Phil. Trans. R. Soc. Lond. B. **276**, 51–61 (1976) [51]
Printed in Great Britain

Capsid transformation during packaging of bacteriophage λ DNA

By T. Hohn, M. Wurtz and Barbara Hohn
Biozentrum der Universität Basel, Klingelbergstrasse 70, *Basel, Switzerland*

[Plates 8–15]

Assembly pathways of complex viruses might not be simple additions of one protein after another with rigid tertiary structure. It might in fact involve shifts in subunit structure, movement of subunits relative to each other to form new arrangements, transient action of proteins and protein segments, involvement of structure forming 'microenvironments' of the host.

Thus morphogenesis of the bacteriophage λ head starts with the formation of a core-containing DNA-free petit λ particle. In a first transition, and dependent on a host function, the core is released, minor protein components of the capsid are processed and the particle's structure is altered, as shown by a change of its hydrodynamic properties.

The resulting 'prehead' undergoes a second transition triggered by a complex of DNA and recognition protein (*A*-protein). This transition is more drastic than the first one. The particle doubles its volume without increasing in protein mass, the shell becomes thinner, and the surface structure is changed. Concomitantly with this process, the DNA becomes packaged and the particle becomes able to bind the small '*D*-protein' in amounts equimolar to the capsid protein, which it could not do before.

The *D*-protein addition probably causes another shift of the capsid structure. DNA packaging is completed, and the DNA is cut from concatemeric precursors to unit length molecules. Binding sites are created for the tail connector molecules which in turn allow the independently assembled tail to attach.

Research on these processes proceeds along several lines: comparison of physical and chemical properties of particles accumulating in mutants; pulse-chase experiments on assembly precursors; morphogenesis *in vitro*; and model transitions of aberrant λ polyheads.

Introduction

In the course of the assembly of the bacteriophage λ head three steps can be differentiated (figure 1).

(1) In the prehead formation process several protein species assemble, some of which become chemically modified: with the help of the product of the *Nu3* gene, which probably serves as a core and carrier for the minor proteins p*C*† and p*B* (Ray & Murialdo 1975; Hohn, Flick & Hohn 1975) 420 copies of the main capsid protein p*E*, mol. mass 38000, assemble. The resulting structure constitutes one of the many possible petit λ forms. We call it 'unprocessed prehead'. Upon the action of the host gene *groE* a series of cleavage and fusion reactions occur: the *C* protein molecules (about 12) become fused to an equal amount of *E* protein molecules. This fusion product is cleaved at both ends to yield the processed proteins X1 and X2, in about equimolar amounts (Hendrix & Casjens 1974). The core protein p*Nu3* is removed. Dependent on the p*E*–p*C* fusion-cutting reaction proteolytic cleavage of the *B* protein molecules occurs. The resulting particle, also belonging to the petit λ family, is termed prehead.

† p*A*, p*B* are products of the genes *A*, *B*; p*B** is the cleaved product of the *B* gene.

(2) The DNA packaging step requires the presence of the products of the genes *A*, *NuI* and *FI* and DNA. DNA becomes packaged concomitantly with the phage head expanding. The resulting structure is stabilized by the addition of *D* protein (mol. mass 12000) in amounts equimolar to the *E* protein (Hohn *et al.* 1975 and references therein).

(3) The packaged phage head is completed, i.e. it acquires the capacity to bind the tail by subsequent reaction with the *W* protein and addition of about 6 molecules of *FII* protein (Casjens, Hohn & Kaiser 1972; Casjens 1974). The independently assembled tail is attached without the further help of a protein (Weigle 1966).

It is on step two that we will concentrate our attention. It contains the DNA packaging step which of course is the biological purpose of the assembly chain. A mechanism ensuring the packaging of the proper DNA at the right time and possessing the correct size must exist. It is this step also that contains the transition from the petit λ sized head to the mature sized head.

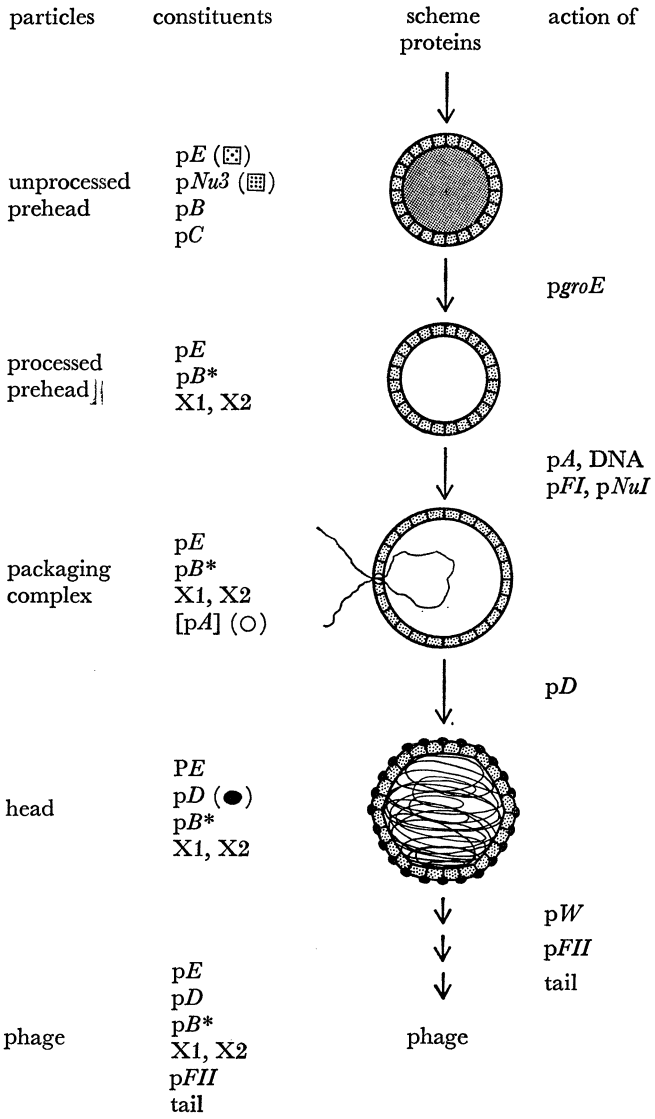

FIGURE 1. Pathway of head morphogenesis of bacteriophage λ. For explanation see text.

This step can be assayed *in vitro*. Thereby with high efficiency preheads are filled with DNA and transformed to plaque forming particles (Hohn & Hohn 1974). Figure 2 shows the speed of this process, namely the conversion of the DNA from a DNase sensitive to a DNase resistant, i.e. packaged, form and the conversion of preheads and DNA to plaque forming particles. This *in vitro* assay allows the identification, isolation and physical, chemical and electron microscopical characterization of the reaction partners involved.

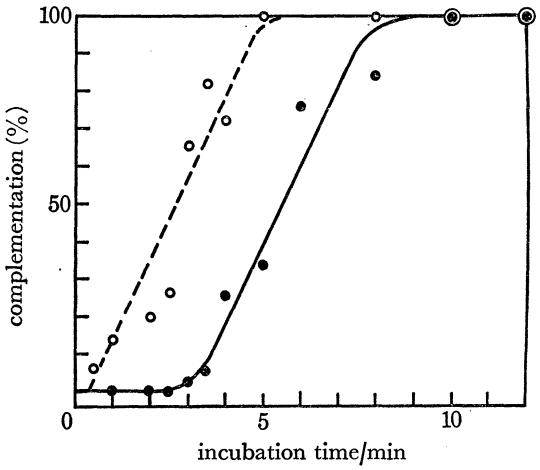

FIGURE 2. Kinetics of *in vitro* complementation of an A^- with an E^- lysate. Aliquots of a complementation mixture containing 500-fold concentrated induced lysogens, 0.01 M spermidine, 1.5 mM ATP, 0.01 M $MgCl_2$, 0.01 M NaN_3, 0.04 M tris HCl, pH 8 and 10% (by vol.) dimethylsulphoxide were put in separate tubes and lysed by freezing in liquid N_2 and thawing and placed in a 37 °C shaking waterbath. For curve -O---O-, at times indicated DNase I was added to separate tubes to give a final concentration of 20 μg/ml and the complementation continued until 60 min when a 50-fold dilution with SMC (Hohn & Hohn 1974) and a drop of $CHCl_3$ stopped the reaction. The number of plaque forming particles expressed as a percentage of the maximal yield is plotted against the time of DNase addition. The curve therefore shows the kinetics of transformation of the DNA contained in both lysates from the DNase sensitive to a DNase resistant, i.e. packaged, form. Curve -●——●- shows the kinetics of *in vitro* complementation, starting from preheads (as present in the A^- lysate), λ DNA (as present in both lysates), *A* protein (as present in the E^- lysate), other proteins and tails (as present in both lysates) to finished plaque forming particles. Complementation was stopped at the indicated time points by a 50-fold dilution with SMC containing 1 μg/ml DNase and a drop of $CHCl_3$.

1. THE PREHEAD EXPANDS

Negatively stained preheads have a diameter of 45 nm, heads of 54 nm (figure 3, plate 8, see also table 3). Thus the size difference is 20% in one dimension, but a 100% increase o the inner volume is achieved. The same difference in diameter can be measured on intracellular particles visualized in thin sections of induced wild type λ producing bacteria (figure 4, plate 9; Lickfeld *et al.* 1976); absolute values in this case are 39 and 47 nm.

Is the process underlying this size increase a real transition or rather a breakdown and reassembly of the particles? To answer this question, the following experiment was performed: Preheads were density labelled by growing and inducing a λ prehead producing lysogen in medium containing D_2O and $^{15}NH_4Cl$. Preheads isolated and purified from this lysate, preheads purified from a normal 'light' lysate and a mixture of these two particles were complemented *in vitro* and the resulting phage particles centrifuged in very shallow CsCl gradients (Hohn *et al.* 1974; Kaiser, Syvanen & Masuda 1975). The banding profile of the mixed and

complemented particles looks exactly like a superposition of the profiles of the heavy and light complemented preheads (figure 5). No material of intermediate density is present. This experiment rules out the possibility that the prehead–head transition is a nonconservative one and also invalidates any model that explains the expansion of the prehead to the head size by the addition of more *E* protein molecules. The latter idea was unlikely *a priori* because the transition can be shown not to require additional *E* protein subunits since *in vitro* it can take place on purified preheads in an E^- extract.

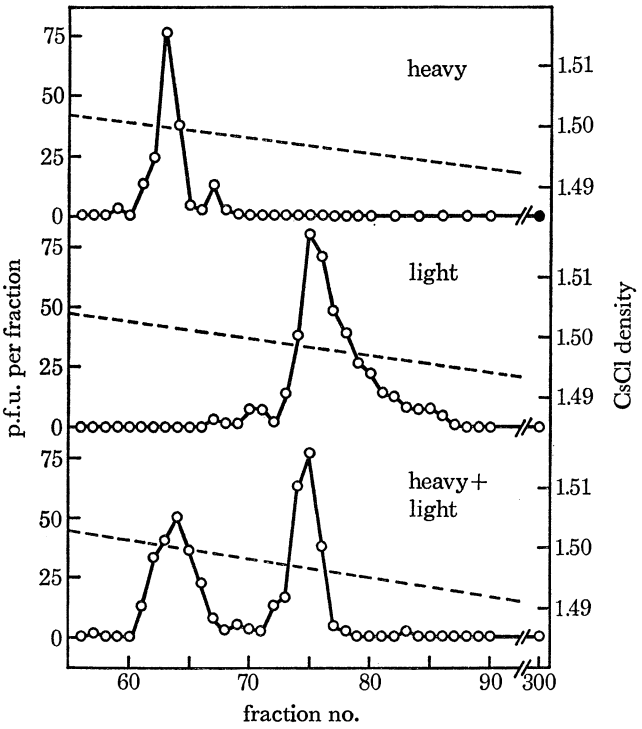

FIGURE 5. CsCl gradients of bacteriophage λ produced by *in vitro* complementation of heavy, light and a mixture of heavy and light preheads. Heavy preheads were isolated according to Hohn *et al.* (1975) from an induced A^- lysogen grown in D_2O-$^{15}NH_4Cl$ medium (Bijlenga, v. d. Broek & Kellenberger 1974). Light preheads were isolated from an induced A^- lysogen grown in H_2O-$^{14}NH_4Cl$ medium. *In vitro* complementation was performed with a light E^- lysate. The CsCl gradients were spun in an A 321 fixed angle rotor, for 60 h at 30000 rev./min at 4 °C in an International Centrifuge. About 300 fractions were collected and assayed for plaque forming units.

