STRUCTURE OF TYPE 5 ADENOVIRUS

II. Fine Structure of Virus Subunits. Morphologic Relationship of Structural Subunits to Virus-Specific Soluble Antigens from Infected Cells*

By WESLEY C. WILCOX,‡ PH.D., HAROLD S. GINSBERG, M.D., AND THOMAS F. ANDERSON, PH.D.

(From the Department of Microbiology, University of Pennsylvania, and the Institute for Cancer Research, Philadelphia)

Plates 26 to 30

Type 5 adenovirus was observed by Horne and coworkers to be an icosahedron with a capsid comprised of 252 roughly spherical subunits or capsomeres, each approximately 70 A in diameter (1). It was demonstrated that the structural subunits of the type 5 virus are antigenically similar to the group- (L) and type-specific (E) soluble antigens (2). Consequently, studies were initiated to investigate the relationship of the structural or antigenic subunits of the virus to the capsomeres or morphological subunits described by Horne *et al.* (1). It is the purpose of this communication to present evidence that purified type 5 adenovirus contains two demonstrable morphological subunits which are structurally and immunologically identical with the virus-specific soluble antigens (L and E) which accumulate in adenovirus-infected cells.

Materials and Methods

Preparation of Purified and Degraded Virus.—Highly purified (3 times CsCl-banded) type 5 adenovirus was prepared as described in the preceding paper (2). Purified virus was disrupted by dialyzing for 4 days against 0.1 M carbonate-bicarbonate buffer at pH 10.5. Prior to electron microscopy these preparations were dialyzed against 0.01 M phosphate buffer, pH 7.5, and assayed for infectivity and complement-fixing activity as described previously (3).

Preparation of Purified Soluble Antigens.—Soluble antigen preparations for electron microscopy were made in two ways: (a) Highly purified group-specific (L) antigen was prepared

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from infected cell homogenates by two cycles of chromatography on DEAE cellulose columns as described previously (3). A mixture of type-specific (E) antigen and toxin (E-T) was also obtained from DEAE columns. This latter material was not contaminated by L antigen or normal host cell materials. (b) The soluble antigen band, which develops with centrifugation of freon 113 treated virus in CsCl (2), was carefully collected and banded two additional times by centrifugation in a CsCl equilibrium density gradient. The final material obtained consisted of a mixture of E and L antigens and toxin. Prior to use, these preparations were dialyzed against 0.01 M phosphate buffer at pH 7.5 and the quantity of infectious virus, complement-fixing antigens, and toxin determined by appropriate titration procedures (3).

Assays.—Infectivity, toxin, and complement-fixation titrations were done as described previously (2).

TABLE I

Infectivity Titers and Antigenic Characteristics of Preparations Studied by Electron Microscopy

Description of sample	Depicted in Fig.	Infectious virus ID_{50}/ml	CF titers* vs.	
			Anti-E	Anti-L
		log		
Purified type 5 virus	1	9.5	2	64
Disrupted‡ type 5 virus	2	<1.0	4	64
Soluble antigen mixture§	3	<2.0	2048	2048
Purified L antigen‖	4	<1.0	32	16,384
Purified E-T antigens¶	5	<1.0	8192	<10

* Expressed as reciprocal of the highest antigen dilution resulting in complete complement-fixation.

‡ Purified type 5 virus disrupted by prolonged dialysis at pH 10.5.

§ A 3 times CsCl-banded preparation containing E and L antigens and toxin.

‖ DEAE-purified (rechromatographed 2 times) L antigen from infected cells.

¶ DEAE-purified (rechromatographed 2 times) E antigen from infected cells; contaminated to a slight extent with toxin (T).

Electron Microscopy of Virus and Soluble Antigen Preparations.—Preparations dialyzed against 0.01 M phosphate buffer (pH 7.2) were mixed with 4 per cent potassium silicotungstate on carbon-coated copper grids. The excess liquid was removed with filter paper and before the residual liquid had dried the specimen was placed in a Siemans elmskop I electron microscope for examination (4).

