BACTERIOLOGICAL REVIEWS, June 1975, p. 314-359
Vol. 40, No. 2

Printed in U.S.A.

Head Length Control in T4 Bacteriophage Morphogenesis: Effect of Canavanine on Assembly

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INTRODUCTION

The assembly of macromolecules into subcellular structures has recently been the subject of several reviews (5, 20, 54, 76). Included for discussion were microtubules, flagella, ribosomes, and, not surprisingly, viruses. Almost from the time they were first visualized in the electron microscope, viruses have generated a great deal of excitement with regard to their structure and mechanisms of assembly. In 1950, Crane (27) predicted that most rod-shaped structures would have helical symmetry. He further forecasted that stages of subassembly intermediates would be essential in achieving the final product. These predictions have been amply borne out with regard to tobacco mosaic virus (75). Later, Crick and Watson (28) considered those viruses described as having spherical or cubic symmetry. By analyzing the properties of tetrahedral, cubic, octahedral, and icosahedral crystallographic forms, Crick and Watson calculated that each of these forms had some multiple of 12 asymmetric units and predicted that spherical viruses would have a similar arrangement. These considerations have proved to be quite accurate. Williams and Smith (116) were able to show, using double shadowing techniques, that Tipula iridescent virus clearly had icosahedral symmetry. Caspar and Klug (21), utilizing the mathematical relationships derived by Goldberg (51), presented a comprehensive theory of spherical virus structure which preserved the basic elements of icosahedral symmetry and allowed for the enormous size range among the spherical viruses. Their theory stated that all spherical viruses are composed of $20T$ triangular faces, where T was defined by the sum of $H^2 + HK + K^2$, and H and K can be any two integers. Further, they determined that all icosahedral viruses would have 12 and only 12 pentamer vertices of triangular faces. Additional faces would necessarily have hexamer vertices. The total number of pentamer and hexamer vertices would be given by the quantity $10T + 2$. Obviously, certain multiples of the 20 triangular faces contained in the simple icosahedron were excluded. This theory has withstood the test of time. Even in cases where some viruses were thought not to have icosahedral symmetry (e.g., Bradley [17] presented micrographs which suggested that the head of bacteriophage T5 possessed octahedral symmetry), later work indicated icosahedral

symmetry (119). This has also been found to be true for some of the non-isometric bacteriophage heads (18, 92).

A real conceptual advantage of reducing the structure of viruses to either helical or icosahedral symmetry was the hope that self-assembly of the component molecules would be the essential mechanism of construction. Although much success on in vitro assembly has been achieved with some of the more simple viruses (see Klug [75] for the assembly of tobacco mosaic virus, and Hohn and Hohn [56] and Bancroft [6] for discussions of the in vitro assembly of many simple ribonucleic acid [RNA] viruses), Thompson (110) and Bernal (8) recognized that there was a radical difference between symmetry observed in organisms (and viruses) and that determined in crystallographic structures. In the case of biological entities, symmetry should be considered not so much a product of the shapes of molecules but rather of the rate of growth of the structure. Moreover, the enormous complexity of the structures of some viruses was not appreciated until better techniques for visualization were developed. It has become increasingly evident that assembly of the complex viruses involves interactions between many gene products, some of which are contained within the final capsid and some which are not (20, 54). Assembly is now thought to be a pathway of events that may involve the maturation of intermediate capsid-like structures (proviruses) leading eventually to the completed infectious virion. Morphogenetic cleavage reactions, packaging of the nucleic acid, and capsid size determination are processes that are only now becoming clarified and understood. In this review, we will concentrate on these processes as they occur in T-even bacteriophages of *Escherichia coli*, with a special emphasis on capsid size determination. Other viruses will be discussed mainly for comparison with the T-even bacteriophage.

Preamble to Capsid Size Determination

In 1972, Kellenberger (68) summarized some of the possible molecular mechanisms by which the size or length of viral capsids and their substructures could be determined. Three general models were presented.