Can a change in the primary structure of the *E* protein be made responsible for the physical change of the particle? Chemical analysis has revealed that the amino acid composition stays the same, the amino- and carboxy-terminal amino acids are the same and no phosphorylation or glucosylation takes place during the transition (Hohn, Morimasa & Tsugita 1976). Furthermore, both the amount per particle and the molecular mass (as determined in SDS acrylamide gels) of the minor protein components are identical in the prehead and the head, thus ruling out their playing a direct and active role in the transition. However, the minor proteins must play a role as a recognition device on the particle for the transition-triggering *A* protein-DNA complex because *in vivo* and *in vitro* particles lacking the minor proteins are not transformed to mature sized capsids.

From all these data the conclusion necessarily emerges that it is the main capsid protein p*E* itself that, upon a certain signal (see below) changes its conformation such that a bigger head with new properties arises.

2. What triggers the prehead expansion?

(*a*) *Biological transition*

Table 1 lists the biological requirements for the prehead–head transition. In the following discussion we will concentrate on each individual component.

Table 1. Requirements for the biological prehead–head transition

biological condition	component present: prehead	p*A*	p*FI*	p*D*	DNA	head expansion	stable DNA packaging
B⁻, *C*⁻	−	+	+	+	+	−	−
thy⁻	+	+	+	+	−	−	−
A⁻	+	−	+	+	+	−	−
FI⁻	+	+	−	+	+	−	−
D⁻	+	+	+	−	+	+	−
wild type	+	+	+	+	+	+	+

A⁻, *B*⁻: condition lacking *A* protein, *B* protein... due to an amber mutation in the *A*, *B*... gene. *B*⁻, *C*⁻ lysates contain petit λ (Murialdo & Siminovitch 1972) but not preheads (Hohn *et al.* 1975). *A*⁻, *FI*⁻, *D*⁻ and wild type lysates do contain preheads, *D*⁻ lysates contain some enlarged preheads in addition, but no full heads (Murialdo & Siminovitch 1972; Hohn *et al.* 1975). Thy⁻: induced thymine auxotrophic, thymine deprived λ wild type lysogen; no phage DNA is synthesized.

(i) *The prehead*

It is a particle belonging to the petit λ class. The different petit λ particles known, each produced under conditions in which one or two proteins are not allowed to be produced in their entire sequence (amber mutants), are distinguished from each other by their different, or even lacking, set of minor proteins, processed or unprocessed, and by sedimentation However, their size to the limit of electron microscopical resolution appears to be constant. Only one of these particles is able to accept DNA for packaging and to increase its size, namely the prehead (Hohn *et al.* 1975; Hendrix & Casjens 1975).

(ii) *The DNA*

In the absence of λ DNA only petit λ particles are produced (Karamata, Kellenberger, Kellenberger & Terzi 1962). Since for a long time petit λ particles were considered to be nonfunctional byproducts of phage assembly because they appeared under all conditions in which *E* protein could assemble at all, this result could be taken to indicate a direct involvement of DNA in the production of a correctly assembled structure. We have tested the petit λ particles produced under thy⁻ conditions and found that they are, in fact, preheads (Hohn *et al.* 1975). This finding now places the role of the DNA in head assembly more precisely at the stage of the prehead–head transition, and not – apart from coding functions of course – earlier. Thus λ DNA is absolutely required for the biological prehead–head transition. Conversely, only the right precursor head allows DNA packaging and cleavage into mature size pieces out of the concatemeric precursor DNA molecule.

The mutual dependence of these processes can of course best be proven by *in vitro* packaging of pure isolated λ DNA into pure isolated preheads in an extract that provides the other proteins. This can in fact be demonstrated (table 2). This result taken together with the observation that one can block DNA synthesis in the *in vitro* complementation mixture without changing the packing efficiency (Hohn & Hohn 1974) clearly indicates that DNA synthesis is not required during the packaging.

TABLE 2. *IN VITRO* PACKAGING OF PURIFIED λ DNA INTO ISOLATED PREHEADS

reaction partner			packaging of mature λ DNA (% of DNA molecules transformed into plaque forming particles)
preheads	E^- lysate	mature λ DNA	
−	+	+	$< 2 \times 10^{-7}$
+	−	+	$< 2 \times 10^{-7}$
+	+	+	10^{-2}

Complementation was performed as described for figure 2 except that purified preheads (Hohn *et al.* 1975) were used instead of the A^- lysate and that λ DNA isolated from purified phage was added. Plating was done on indicator bacteria on which phage containing the exogenous λ DNA could be assayed selectively.

The natural source for DNA packaging is concatemeric DNA, a species containing several units of λ DNA but lacking the cohesive ends characteristic of mature linear λ DNA. Only upon packaging are these cohesive ends produced (Wake, Kaiser & Inman 1972). *In vitro* however, linear mature DNA which does not exist as an intracellular species is also packaged. Thus the production of the cohesive ends is not a requirement for the packaging to occur; packaging is, however, required for DNA maturation. This must be a safety mechanism to protect mature DNA.

(iii) *A protein*

This protein has been shown to be responsible for the production of the λ cohesive ends (Wang & Kaiser 1973; Becker & Gold 1975), when it requires, as cofactors or activators preheads, ATP and *D* protein. However, *A* protein is also needed for the production of full λ heads. It performs this latter function by complexing to DNA and thereby mediating the proper spatial relationship between DNA and prehead. Figure 6 shows a direct demonstration of a p*A*–DNA complex (*a*) and a p*A*–DNA–prehead complex (*b*) by sucrose gradient centrifugation. The *A* protein–DNA complex, recognized by its activity in an *A* protein requiring *in vitro* packaging assay, sediments slightly faster than mature DNA, probably because it consists of concatemeric DNA. The DNA–*A* protein–prehead complex sediments in a broad peak about 40–65S, which is about twice as fast as mature linear DNA but much slower than preheads alone (which sediment at 135S) so that a retardation effect of the DNA on the prehead has to be assumed. Similar kinds of complexes can be detected after incubation of purified linear monomeric DNA, with *A* protein containing and *A* protein and prehead containing extracts.

Tests for the specificity of the DNA forming this complex have revealed that the *A* protein binding site is most likely located within 1% from the left end of the DNA (Hohn 1975). The DNA cleaving function of the *A* protein is established only after packaging has been completed, *D* protein has been added to the capsid and two precohesive ends (called cohesive

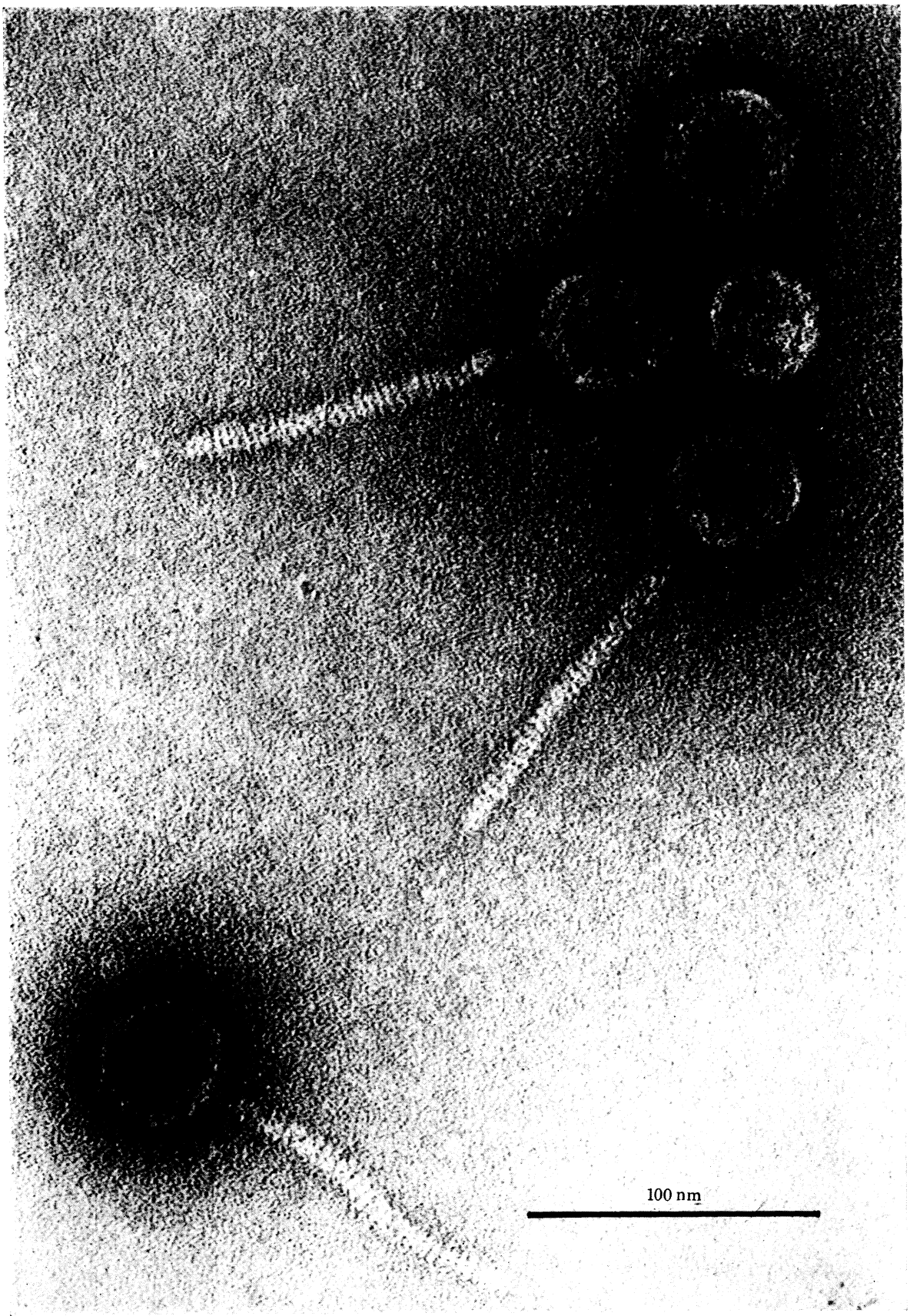

FIGURE 3. Preheads and finished phage. Negative stain with uranyl acetate.