EXPERIMENTAL

Characteristics of the Preparations Examined by Electron Microscopy.—Infectivity titers and immunological properties of the purified virus and antigen preparations employed are summarized in Table I. The quantitative and qualitative characteristics of these materials indicate that: (a) Purified virus exhibited a characteristic preponderance of group-specific (L) antigen as noted previously (2); (b) Virus disrupted at pH 10.5 showed a total loss of infectivity but disruption caused no significant alteration in the relative titers of E and L antigens; (c) The soluble-antigen mixture, obtained from freon-treated virus

suspensions and subsequently separated from virus particles by banding 3 times in CsCl density gradients, exhibited equal titers of E and L antigens and a relatively small amount of infectious virus; and (d) Neither the DEAE-purified L antigen nor the E-T (type-specific antigen plus toxin) mixture contained detectable infectious virus; furthermore each was relatively free of cross-contamination with the other soluble antigen as revealed by the complement-fixation test with antigen-specific antisera.

Electron Microscopy of Highly Purified Type 5 Adenovirus.—The fine structure of type 5 adenovirus, illustrated in Fig. 1, confirms in large part the basic morphological observations of Horne and coworkers (1). In addition, this electronmicrograph reveals that the surface subunits, or capsomeres, are not solid spheres as was previously proposed (1), but rather that each capsomere appears to be a short polygonal rod with a central hole. This is especially evident in the appearance of the free capsomeres. Inspection of the periphery of the virus particles suggests the presence of a regular array of minute surface projections. Characteristically, few fibrous or thread-like strands are observed near the virus particles and free capsomeres (Fig. 1, arrow).

Electron Microscopy of Disrupted Virus.—Purified virus disrupted at pH 10.5 as described in the section on methods, was examined by electron microscopy (Fig. 2). Examination of this material confirmed the evidence obtained by infectivity assay and equilibrium density gradient centrifugation which indicated that virus particles disintegrated when treated for prolonged periods at high pH. Two structures were visible in this preparation: (a) Hollow polygonal structures which corresponded to the virus capsomeres seen in preparations of purified virus; and (b) thread- or fiber-like strands which were also characteristically observed in preparations of purified virus. Of these structures, the former (polygonal capsomeres) were greatly predominant in number. The capsomeres measured approximately 70 to 80 A in greatest dimension with a central hole of about 25 A in diameter. Widths of the fibers ranged from 10 to 25 A, and the average length of the fibers was about 250 A.

These data indicate that during prolonged dialysis at pH 10.5 virus particles dissociate into at least two protein subunits and DNA. This interpretation is in agreement with the data presented in the accompanying paper which showed that degradation of the virus particle resulted in release of two soluble protein antigens, the type-specific (E) and the group-specific (L) antigens. On this basis one might postulate that the two units of virus structure seen in Fig. 2 might represent the E and L soluble antigens.

Electron Microscopic Examination of a Soluble Antigen Mixture from Virus-Infected Cells.—A soluble antigen mixture containing E, L, and toxin from an infected cell homogenate was freon-treated, banded 3 times by centrifugation in a CsCl density gradient and examined (Fig. 3). The infectivity and soluble antigen titers of this preparation are presented in Table I. Three types of

structures were readily identified in this micrograph: (a) Partially degraded or "incomplete" virus particles; (b) Polygonal, hollow rods, the virus capsomeres; and (c) Thread-like strands previously seen in small numbers in preparations of intact and disrupted virus. The majority of capsomeres appear to have fibers associated with them. It is notable that in this preparation there was no preponderance of capsomeres over the thread-like fibers as was observed in the disrupted virus preparation (Fig. 2). In keeping with this finding, the data summarized in Table I indicate that the titers of E and L antigens were essentially equal in the sample containing a mixture of antigens. This evidence suggested that the capsomeres and fibers are the E and L antigens although one cannot discount the possibility that one of these structures may represent the toxic moiety.

Electron Microscopy of Purified Group-Specific (L) Antigen.—To identify a given antigen with a particular morphological structure, purified, isolated antigens were desired. Therefore, a purified preparation of L antigen, that cross-reacted only weakly with anti-E serum (Table I), was examined with the electron microscope. The L antigen preparation was composed of an almost homogeneous population of virus capsomeres (Fig. 4). It may be concluded from this evidence that the polygonal virus capsomeres represent the group-specific (L) antigen. This finding and the resultant interpretations are consistent with the data presented in the previous paper (2) and in Table I which indicated that the L antigen represented the predominant virus protein. This micrograph also vouches for the homogeneous nature of highly purified L antigen preparations (they contained neither detectable E antigen nor infectious virus) that have been employed to produce antigen-specific antisera (2, 3).