(i) Template model: the length of a rod-shaped or tubular structure is determined by a template, for example, a given length of nucleic acid or of a fibrous protein. Certainly, the best-known example of this type of length control is that of tobacco mosaic virus: the length of the RNA determines the length of the helical rod-like virus (44). More recently, the template model has been implicated in the length determination of bacteriophage λ tails (64). The tail normally is about 150 nm long and is attached at one end to the icosahedral head and at the other to a small basal protein structure and a tail fiber. At least 11 genes control λ tail morphogenesis and one of these, V, codes for the major tail protein. In lysates obtained from the growth of λ defective in gene U, polytails are found (Fig. 1), up to 5 μm in length. Katsura and Kuhl (64) propose that tails are assembled via a complex between pU and pV (throughout this review, the standard procedure for identifying gene products will be used: the gene symbol will be preceded by lower case p, i.e., pU is the product of gene U), via a fibrous material of precise length attached to the basal structure. When pU is defective, the interaction is altered such that pV continues to polymerize. Similarly, in T4 bacteriophage tail tube assembly, King (72) has proposed that the length of tail tubes is determined by a fibrous substance protruding from the baseplate which interacts with the single-tube protein subunit (p19). Alternatively, the length of the tube could be controlled by "induced strain" of the subunits (see below). King based his model on the observation that polytail tubes are rare (but see below, the description of polytail tubes induced by an amino acid analogue) and, therefore, the prescribed length must be under strict genetic control, possibly in genes 48 and 54, genes known to be essential for T4 baseplate assembly (72).

(ii) The vernier model: well before it was known that separate pathways for the assembly of head and tail structures existed, Anderson and Stephens (4) speculated on the length determination of T6 bacteriophage tails. Based on decomposition studies, they proposed that if sheath and tail were assembled together but that each were to be ultimately composed of a different number of subunits n for one and m for the other, and n and m had no common denominator, then the precise length of the tail (sheath and tube) would be determined when n and m were in register, like the lines of a vernier, to fit the baseplate. Length at the other end would be similarly determined by attachment of the head. No explicit examples are currently known which fit this tube-within-a-tube model, but one could imagine a vernier calibration for completing the closure of an icosahedral capsid. This may be especially relevant in those viruses (T4, P22) in which a core is known to occur within the viral structure. From a slightly different perspective, the model of Kirschner and Williams (74) for microtubule assembly resembles the vernier model. Micro-

