mouse Elberfeld virus polypeptides*[a]

Amino acid residue	Alpha chain (mol wt 33,000) (1)[b]	Beta chain (mol wt 30,500) (2)	Gamma chain (mol wt 25,800) (3)	Delta chain (mol wt 7,300) (4)	Amino acid residues per protomer (5)[c]	(6)[d]
Asx	28	28	19	13	88	86
Thr	24	27	25	3	79	83
Ser	31	23	25	11	90	69
Glx	30	28	18	6	82	67
Pro	22	14	21	3	60	71
Gly	33	25	23	6	87	60
Ala	19	22	22	5	68	65
Cys[e]	5	2	3	0	10	9
Val	21	19	14	2	56	60
Met[e]	3	4	7	1	15	16
Ile	9	10	13	3	35	32
Leu	22	22	17	6	67	65
Tyr	9	8	10	2	29	35
Phe	18	11	11	3	43	46
Lys	15	10	10	1	36	32
His	6	8	3	0.2	17	16
Arg	11	14	7	1	33	30
Trp	ND[f]	ND	4	ND	ND	
Total	306	275	252	66	899	842

[a] Values were calculated from the amino acid composition of each chain (Table 1) using the electrophoretically determined (6) molecular weights of the alpha, beta, and gamma chains, and the chromatographically determined molecular weight for the delta chain (Table 2).

[b] Numbers in parentheses indicate column numbers.

[c] Calculated by summing "residues per chain" in alpha, beta, gamma, and delta.

[d] Calculated from data of Rueckert (22), assuming the protomer has a molecular weight of 96,000 (23).

[e] Calculated from the distribution of radioactivity in gel electropherograms of disrupted virus which had been labeled by growth in the presence of the indicated radiolabeled amino acid (see Tables 5, 6).

[f] ND, no data.

TABLE 4. *Relative chain ratios of polypeptides from mouse Elberfeld virus based on leucine composition*

Chain	Fraction of total Leu[a]		Leucine residues per chain[b]	Relative chain ratio[c]		
	^{14}C	^{3}H		^{14}C	^{3}H	Avg
	(1)[d]	(2)	(3)	(4)	(5)	(6)
Alpha	0.313	0.315	22	0.93	0.99	0.96
Beta	0.321	0.327	22	0.95	1.02	0.98
Gamma	0.261	0.246	17	1.00	1.00	1.00
Delta	0.093	0.094	6	1.01	1.07	1.04
Epsilon	0.012	0.018	28	0.028	0.044	0.036

[a] Results based on average of two determinations on virus labeled with ^{14}C-leucine and one determination on virus labeled with ^{3}H-leucine. Greater than 97% of the radioactivity applied to the polyacrylamide gel was recovered in the five virion chains using previously described procedures to fractionate and count the gels (2, 10).

[b] Calculated from amino acid compositions in Table 1 and assuming alpha, beta, and gamma weigh 33,000, 30,500, and 25,800, respectively (6), and the delta chain weighs 7,300 (Table 2). The result has been expressed to the nearest whole number. The number of leucine residues in epsilon was assumed to be the sum of the number of leucine residues in beta and delta.

[c] Obtained by dividing columns 1 or 2 by column 3 and normalizing with respect to the gamma chain.

[d] Numbers in parentheses indicate column numbers.

60 gamma chains per virion, one calculates from Table 4 an epsilon content of 2.2 chains/average virion.

A similar experiment using ^{14}C-methyl-labeled methionine indicated that the alpha, beta, gamma, and delta chains contain 3, 4, 7, and 1 methionine residue, respectively (Table 5, column 4). These values are in reasonable agreement with values of 2.9, 4.1, 5.1, and 0.7 computed from the acid hydrolysis data (Table 5, column 3).