Electron Microscopy of a Purified Mixture of Type-Specific (E) and Toxin Antigens.—A DEAE-purified preparation of E antigen was prepared (3). This antigen was contaminated only to a slight extent with toxin (titer = 1:60); no group-specific (L) antigen could be detected (Table I). Examination of this preparation with the electron microscope revealed many fiber-like strands and a few irregularly shaped globular structures (Fig. 5). Because the toxin represented only a minor reactive component of this mixture it seemed highly probably that the fibers represented type-specific (E) antigen. Efforts are being made to obtain a pure toxin preparation in order to ascertain whether or not a structural unit can be assigned to this antigen. When one reexamines Figs. 1 and 2 with the preceding data in mind it is apparent, from morphological considerations alone, that the virus particle contains relatively small amounts of the type-specific or E antigen. This is compatible with the observations described in the preceding paper, based on immunological evidence, indicating that the E antigen represented a minor virus component or subunit (2).

DISCUSSION

Type 5 adenovirus was demonstrated to be an icosahedron comprised of 252 subunits arranged in 5:3:2 symmetry (1). Observations described in this communication confirmed the original description by Horne *et al.* (1) with the exception that the virus capsomeres were shown to be short, hollow, polygonal rods rather than solid spheres. Thus, the capsomeres of type 5 adenovirus are similar to those of the so-called avian adenovirus, GAL virus (5, 6), and strengthens the notion that the avian agent is an adenovirus although the characteristic common group antigen is not present (7). It is striking that the capsomeres of type 5 adenovirus also resemble the capsomeres of herpes simplex (8) and polyoma viruses (9).

Adenoviruses are rapidly inactivated at a pH greater than 9.0–9.5 (10). This characteristic suggested that the virus particles might disintegrate under alkaline conditions. As described in the previous paper, prolonged dialysis at pH 10.5 disrupts adenovirus particles and liberates the virus antigens (2). The capsomere is the major surface, structural subunit and has been identified immunologically to be the group antigen. The soluble, group-specific antigen has been shown to be morphologically identical with the capsomere. These data furnish an explanation for the finding that type 5 adenovirus is neutralized by antisera obtained from rabbits immunized with purified type 5 soluble, group-specific antigen free from virus particles and type-specific antigen (11).

In addition to the polygonal capsomeres, a fiber-like particle of heterogenous length was liberated by disrupting type 5 adenovirus particles. The fiber-like structure was identified as the type-specific antigen. It is important to emphasize that immunologically the group antigen was the predominant antigen measured in preparations of purified virus (Table I). Similarly, when purified virus was dissociated, 15 to 20 times more group-specific antigen than type-specific antigen was solubilized. Electron microscopic observations confirmed the finding that the group antigen was predominant.

The exact location and the structural role of the fiber-like, type-specific antigen in the intact virus particle is not apparent. In certain views, the virus particles appear to possess fibrillar projections, which could possibly be the type-specific antigen. This suggests that the fiber-like structures project from the virus particle and are located either between or in the central hole of the capsomeres. Quantitative considerations imply that a type-specific antigen is not associated with each capsomere but rather that the fibers are distributed at strategic points on the icosahedron. It should be recalled that the soluble type-specific antigen is the major hemagglutin in type 5 adenovirus preparations (12).

The toxin-like material associated with type 5 adenovirus soluble antigens has not yet been observed as a distinct morphological structure. Nor has the

toxin been detected in preparations of intact or disrupted virus particles. However, it is now clear that the other characterized specific, soluble antigens (the group- and type-specific antigens) are distinct morphological units which are identical with structural subunits of the virus particle. Therefore, the soluble antigens, which are synthesized before infectious virus particles are assembled (13, 14), correctly can be considered to be precursors of the mature particle.

SUMMARY

Purified type 5 adenovirus was disrupted at pH 10.5 and the capsid shown to be comprised of two characteristic morphological subunits: (a) Hollow, polygonal structures corresponding to the virus capsomeres seen in preparation of purified virus and (b) thread-like strands also identifiable in preparations of purified virus. These structures were compared morphologically with purified preparations of the group- and type-specific soluble antigens characteristically produced in mammalian cells infected with adenoviruses. The group-specific soluble antigen was a homogeneous preparation of hollow, polygonal rods identical with the virus capsomeres. The type-specific soluble antigen corresponded to the thread- or fiber-like components of the purified virus particle. Inspection of disrupted virus preparations confirmed earlier immunological data which indicated that the major virus antigen was the group-specific soluble antigen. These data provide convincing evidence for the hypothesis that the adenovirus-induced soluble antigens represent virus subunits produced in excess.