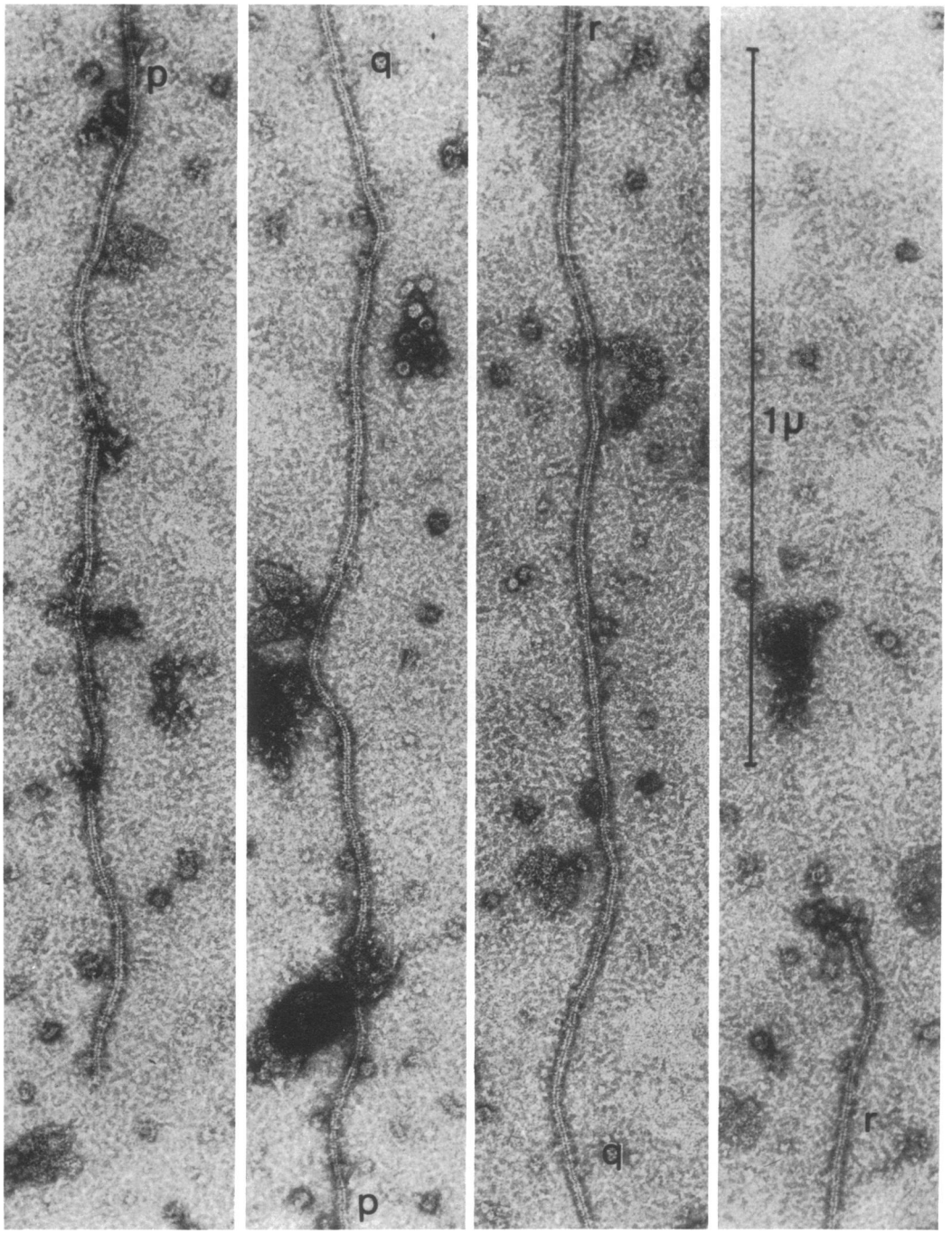

FIG. 1. *Electron micrographs of a giant λ polytube in a U⁻H⁻ lysate. This polytube is one of the shortest in U⁻H⁻ lysates. However, it is still so long that four micrographs were necessary to show the complete structure. Normal λ tails are only 150 nm long. The letters on the micrographs show the corresponding positions of the polytube. (Kindly supplied by I. Katsura [64] and reproduced here with the permission of the publisher, Alan R. Liss, Inc., New York.)*

tubules (Fig. 2) consist of two types of subunits and, upon depolymerization and polymerization, specific structures are observed. Kirschner and Williams depicted microtubules as an array of parallel protofilaments (Fig. 3) that are capable of forming ringlike structures. Whether the double ring illustrated is an actual intermediate in assembly is not known, but the parallel with the venier model is obvious.

(iii) The model of cumulated strain: this model is based on the concept that interacting subunits "deform" each other by virtue of their interaction. During assembly, protein subunits induce conformational changes in each other