(*Facing p.* 56)

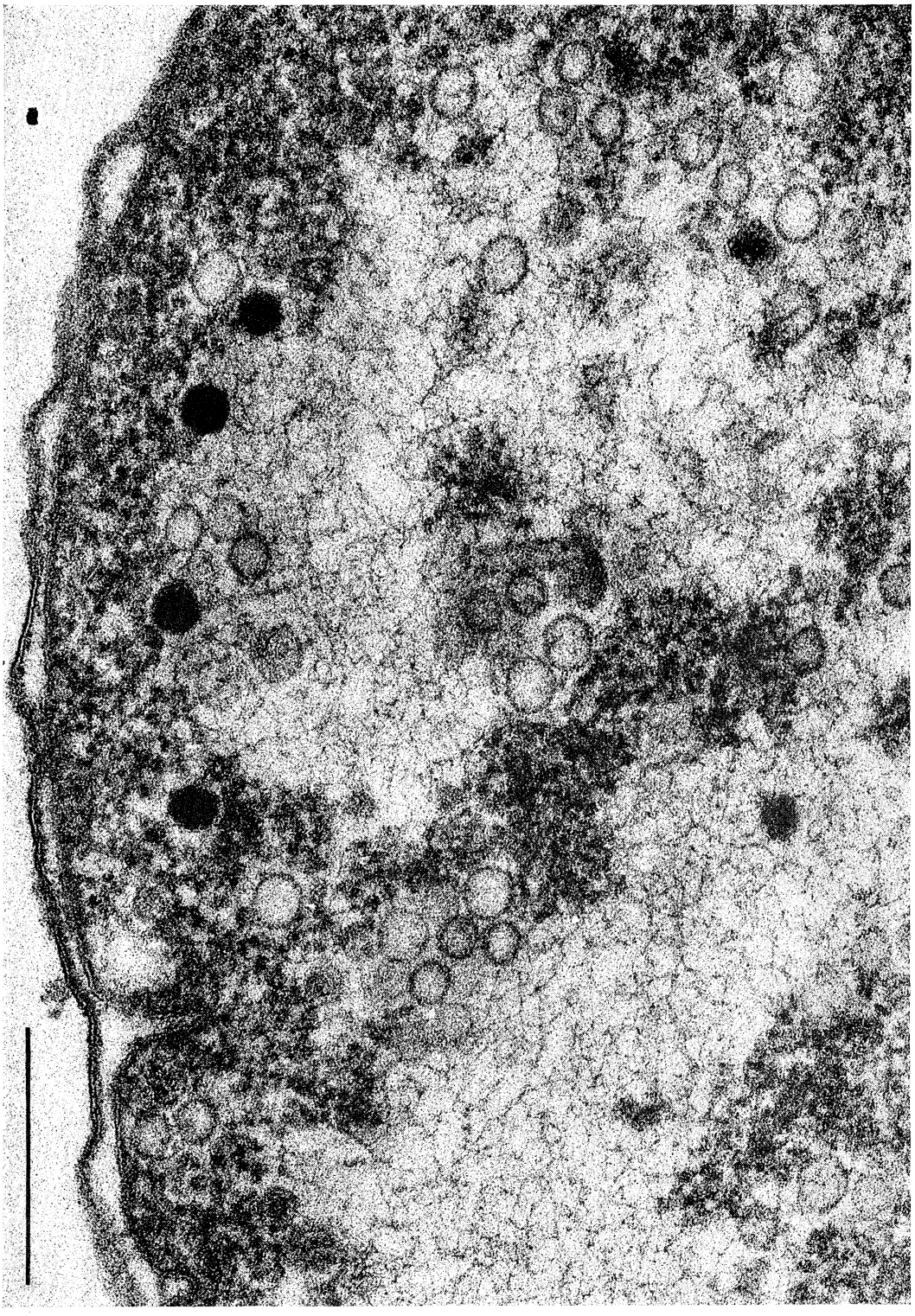

FIGURE 4. Thin sections of a λ wild type lysogen, 120 min after induction. A detailed procedure will be published elsewhere (Lickfield *et al.* 1976). Petit λ (the small white particles), empty heads (the large white particles) and phage heads (the black particles), one of it with tail. (Scale: 250 nm. Electron micrograph by K. Lickfeld & B. Menge.)

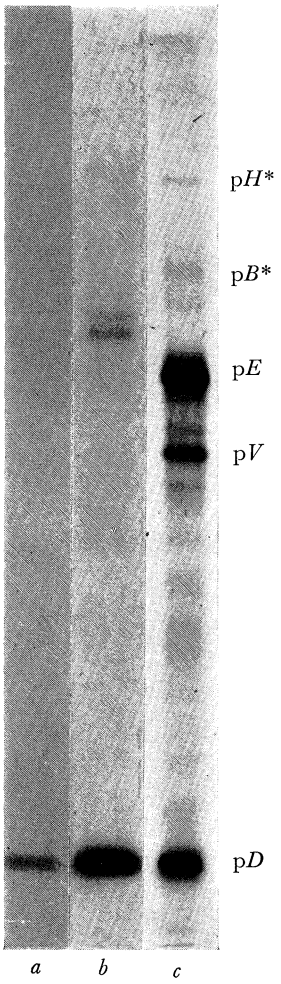

FIGURE 7. Binding of radioactive proteins to preheads and artificially expanded preheads. A^- preheads and the enlarged form thereof, obtained after treatment with 4 M urea and subsequent dialysis in buffer, were reacted with an E^- extract radioactively labelled with ^{35}S according to the procedure described (Hohn *et al.* 1975), freed from large molecular mass material by 16 h centrifugation in an International Centrifuge A321 rotor, 55000 rev./min, dialysis against H_2O, lyophilization and solution in buffer. After this reaction particles were freed from excess radioactive material by sucrose gradient centrifugation and assayed on a 12% SDS acrylamide gel as described by Hohn *et al.* (1975). (*a*) preheads + ^{35}S proteins; (*b*) enlarged preheads + ^{35}S proteins; (*c*) totally labelled ^{35}S phage.

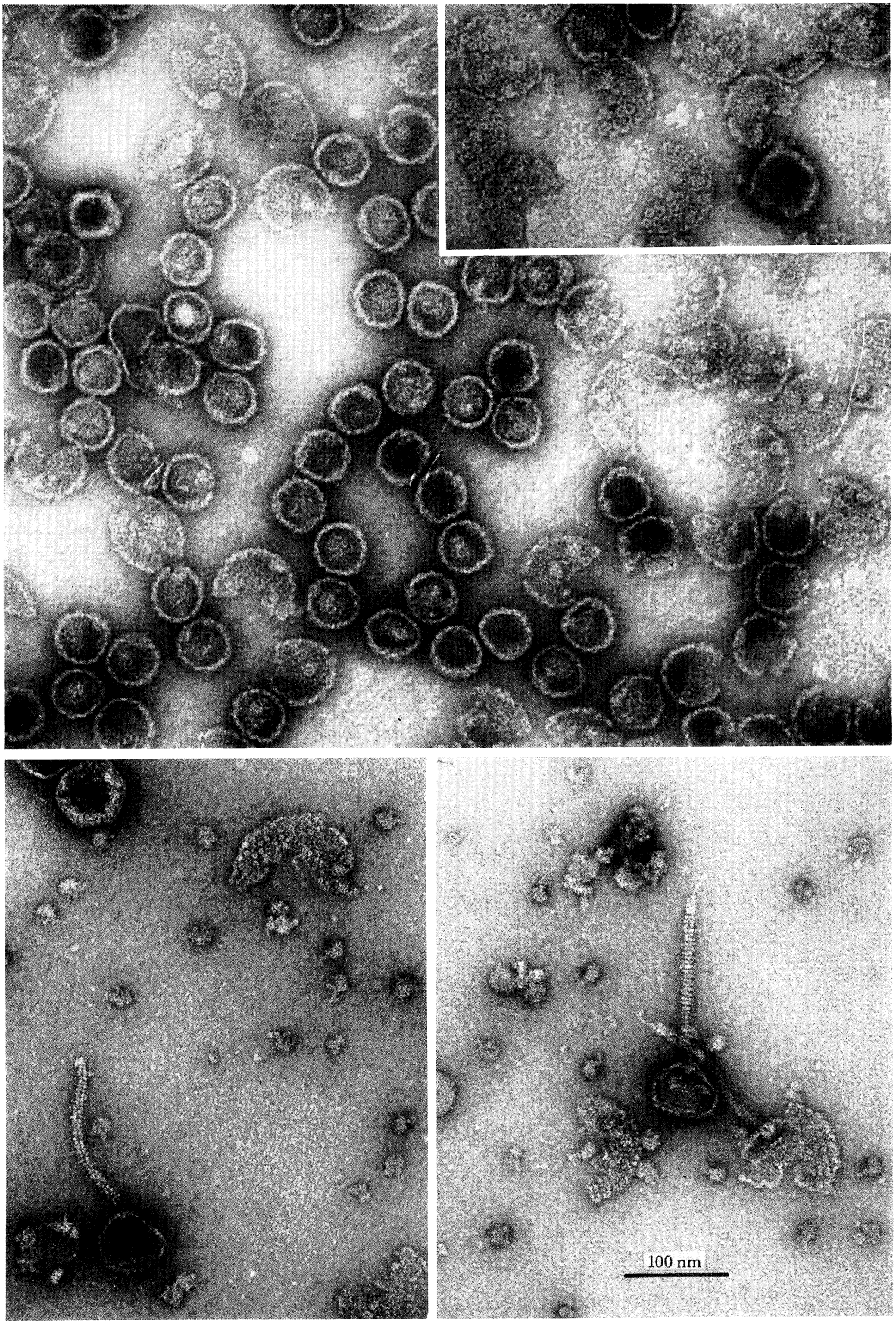

FIGURE 8. Subunit clustering visible on squashed petit λ (upper part) and squashed heads (lower part).

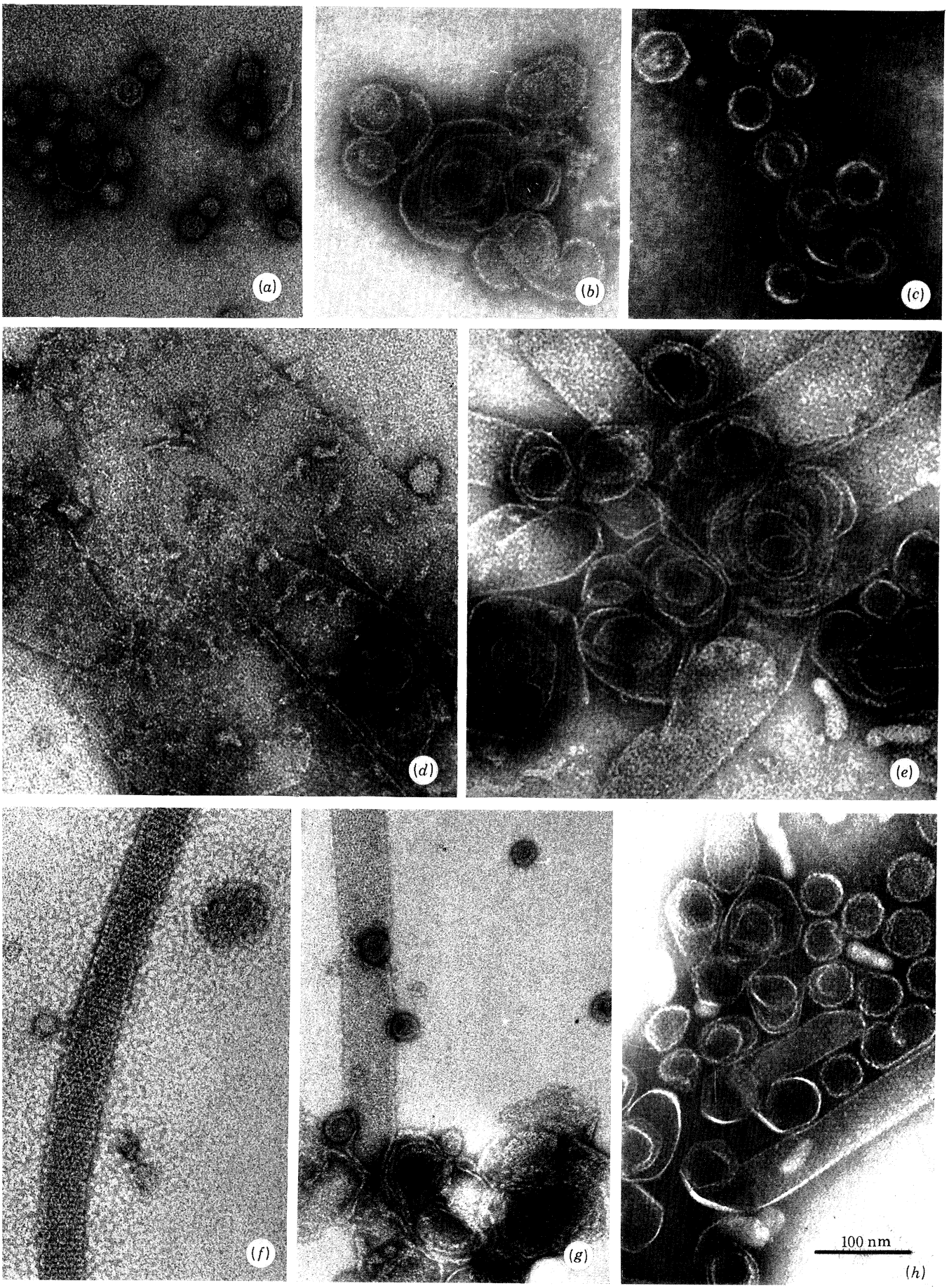

FIGURE 9. Total mixture of reassembled particles showing petit λ (*c*, *h*), 'petit petit λ' (*a*, *g*), empty heads (for example larger hexagonal particle in *c*), sheets (*d*, *e*), spirals (*b*, *e*, *g*), 'peanuts' (*h*) and polyheads (*f*, *g*, *h*). DNA free phage heads were disassociated with guanidinium hydrochloride and reassembly allowed by dilution with buffer followed by pressure dialysis, as described by Wurtz *et al.* 1976.