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EXPLANATION OF PLATES

PLATE 26

FIG. 1. Electron micrograph of purified particles of type 5 adenovirus embedded in sodium silicotungstate. Many hollow capsomeres can be seen scattered about the field. The contrast is so great in the original negative that when it was enlarged to show the isolated capsomeres, the virus particles were quite black. The plate was therefore "dodged" by placing an underexposed, positive, out-of-focus print in contact with it in the enlarger. This enables one to see the capsomeres on the surfaces of the virus particles and, as pointed out by arrows, a number of fine fibers extending from them. In this and subsequent plates the bar represent. 1000 A. EMG 30. × .62.3 × 440,000.
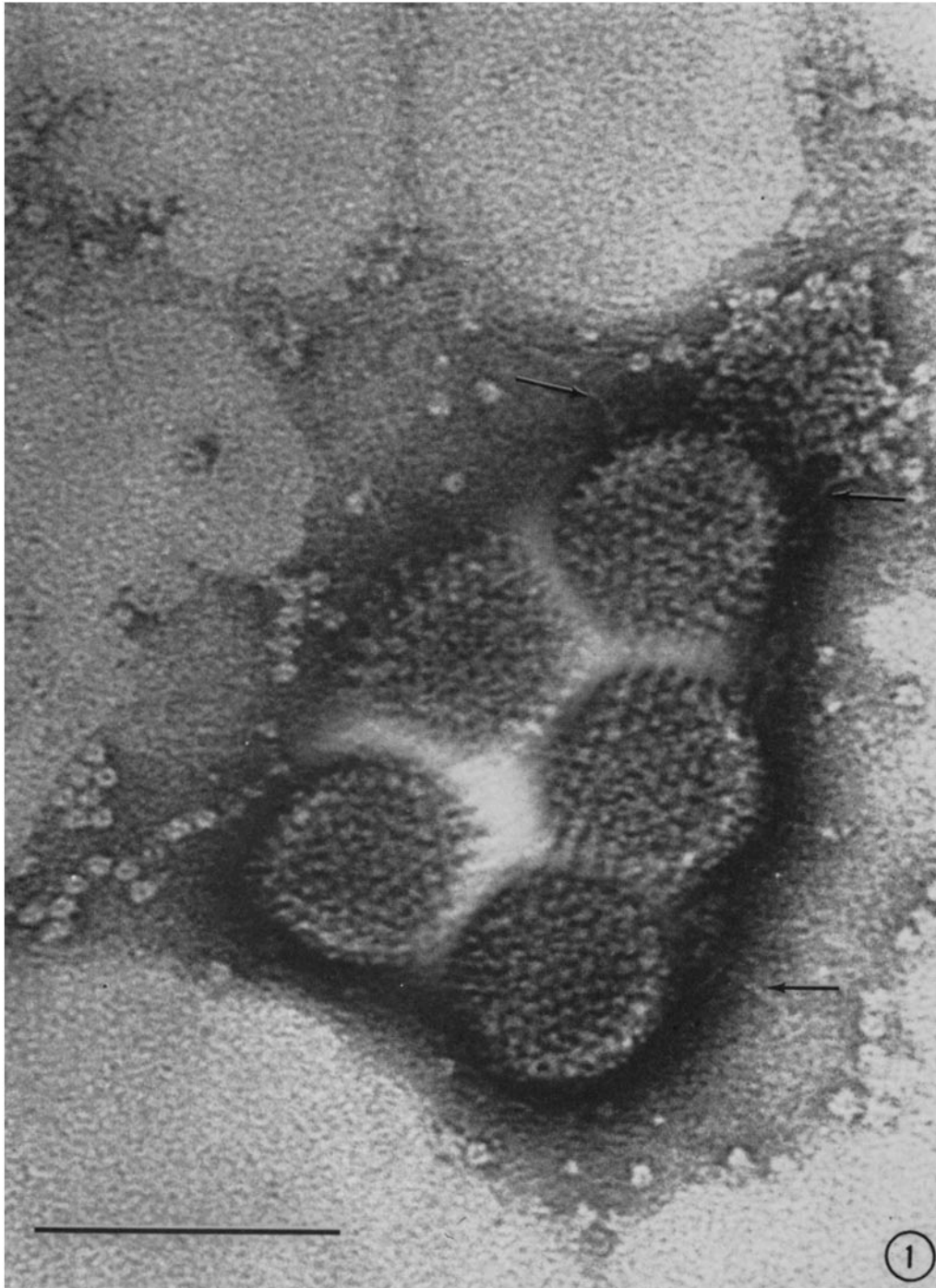
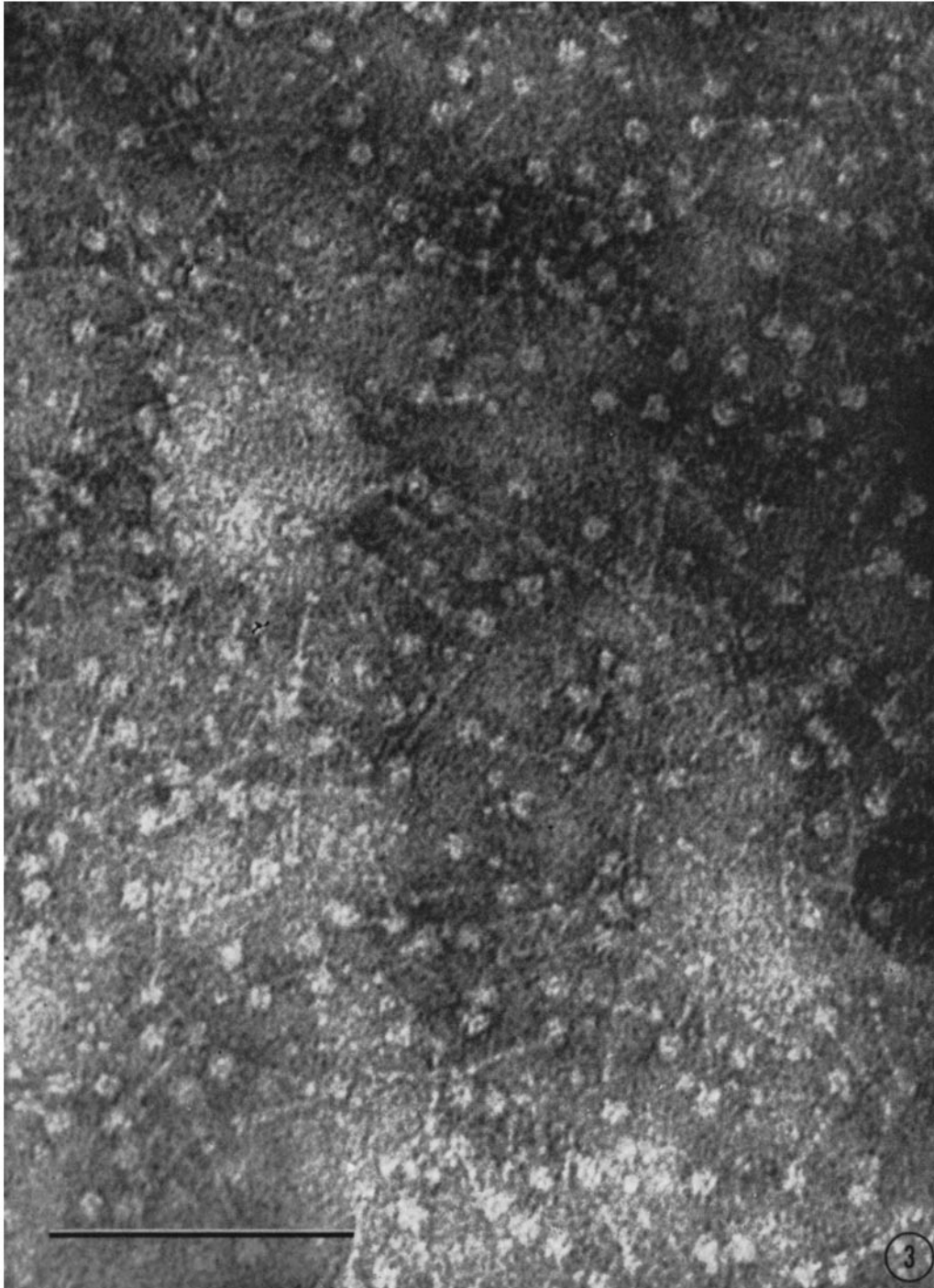(Wilcox *et al.*: Fine structure of adenovirus subunits)