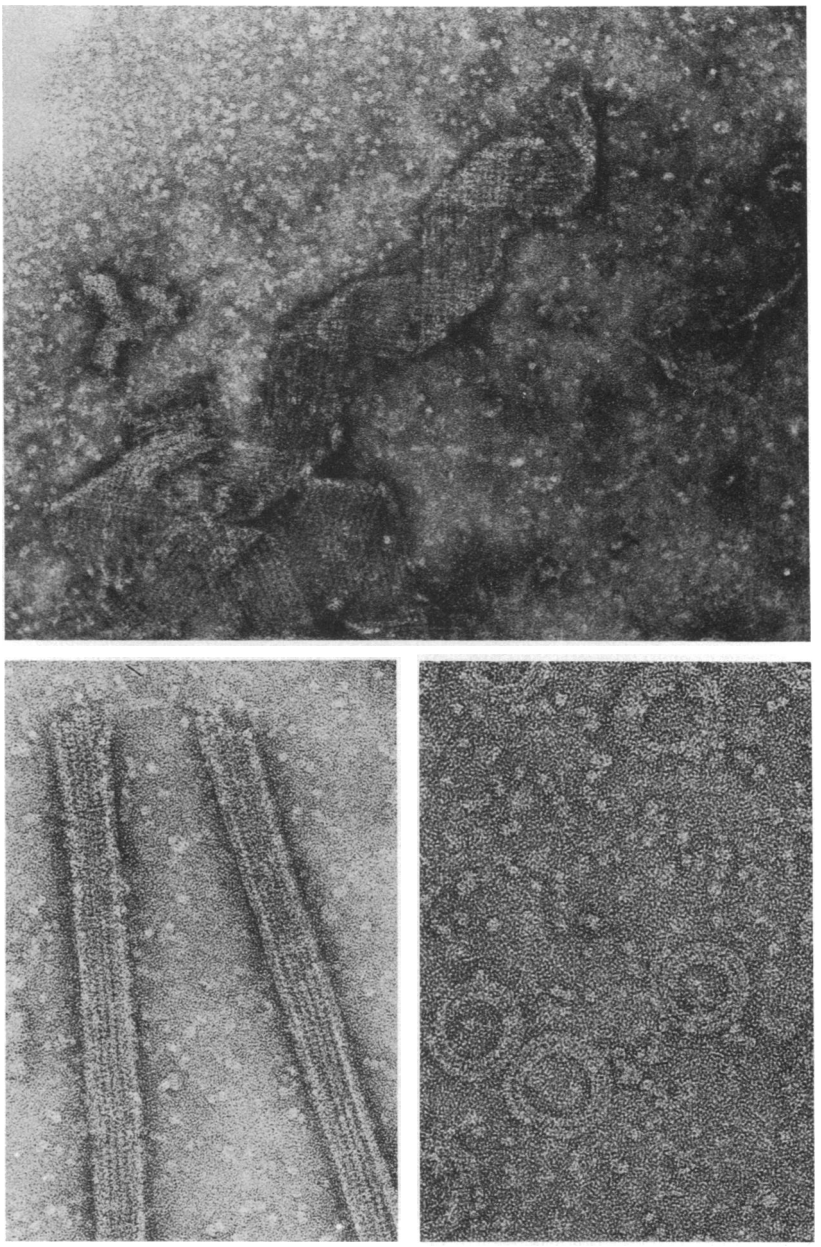

FIG. 2. *Electron micrographs of forms exhibited by microtubules. Lower left, normal tubes; lower right, repolymerized rings or spirals; and upper panel, repolymerized ribbons. (Kindly supplied by M. W. Kirschner [74] and reproduced here with the permission of the publisher, Alan R. Liss, Inc., New York.)*

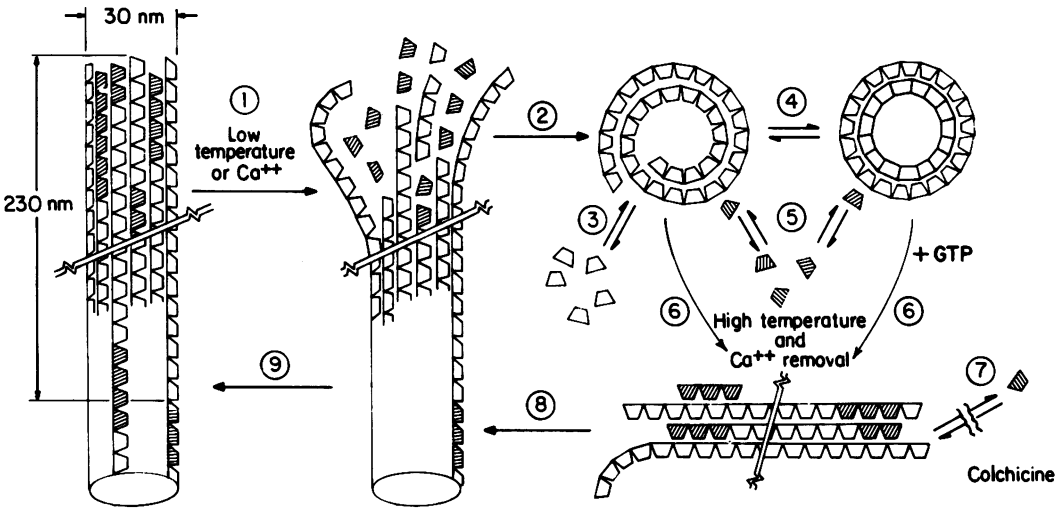

FIG. 3. *Model for microtubule polymerization and repolymerization. Note the two types of subunits and the concentric rings. (Kindly supplied by M. W. Kirschner [74] and reproduced here with the permission of the publisher, Alan R. Liss, Inc., New York.)*