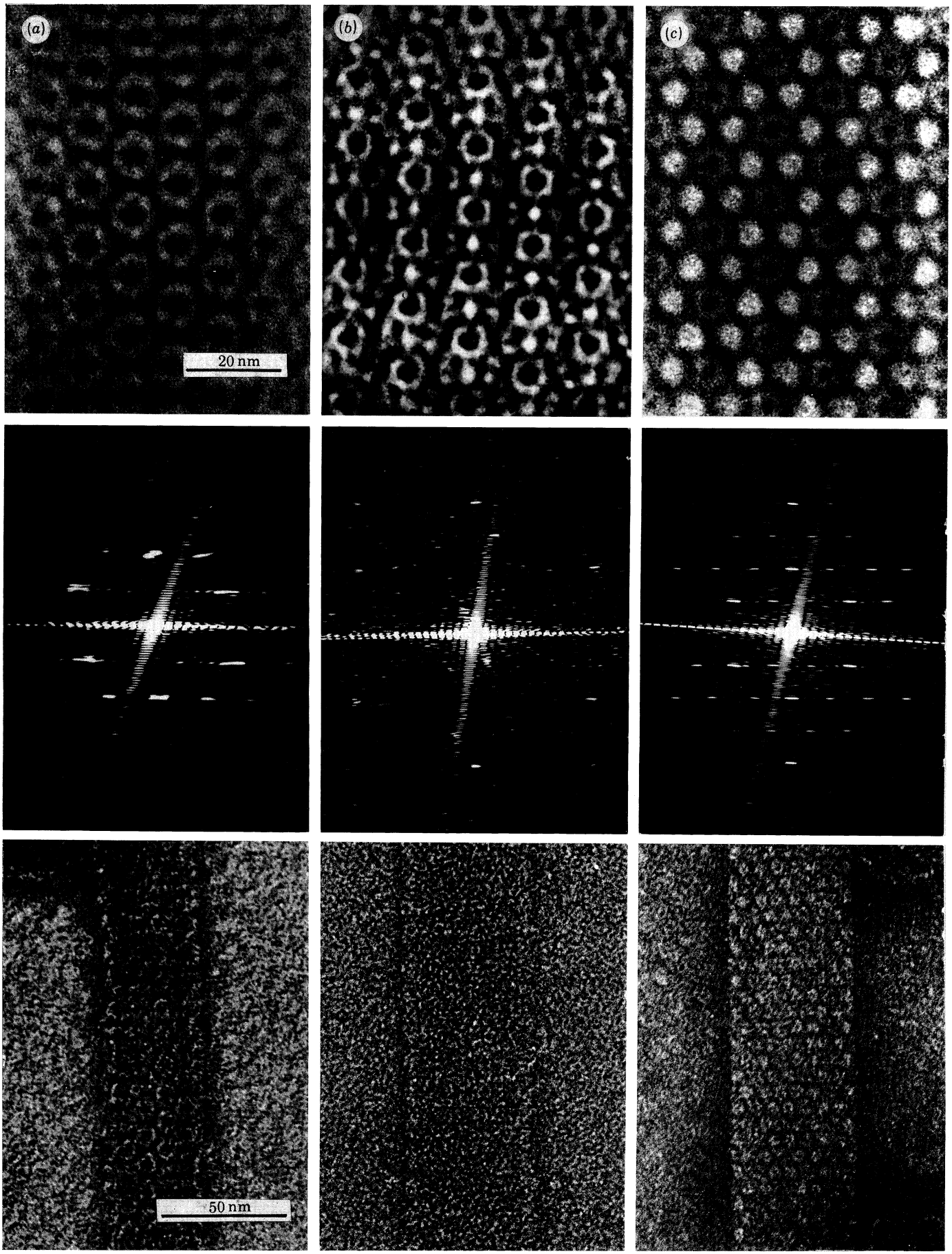

FIGURE 10. A-, B- and C-type polyheads, negatively stained (bottom row), diffracted (middle row) and optically filtered (top row).

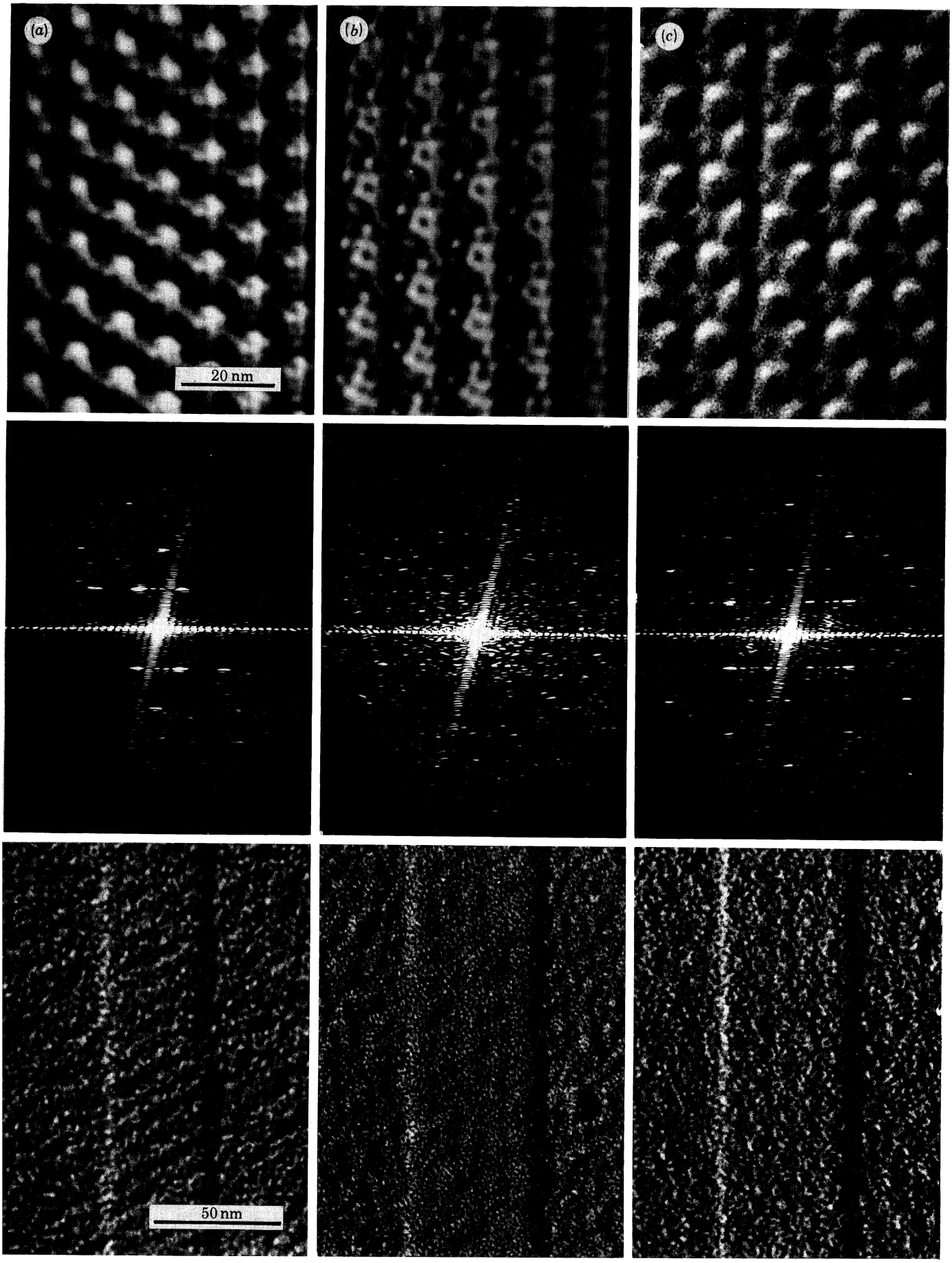

FIGURE 11. A-, B- and C-type polyheads, freeze dried and shadowed (bottom row, electron micrographs by J. Kistler). Diffraction in middle row and image reconstruction in top row.

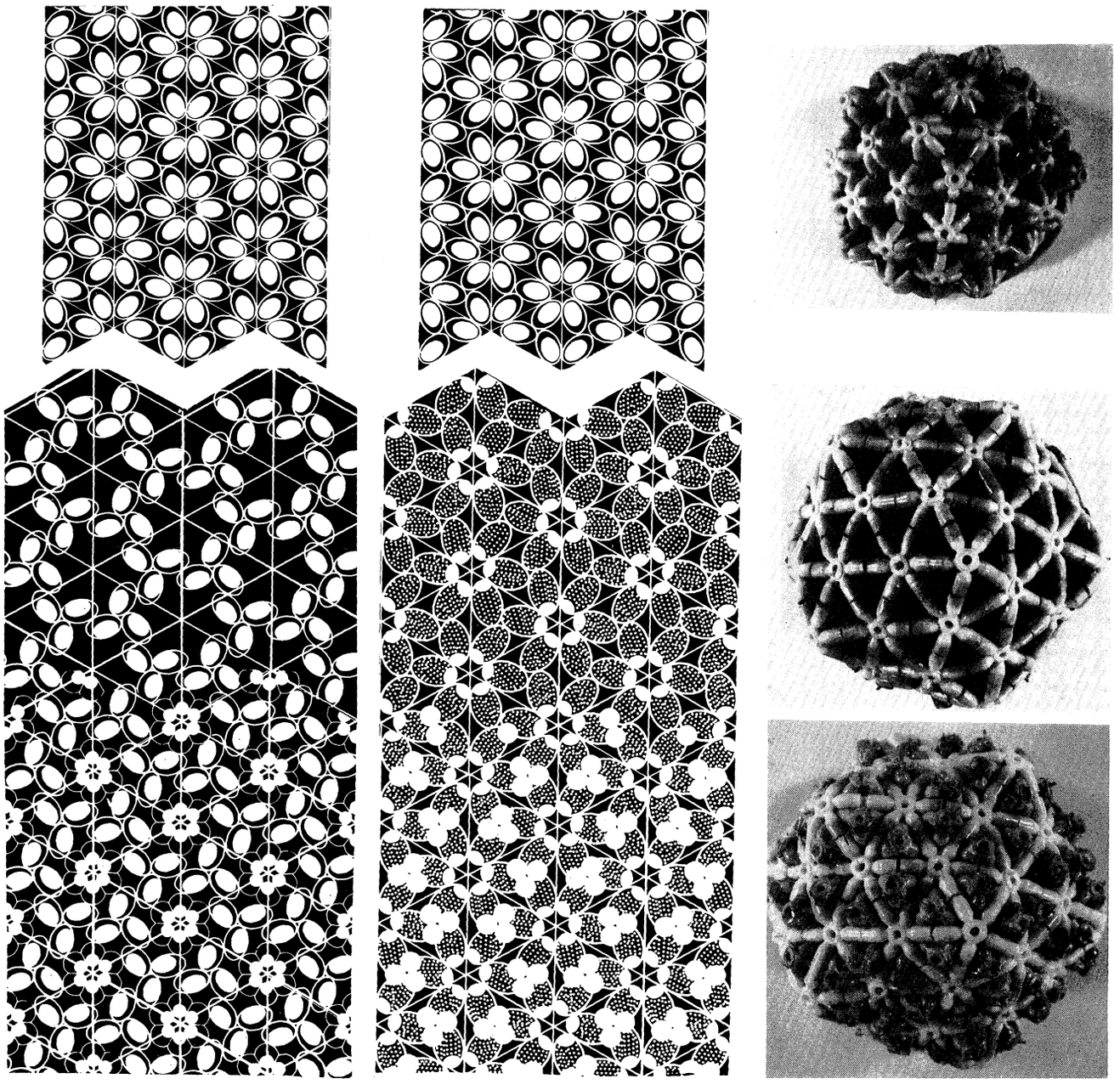

FIGURE 12. Hypothetical lattices (left two columns) and geodesic models (right column) of *E* protein subunits in preheads (top line), *E* protein subunits in expanded preheads (middle line) and *E* and *D* protein subunits in heads (lower line). The black areas of the molecules in the lattice, should represent the protruding parts of the molecules whereas the thin lines should illustrate the molecule contours and thus the intermolecular contacts. The orientation angle of the capsomer in respect to the lattice lines is arbitrary. See text for discussion.

sites) are brought close together (Wang & Brezinsky 1973, and references therein; Hohn *et al.* 1974). This at least is the simplest explanation for the finding, that with the exception of the production of certain defective particles (Sternberg & Weisberg 1975) two cohesive sites are required for a successive cutting event by the *A* protein.