PLATE 27

FIG. 2. Purified type adenovirus disrupted at pH 10.5. Many hollow capsomeres and a few fibers can be seen. EMG 30. X .62.23; X 440,000.
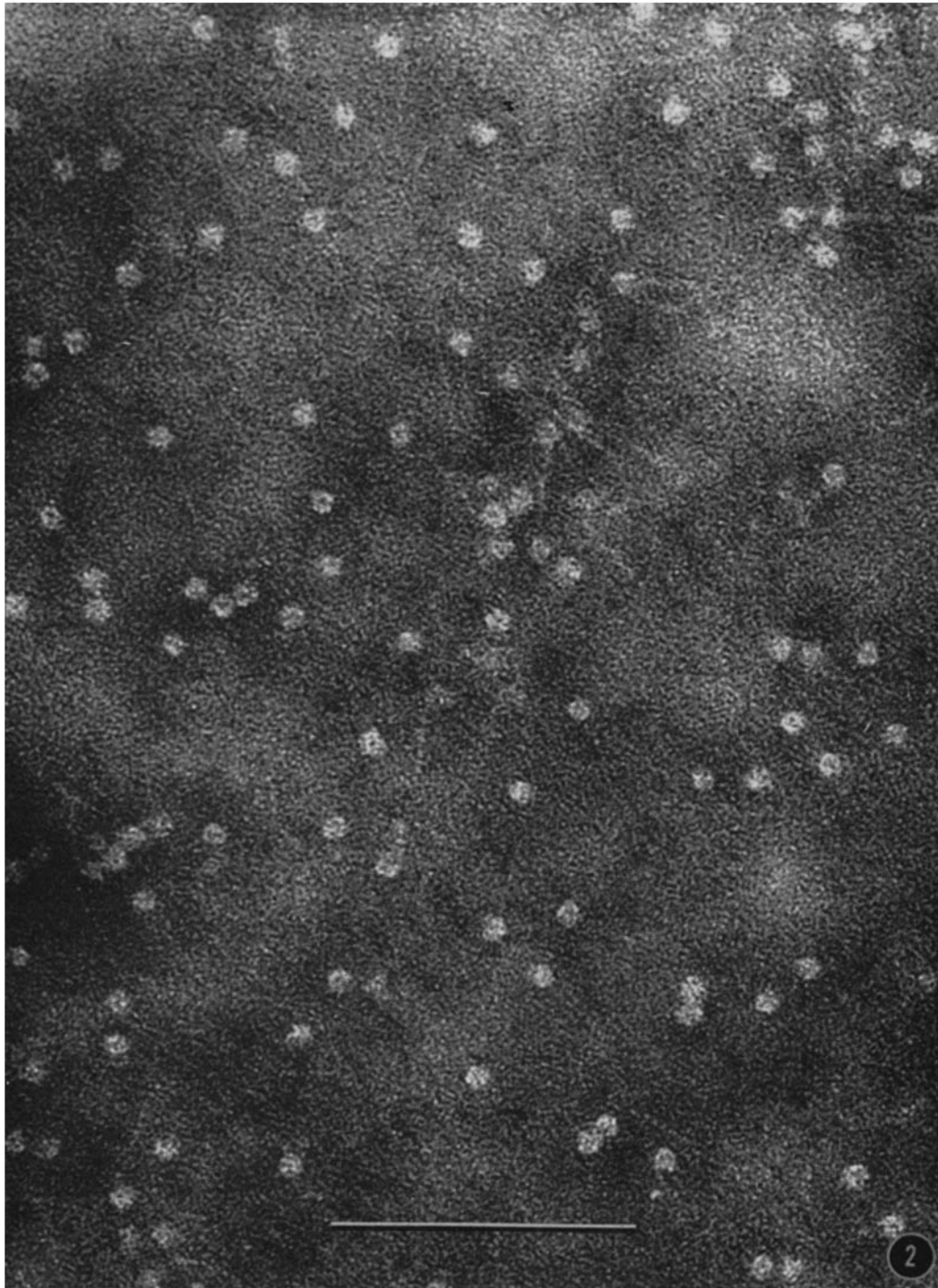
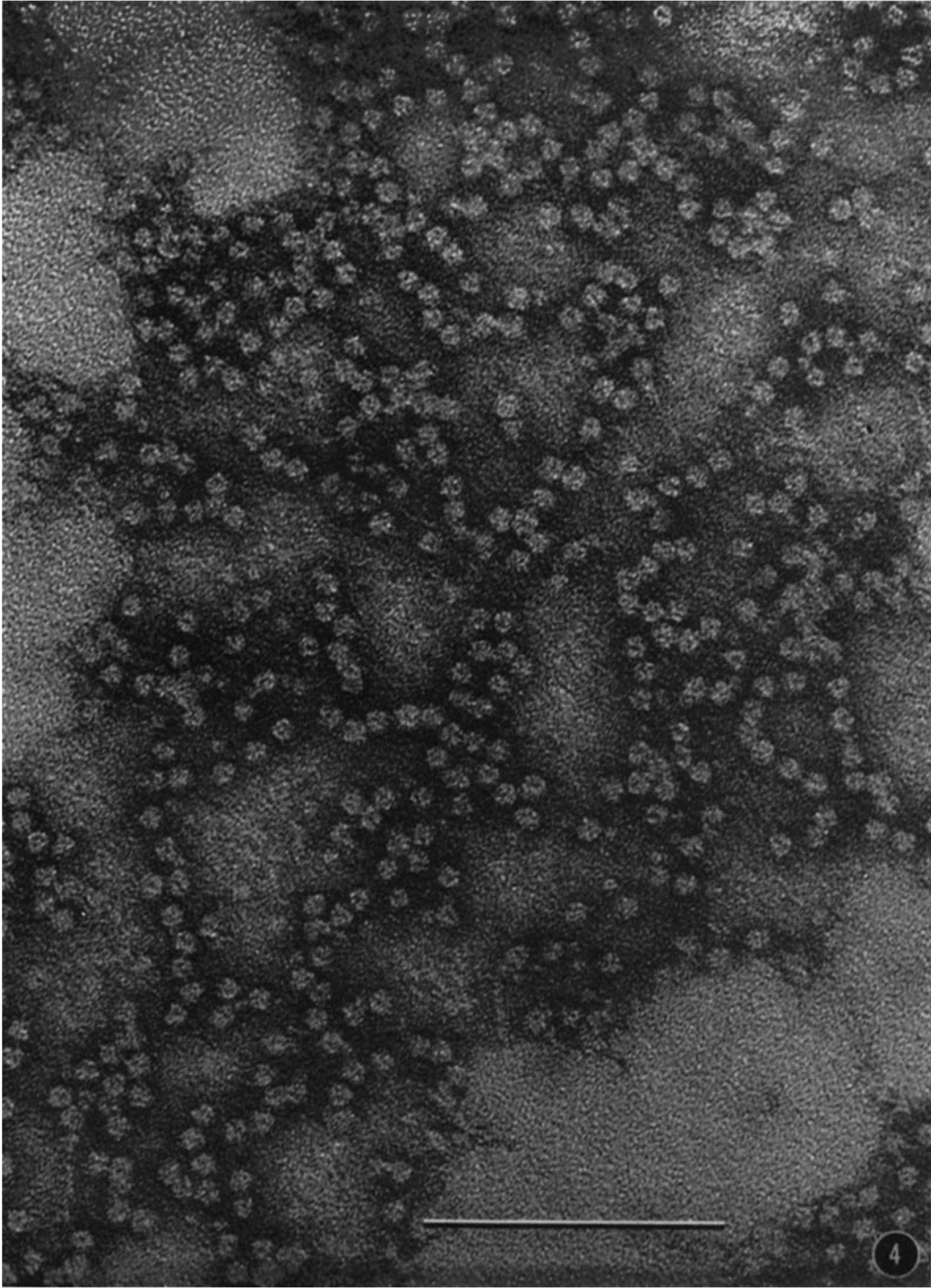(Wilcox *et al*.: Fine structure of adenovirus subunits)