Virus labeled with ^{35}S-cystine yielded values of 5, 2, 3, and 0 cysteine residues in the alpha, beta, gamma, and delta chains, respectively (Table 6). These values confirm an earlier report that the delta chain of ME virus lacks cysteine (24). Independent evidence corroborating this result was obtained by labeling purified ME virus by disulfide interchange with ^{35}S-*N,N'*-diacetyl cystamine (24; and *manuscript in preparation*). The almost integral values obtained between chains for these two amino acids provide further support of our conclusion that all four chains are present in approximately equimolar proportions.

The amino acid composition of the viral protomer (alpha, beta, gamma, delta) computed by summing the number of amino acid residues in all four chains is shown in Table 3, column 5. This is compared to the number of residues per protomer, i.e. per 96,000 daltons, calculated from a previously reported (22) analysis on total capsid protein of ME virus. The two results are in good agreement. Exceptions are serine, glycine, and glutamic acid which reveal discrepancies of the order of 25 to 30%. The reason for these discrepancies will have to be clarified by further investi-

TABLE 5. *Number of methionine residues per chain*

Chain	Fraction of counts/min[a]	Number of residues per chain			
		(1)[b]	(2)[c]	(3)[d]	(4)[e]
Alpha	0.202	3.2	3.0	2.9	3
Beta	0.259	4.1	3.9	4.1	4
Gamma	0.461	7.3	6.9	5.1	7
Delta	0.063	1.0	0.94	0.7	1
Epsilon	0.014	0.2	0.21	ND[f]	5 to 6

[a] Average of four determinations on virus grown in the presence of [methyl-^{14}C]methionine.

[b] Assuming 1 methionine residue per delta chain and equimolar chain ratios.

[c] Assuming 15 methionine residues per (alpha, beta, gamma, delta) protomer (from column 1).

[d] Based on amino acid analysis of acid hydrolysate (Table 1) and assuming delta has a molecular weight of 7,300.

[e] Probable number of residues per chain (by inspection of columns 1, 2, and 3).

[f] ND, no data.

TABLE 6. *Number of cysteine residues per chain*

Chain	Fraction of counts/min[a]	No. of residues per chain[b]
Alpha	0.496	5.0
Beta	0.202	2.0
Gamma	0.298	3.0
Delta	0.000	0
Epsilon	0.010	2.0

[a] Average of two determinations.

[b] Assuming 10 cysteine residues per (alpha, beta, gamma, delta) protomer.

gation. However, it may be significant that the glutamic acid content of lysozyme isolated by the preparative electrophoresis technique was also high (Table 1).

DISCUSSION

The evidence reported here further supports our earlier proposal that the capsid of ME virus is determined by 60 identical protein subunits (24). According to this model, each subunit, here designated an immature protomer (epsilon, alpha, gamma), is cleaved in the epsilon region during viral maturation to generate the mature four-chain subunit (delta, beta, gamma, alpha). The results reported here remove previous uncertainty about the fate of the delta chains and render unnecessary additional hypotheses involving obligatory removal of delta chains at some step in viral assembly or maturation. Hence we believe that the simplest interpretation of available evidence, taking into account the small amount of epsilon chain which always accompanies ME virions (6, 7, 24) is that the average capsid consists of 58 mature (delta, beta, gamma, alpha) and two immature subunits, each having a molecular weight of about 96,000.

Since the virus contains 31% RNA (25), the 60-protomer model predicts a molecular weight of 8.4×10^6 for the virion, a value which has been verified experimentally (1, 27) and 2.6×10^6 for the molecular weight of the RNA (7, 23). A genome of this size is equivalent to about 7,500 nucleotides. Hence, an equal number of positive charges is required to neutralize the electrostatic charge in the virion due to the ionized phosphate residues in the polynucleotide chain. Since each protomer contains 86 basic amino acid residues (36 Lys + 17 His + 33 Arg; see Table 3, column 5), a 60-subunit protein capsid could not neutralize more than (60×86) 5,160 charges which corresponds to only about two-thirds of the genome. This calculation, which is conservative because it ignores the anion contribution of the aspartate and glutamate side chains, suggests that at least one third of the genome must be neutralized by some other cationic species such as polyamines or metal ions.

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