A protein is not a constituent of the finished phage particle (Casjens & King 1975). As measured by its functions it also does not turn over (Hohn 1975). It could be cleaved to an inactive form, thereby constituting an inbuilt regulatory function for the packaging step.

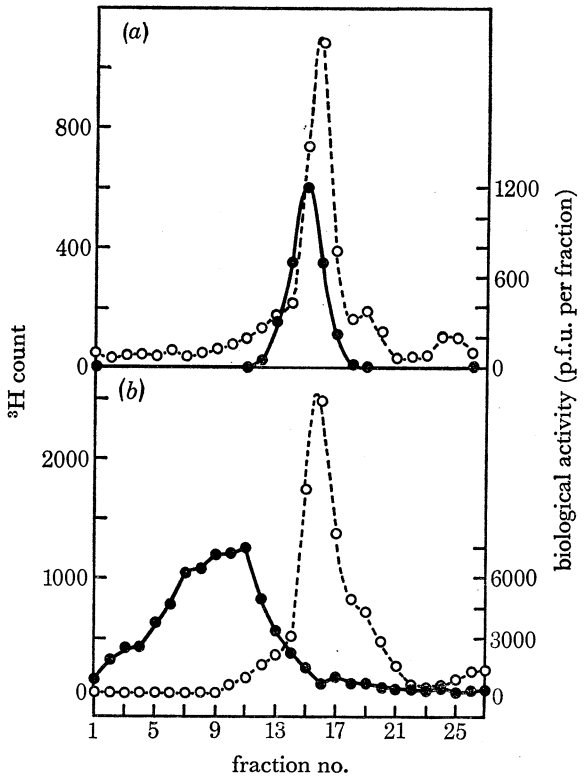

FIGURE 6. Demonstration of a DNA–p*A* (*a*) and DNA–p*A*–prehead complex (*b*). Endogenous λ DNA form a prehead deficient, *A* protein containing (*a*) and prehead and *A* protein containing (*b*) lysate was sedimented together with ^{3}H labelled mature linear DNA as marker in a sucrose gradient (SB 405 rotor, International Centrifuge, 1.5 h 50000 rev./min, 4 °C). ^{3}H count: --○---○--. *In vitro* complementation of an aliquot of each fraction with a heteroimmune A^- lysate was performed. Plating was on indicator bacteria on which complexes transformed to mature phage particles could be assayed selectively: -●——●-.

(iv) *FI protein*

The exact function of this protein in the packaging step has not been determined, nor if it becomes a structural component of the capsid.

(v) *D protein*

D protein is not required for the transition of the prehead to the head since *in vivo* in D^- lysates empty mature heads are found (Murialdo & Siminovitch 1972). However, it is required most probably in an indirect way, as cofactor for the DNA cutting which in turn is necessary for the DNA to be stably packaged in the head. Another function that has been attributed to the *D* protein which, in amounts equimolar to the *E* protein, is located on the capsid's

surface (Casjens & Hendrix 1974) is the loosening of the DNA–*E* protein interaction which had to be as strong as possible in order to get the DNA inside the capsid. Afterwards this interaction has to be weakened, otherwise the DNA could not inject. The role of the large amount of *D* protein might be to exert this effect on the *E* protein (Hohn & Hohn 1973).

(*b*) *Artificial expansion*

This prehead–head transition can also be mimicked artificially. In this case, as in the biological one, the transition is strictly unidirectional. The particles resulting from treatment of preheads with 4 M urea are, in every respect tested, identical to those isolated from D^- lysates. The sedimentation rate, molecular mass and electron microscopically determined radius are the same. Moreover, as shown in figure 7, plate 10, these particles have by their swelling obtained the capacity to bind *D* protein, a property not exhibited by preheads before the treatment.

Also in this case, as in the case of the biological transition, the change from one form to the other is a conservative one, i.e. it happens within the particle and does not involve breakdown and reassembly. This can be demonstrated by examining the swelling reaction by laser light scattering (Kuenzler, unpublished).

One important difference is, however, found between the biological and artificial transitions. The latter is *not* specific for the preheads but can be performed on any petit λ particles. The size of the resulting particles is the same in all cases. From this we can draw two conclusions: (*a*) for the *in vivo* triggering of the reaction specific recognition proteins are involved. These must be the minor proteins on one hand and the *A* protein–DNA complex on the other. In the case of the artificial swelling an agent is needed that helps the protein subunits to overcome an energy barrier. (*b*) *E* protein in its assembled form has 2 (probably more, for the earlier stages of assembly) distinct conformational states. It can, independent of minor protein constituents, exist in one or the other of these states.

3. How is the prehead expanded?

In order to be able to draw some conclusions about the structural changes during the prehead–head transition, the surface structure of the prehead and the head have first to be established. Due to the superposition of the upper and lower layers it is difficult to interpret the structural details visible on intact particles. On damaged phageheads and damaged preheads, subunit clustering can be seen (figure 8, plate 11; Mazza & Felluga 1973; Hohn *et al.* 1974).

A more detailed analysis of the structural arrangements however, can be performed on structures with many identical repeats of the morphological units. In this case image processing can be applied. The amount of polyheads found in lysates is very small, however, and purification thus is very difficult. Therefore conditions were sought and found to assemble polyheads starting from disassembled empty phageheads and petit λ.

Figure 9, plate 12 shows a total mixture of reassembled particles: petit λ, 'petit petit λ', a few empty heads, 'peanuts', sheets, spirals and polyheads. Three kinds of polyheads are assembled depending on the starting material: disassembly and reassembly of petit λ (no matter what genetic origin) as well as of D^- empty heads yields mainly polyheads of type A and a few of type B; whereas from empty heads as starting material exclusively type C poly-

heads are assembled (figure 10, plate 13; Wurtz, Kistler & Hohn 1976). These polyheads can be regarded as structural representations of preheads, enlarged preheads (= D^- heads) and heads, for the following reasons:

(1) the clusters visible on squashed particles and polyheads are similar in arrangement and size,

(2) the relative size difference between prehead and head on the one hand and type A polyheads and type B and C polyheads on the other (as summarized in table 3) is the same,

(3) the same artificial methods can be used to transform the smaller forms into the bigger ones, and

(4) in both cases the small form is unable, but the enlarged form is able, to bind *D* protein.

TABLE 3. SOME PHYSICAL PARAMETERS OF PREHEADS, HEADS AND POLYHEADS

		diameter/nm			
sample	major protein	negative stain†	thin section†	calculated from polyhead lattice constant‡	lattice constant‡ nm
spherical particles					
prehead	p*E*	45	39	48	—
enlarged prehead	p*E*	54	—	57	—
head	p*E*+p*D*	54	47	57	—
polyheads					
type A	p*E*	—	—	—	10.8
type B	p*E*	—	—	—	13.0
type C	p*E*+p*D*	—	—	—	13.0

† Data from Lickfeld *et al.* 1976.
‡ Data from Wurtz *et al.* 1976.

Figure 11, plate 14, shows polyheads of all three types in a freeze dried and shadowed preparation. In this case only the upper surface structure of the polyheads is seen. Diffraction and image reconstruction of these pictures yields results very similar to the ones obtained from the pictures of negatively stained preparations (compare figures 10 and 11), thus ruling out the possibility that the pattern obtained from the negatively stained preparation is actually only an artefact arising by superposition of the lower and upper layers.

For the arrangement of the subunits on the finished phage head Williams & Richards (1974) have proposed a composite lattice, with *E* protein clusters and *D* protein clusters. In petit λ the *E* protein subunits have been shown to cluster as hexamers (Hohn *et al.* 1974). How can we imagine the transition from prehead to head and the addition of the *D* protein subunits? Figure 12, plate 15, presents two extreme models: the left vertical panel shows a rotation of *E* protein subunits during head expansion, resulting in an *E* protein trimer clustering. *D* protein hexamers (and pentamers) come to sit in the middle. The other two models represent, in two and three dimensions, the other extreme, namely flattening out of the *E* protein subunits, thereby causing the expansion. *E* protein subunits remain clustered in hexamers. *D* protein trimers find their place on the threefold local axes of the lattice. By comparison of the surface structure of preheads or type A polyheads with finished heads or type C polyheads, no decision can be made between the two models. However, *E* protein reclustering, if it happens, should occur during head enlargement. Examination of the reconstructed type B polyheads

indicates a persistence of the *E* protein molecules in hexamer clusters in an expanded lattice. Hence the flattening model is preferred.

4. Why is the prehead expanded?

The λ system is not the only one in which an increase of the head size accompanies DNA packaging. T4, T7 and P22 have proheads that are smaller than the finished heads (review: Casjens & King 1975). In all the cases the prohead is devoid of DNA while the mature head contains DNA. It seems likely then that the DNA collapse, that by bending or kinking consumes energy (Crick & Klug 1975), and the head size increase are coupled processes. Consequently the head should be asked to release energy during expansion. This in turn would mean that in the assembly pathway a structure is first built up with a higher energy content. Already these considerations imply that the protein subunits have a different conformation while assembling than in the final state. This certainly is an oversimplification because there probably are a whole series of sequentially induced conformational changes, each coupled to or triggering the binding, release or processing of a component (Showe & Kellenberger 1975; Kellenberger, this volume p. 3).

The energy release coupled to the head transition might, for instance, be expressed as a temporary vacuum building up inside the expanding head (Hohn *et al.* 1974; Serwer 1975). Anything close to the entrance hole, which in the case of the biological transition will, of course, be the DNA that has complexed to the prohead with the help of the *A* protein, would be sucked in. This model of course predicts that, at least during the expansion, no water can enter the shell through pores, a prediction which is being tested. The strong hydrophobic interactions holding together the subunits of the capsid might be able to accomplish this. Otherwise, or possibly in addition to this sucking in process, sites on the inner surface of the shell might become available during the expansion that attract the DNA.

Finally, the DNA packaging may be linked to ATP hydrolysis. The most likely candidate for an ATPase is the *A* protein which is known to require ATP (Wang & Kaiser 1973; Hohn 1975; Becker & Gold 1975) and which is moreover the important transient link between the DNA and the prehead.

DNA packaging is not linked to core release in the λ system – the core leaves the particles earlier in the pathway (Hohn *et al.* 1975) – and no peptides are found in the finished head (Hendrix, personal communication). The DNA thus cannot collapse with the help of internal peptides as was proposed for T4 (Laemmli, Paulson & Hitchins 1974; Wagner & Laemmli, this volume p. 15).