FIG. 3. Purified soluble antigen mixture from homogenized infected cells. Antigenically this preparation consists of E (type-specific), L (group-specific), and T (toxin) antigens. In this micrograph this preparation is seen to contain fibers, capsomeres, and various smaller bodies. Many of the fibers and capsomeres seem to be associated. EMG 30. × .62.12; × 440,000.

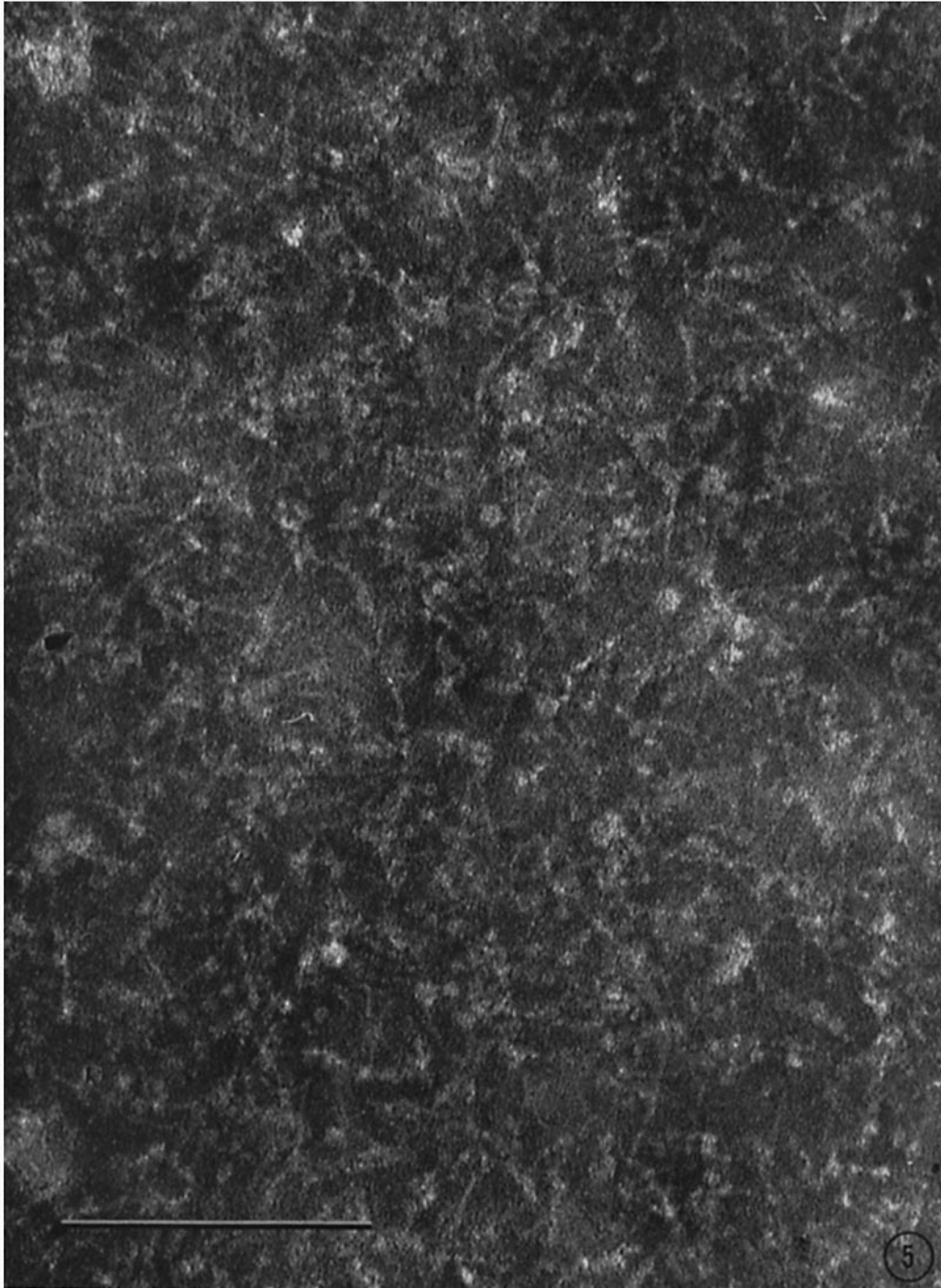(Wilcox *et al.*: Fine structure of adenovirus subunits)

PLATE 29

FIG. 4. Purified group-specific (L) antigen. This preparation is seen to consist almost entirely of virus capsomers. EMG 30.×.62.18; × 440,000.

(Wilcox *et al*.: Fine structure of adenovirus subunits)

PLATE 30

Fig. 5. Purified type-specific (E) antigen containing a relatively small activity of toxin (T). Here the principal component consists of fibers but many irregular globular bodies can also be seen. EMG 30. X .62.16; X 440,000.

(Wilcox *et al.*: Fine structure of adenovirus subunits)