This work was sponsored by the Swiss National Foundation for Scientific Research, no. 30650.73.

References (Hohn, Wurtz & Hohn)

Becker, A. & Gold, M. 1975 *Proc. Natn. Acad. Sci., U.S.A.* **72**, 581–585.
Bijlenga, R., v. d. Broek, R. & Kellenberger, E. 1974 *J. supramolec. Struct.* **2**, 45–59.
Casjens, S. 1974 *J. molec. Biol.* **90**, 1–23.
Casjens, S. & Hendrix, R. 1974 *J. molec. Biol.* **88**, 535–545.
Casjens, S., Hohn, T. & Kaiser, A. D. 1972 *J. molec. Biol.* **64**, 551–563.
Casjens, S. & King, J. 1975 *A. Rev. Biochem.* **44**, 555–611.
Crick, F. & Klug, A. 1975 *Nature, Lond.* **255**, 530–533.
Hendrix, R. & Casjens, S. 1974 *Proc. Natn. Acad. Sci., U.S.A.* **71**, 1451–1455.

Hendrix, R. & Casjens, S. 1975 *J. molec. Biol.* **91**, 187–200.
Hohn, B. 1975 *J. molec. Biol.* **98**, 93–106.
Hohn, B. & Hohn, T. 1974 *Proc. Natn. Acad. Sci., U.S.A.* **71**, 2372–2376.
Hohn, B., Wurtz, M., Klein, B., Lustig, A. & Hohn, T. 1974 *J. supramolec. Struct.* **2**, 302–317.
Hohn, T., Flick, H. & Hohn, B. 1975 *J. molec. Biol.* **98**, 107–120.
Hohn, T. & Hohn, B. 1973 *J. molec. Biol.* **79**, 649–662.
Hohn, T., Morimasa, T. & Tsugita, A. 1976 *J. molec. Biol.* (in the press).
Kaiser, A. D., Syvanen, M. & Masuda, T. 1975 *J. molec. Biol.* **91**, 175–186.
Karamata, D., Kellenberger, E., Kellenberger, G. & Terzi, M. 1962 *Path. Microbiol.* **25**, 575–585.
Kellenberger, E. 1976 *Phil. Trans. R. Soc. Lond.* B **276**, 3–13 (this volume).
Laemmli, U., Paulson, J. & Hitchins, V. 1974 *J. supramolec. Struct.* **2**, 276–301.
Lickfeld, K., Menge, B., Hohn, B. & Hohn, T. 1976 *J. molec. Biol.* **103**, 299–318.
Mazza, A. & Felluga, B. 1973 *J. Ultrastruct. Res.* **45**, 259–278.
Murialdo, H. & Siminovitch, L. 1972 *Virology* **48**, 785–823.
Ray, P. & Murialdo, H. 1975 *Virology* **64**, 247–263.
Serwer, P. 1975 *J. molec. Biol.* **92**, 433–448.
Showe, M. & Kellenberger, E. 1975 In *Control processes in virus multiplication* (ed. D. C. Burke & W. C. Russel), pp. 407–438. Cambridge University Press.
Sternberg, N. & Weisberg, R. 1975 *Nature, Lond.* **256**, 97–103.
Wagner, J. & Laemmli, U. 1976 *Phil. Trans. R. Soc. Lond.* B **276**, 15–28 (this volume).
Wake, R. G., Kaiser, A. D. & Inman, R. B. 1972 *J. molec. Biol.* **64**, 518–540.
Wang, J. C. & Brezinsky, D. P. 1973 *Proc. Natn. Acad. Sci., U.S.A.* **70**, 2667–2670.
Wang, J. C. & Kaiser, A. D. 1973 *Nature New Biol.* **241**, 16–18.
Weigle, J. 1966 *Proc. natn. Acad. Sci. U.S.A.* **55**, 1462–1466.
Williams, R. & Richards, K. 1974 *J. molec. Biol.* **88**, 547–550.
Wurtz, M., Kistler, J. & Hohn, T. 1976 *J. molec. Biol.* **101**, 39–56.

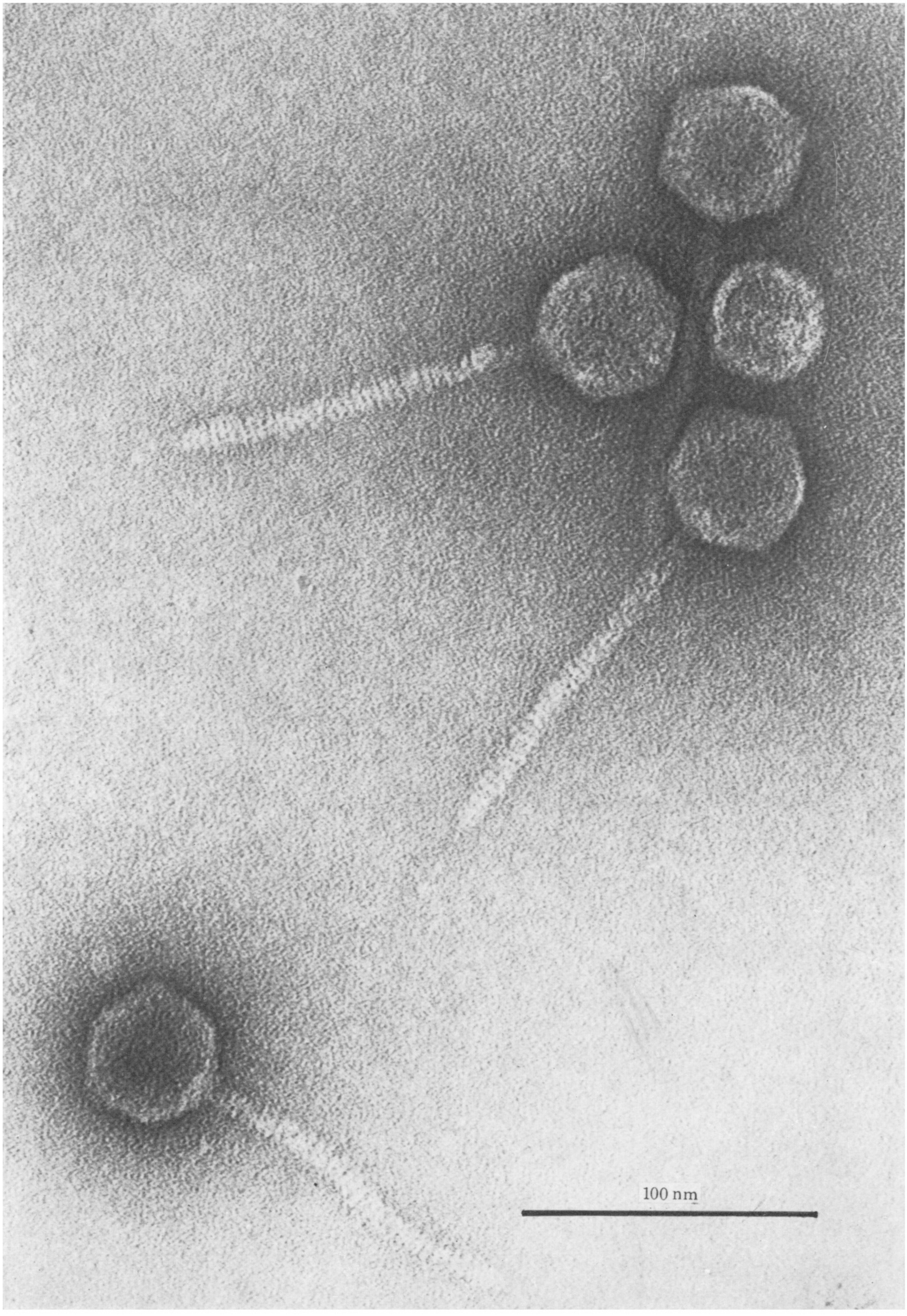

FIGURE 3. Preheads and finished phage. Negative stain with uranyl acetate.

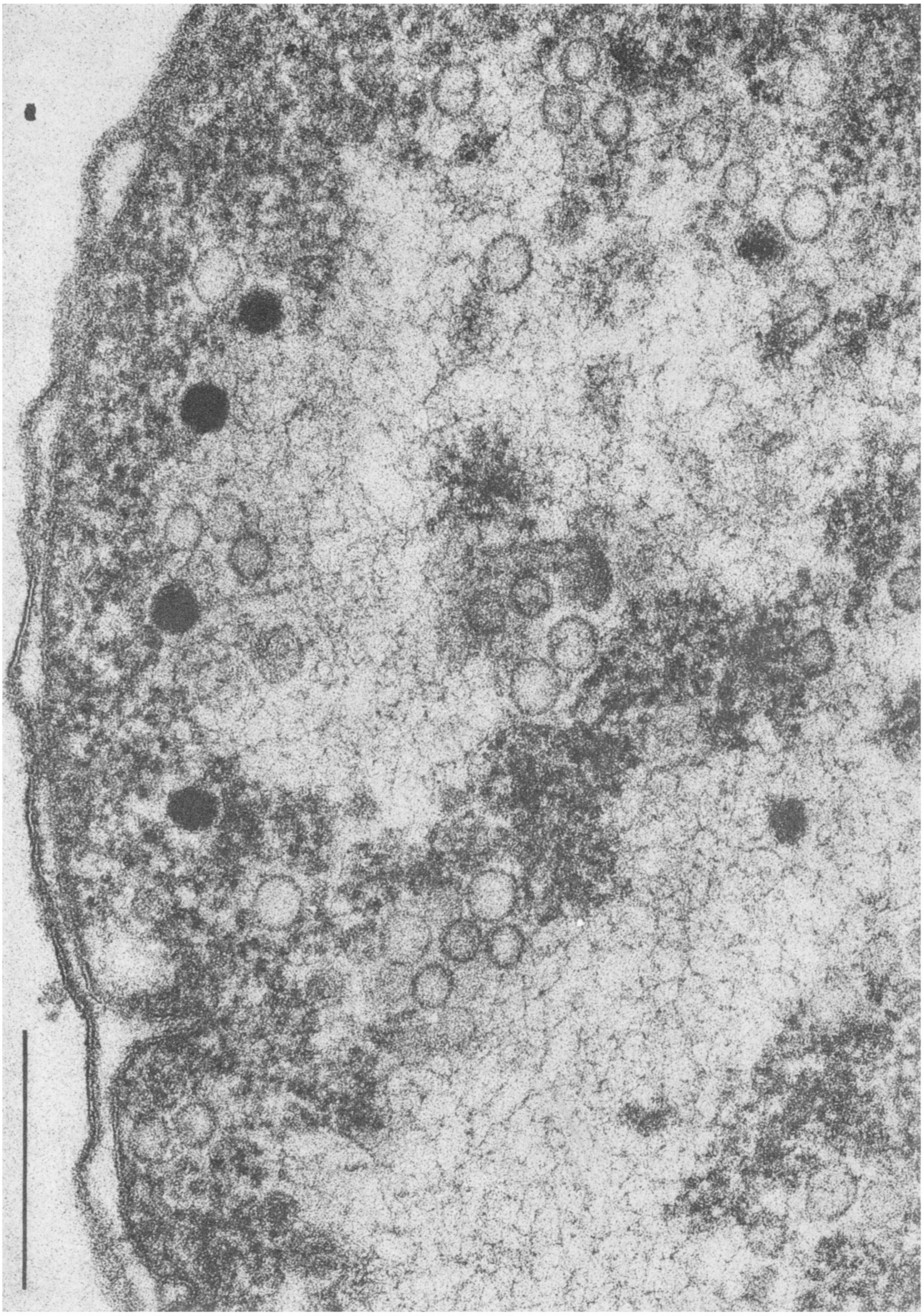

FIGURE 4. Thin sections of a λ wild type lysogen, 120 min after induction. A detailed procedure will be published elsewhere (Lickfield *et al.* 1976). Petit λ (the small white particles), empty heads (the large white particles) and phage heads (the black particles), one of it with tail. (Scale: 250 nm. Electron micrograph by K. Lickfeld & B. Menge.)

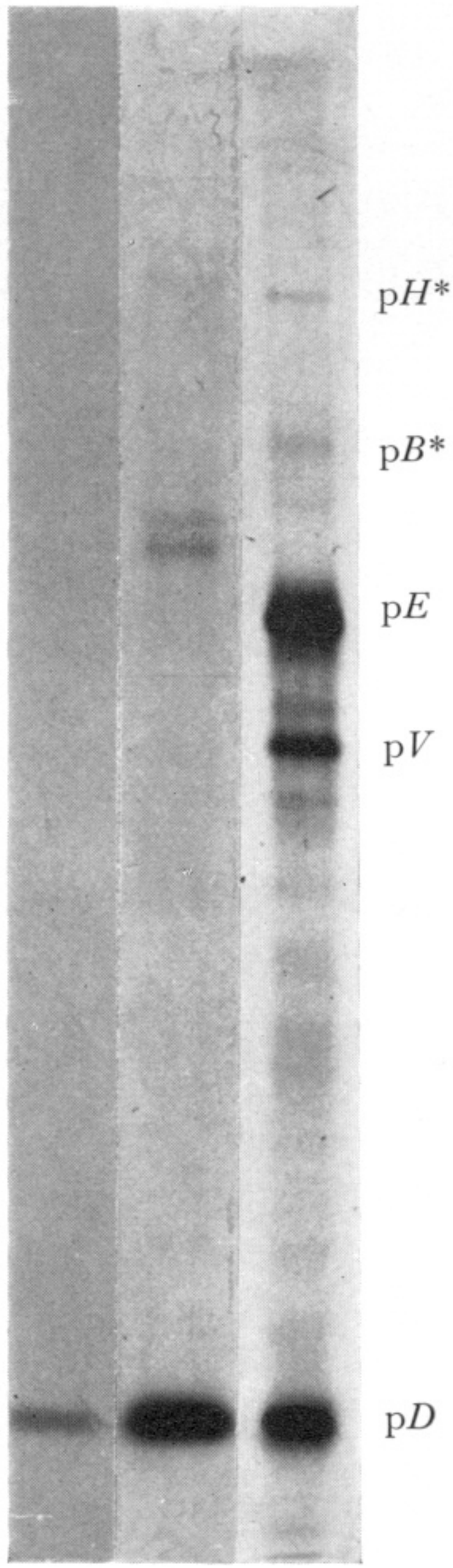

FIGURE 7. Binding of radioactive proteins to preheads and artificially expanded preheads. A^- preheads and the enlarged form thereof, obtained after treatment with 4 M urea and subsequent dialysis in buffer, were reacted with an E^- extract radioactively labelled with ^{35}S according to the procedure described (Hohn *et al.* 1975), freed from large molecular mass material by 16 h centrifugation in an International Centrifuge A321 rotor, 55000 rev./min, dialysis against H_2O, lyophilization and solution in buffer. After this reaction particles were freed from excess radioactive material by sucrose gradient centrifugation and assayed on a 12% SDS acrylamide gel as described by Hohn *et al.* (1975). (*a*) preheads + ^{35}S proteins; (*b*) enlarged preheads + ^{35}S proteins; (*c*) totally labelled ^{35}S phage.

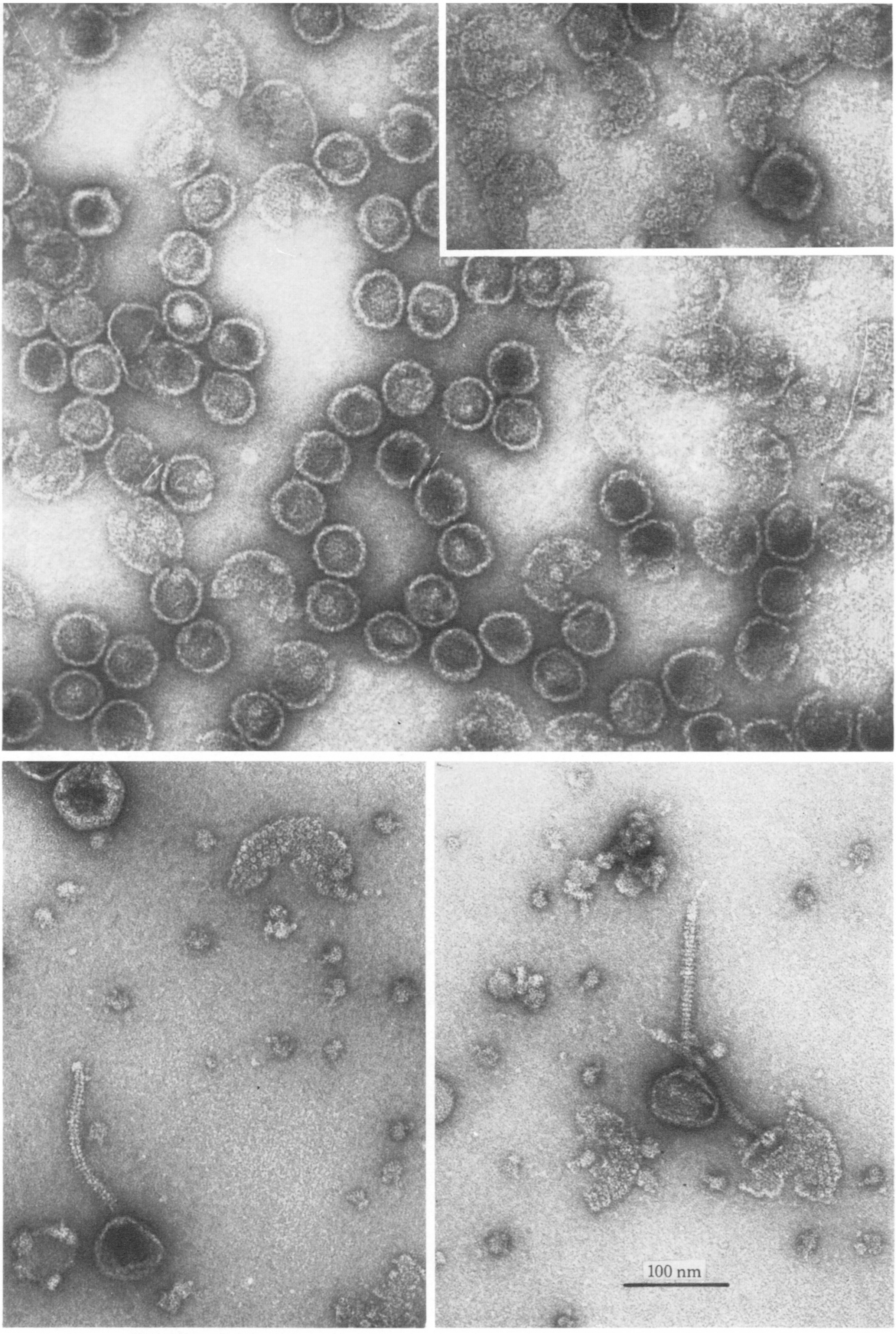

FIGURE 8. Subunit clustering visible on squashed petit λ (upper part) and squashed heads (lower part).

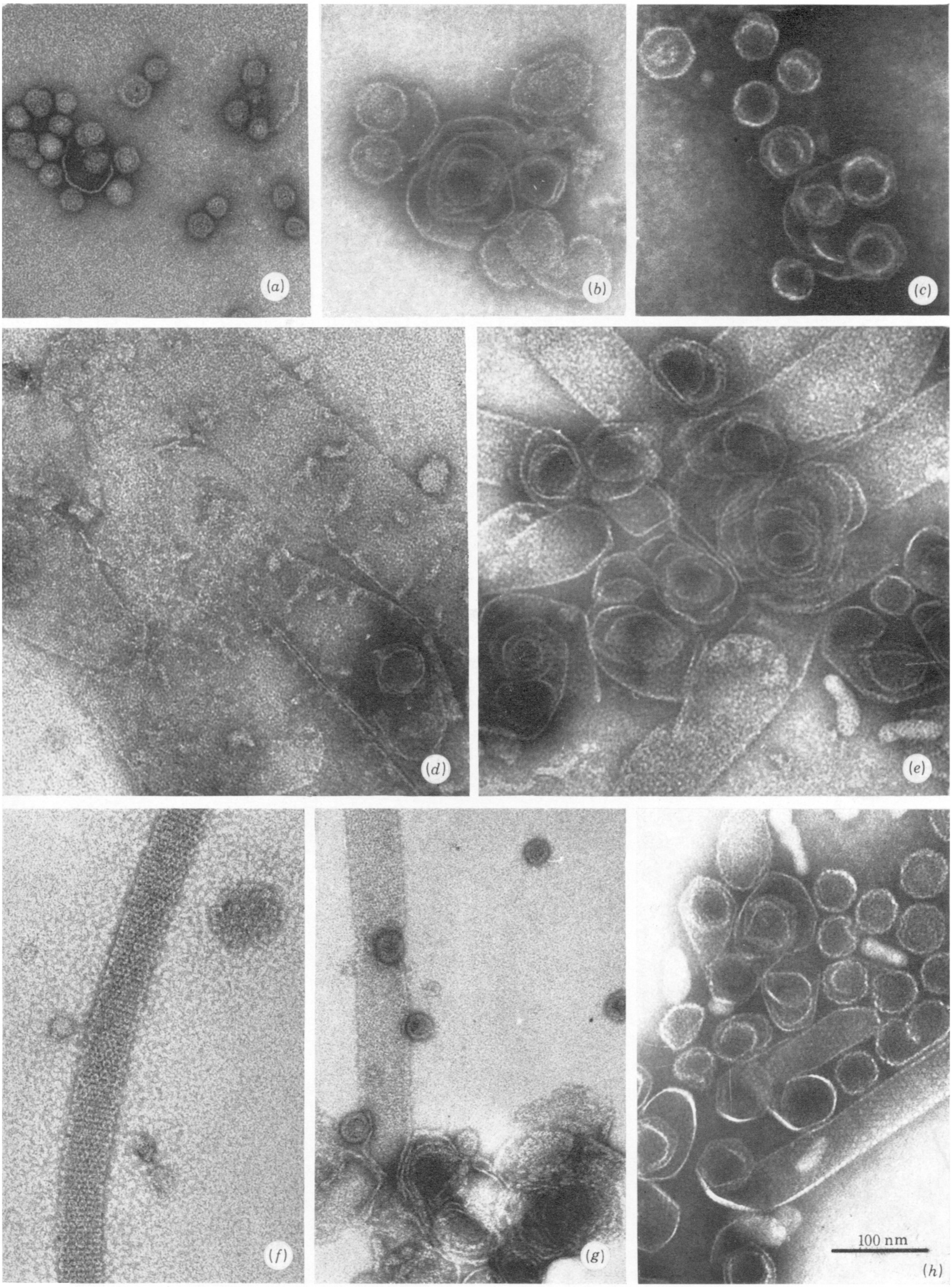

FIGURE 9. Total mixture of reassembled particles showing petit λ (*c*, *h*), 'petit petit λ' (*a*, *g*), empty heads (for example larger hexagonal particle in *c*), sheets (*d*, *e*), spirals (*b*, *e*, *g*), 'peanuts' (*h*) and polyheads (*f*, *g*, *h*). DNA free phage heads were disassociated with guanidinium hydrochloride and reassembly allowed by dilution with buffer followed by pressure dialysis, as described by Wurtz *et al.* 1976.

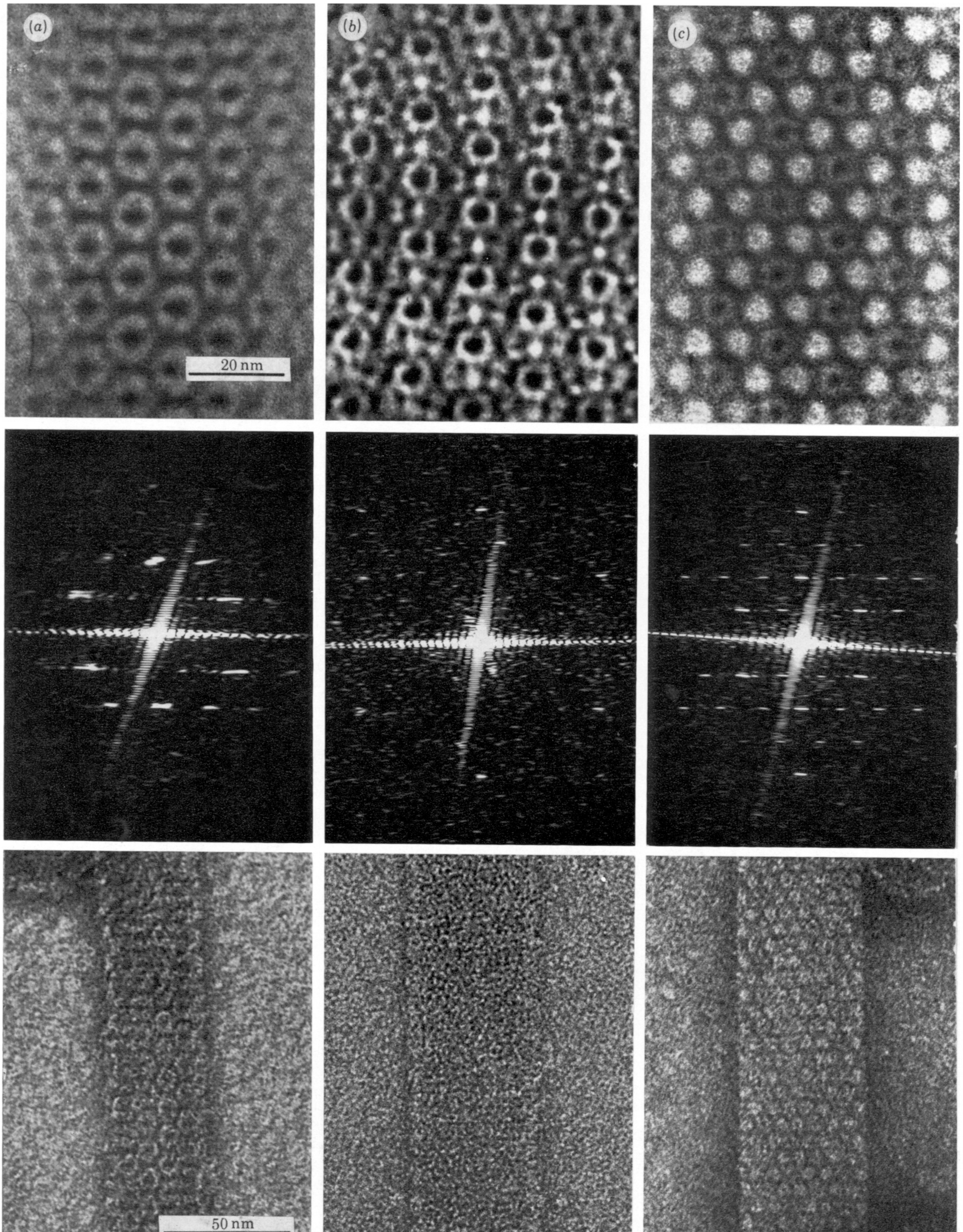

FIGURE 10. A-, B- and C-type polyheads, negatively stained (bottom row), diffracted (middle row) and optically filtered (top row).

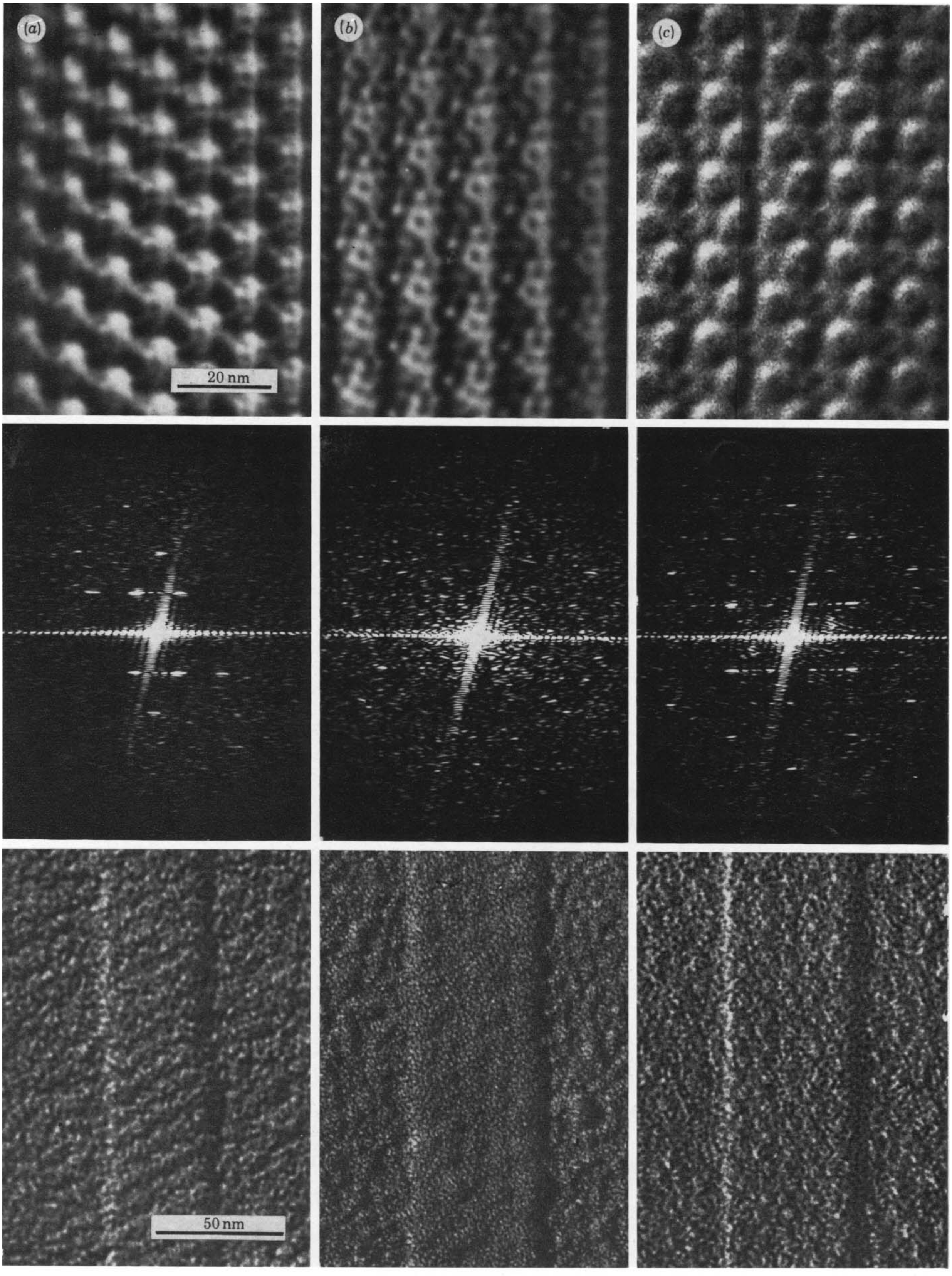

FIGURE 11. A-, B- and C-type polyheads, freeze dried and shadowed (bottom row, electron micrographs by J. Kistler). Diffraction in middle row and image reconstruction in top row.

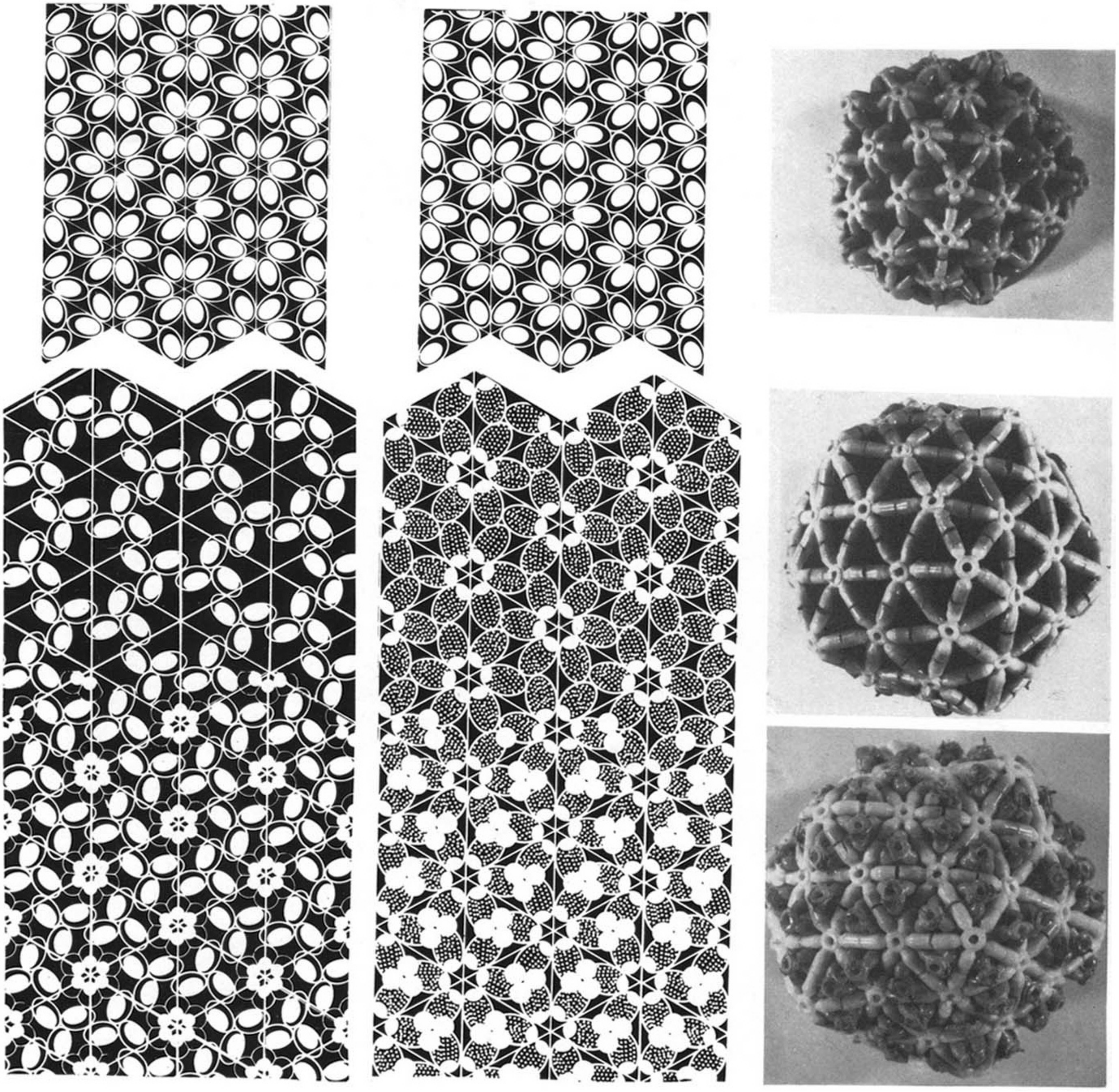

FIGURE 12. Hypothetical lattices (left two columns) and geodesic models (right column) of *E* protein subunits in preheads (top line), *E* protein subunits in expanded preheads (middle line) and *E* and *D* protein subunits in heads (lower line). The black areas of the molecules in the lattice, should represent the protruding parts of the molecules whereas the thin lines should illustrate the molecule contours and thus the intermolecular contacts. The orientation angle of the capsomer in respect to the lattice lines is arbitrary. See text for discussion.