and also distribute strain all about the part of the structure already assembled. As assembly proceeds, this "cumulated strain" could have two effects. (a) In the case of helical structures such as bacteriophage tails or tail tubes, the strain could so deform the final subunit as to make it unavailable for the addition of other subunits and hence bring about termination of the rod. (b) For spherical structures, the "cumulated strain" could compel the partially finished structure to close, and for each virus the shape of the particular gene products would be different and hence different size structures would result. In this respect, the subunits need not all be identical. In fact, interactions between different gene products could contribute to the strain and determine the size of the capsid. There are two recent examples that may illustrate this. Satellite bacteriophage P4 requires a helper phage to achieve viral multiplication (53, 100). P4 relies on all the head and tail genes of the prophage helper in the production of viral particles. This system provides an opportunity for studying capsid size determination since P2 and P4 have different size heads (Fig. 4) even though they contain essentially the same proteins. In deciding between the possible alternatives, Pruss et al. (100) suspected that the different genome sizes might determine head size; to study this, they examined deoxyribonucleic acid (DNA) packaging in vitro. They found that the smaller P4 genome could be packaged into P2 heads but that the P2 genome could not be packed into P4 heads. In fact, their data suggested that multiple copies of the P4 genome could be packaged into P2 heads. They concluded that the size of the genome did not determine the head size. Examination of the protein components of the head by sodium dodecyl sulfate (SDS)-polyacrylamide gels revealed that P4 heads contained a protein not present in the larger P2 heads. They calculated that there were about 45 copies of this protein in each head. Since P4 has a T number of 4, this means that there are 42 vertices ($10T$ + 2 [21]), a number not too dissimilar from the number of copies. Figure 5 illustrates the possible locations of this protein within the icosahedral head of P4. Pruss et al. (100) conclude that the placement of this protein determined head size. Why, in the absence of this protein, P2 has a larger head (T = 9) has not yet been clarified, but one could imagine that a change in the cumulated strain was responsible. Another example again involves bacteriophage λ. This bacteriophage can give rise to two head sizes, normal and petite. The petite heads are devoid of DNA and are not attached to tails. Similar head structures (Fig. 6) have been seen in lysates prepared using bacteriophage Mu-1 (91), but these have not yet been analyzed. The aberrant λ heads are composed of the major λ head protein, pE, but lack pD, the second major component of normal heads (19). Howatson and Kemp (60), by studying tubular forms of these structures, postulate that both types of heads have a T number of 7 and that petite λ heads serve as precursors to mature heads. Upon the

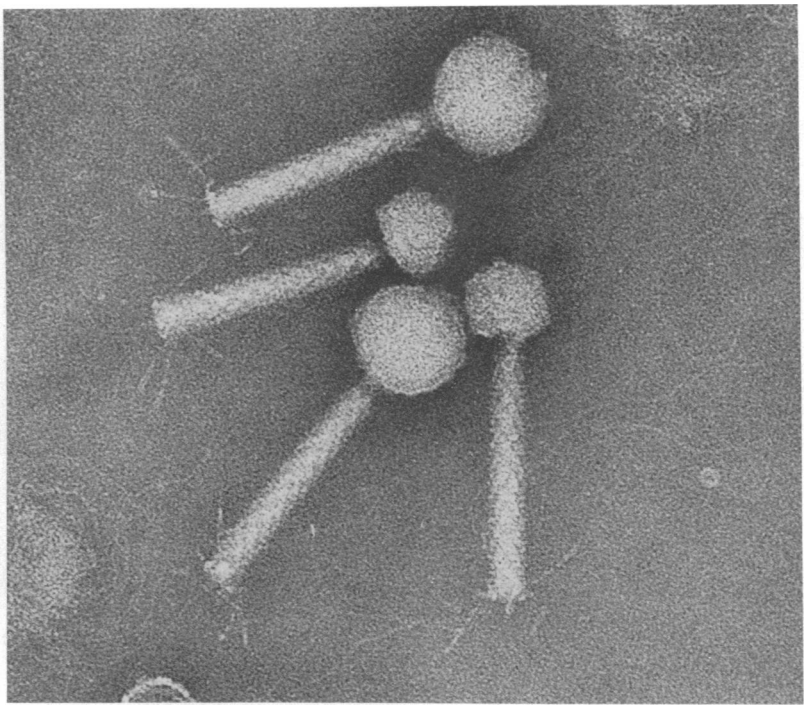

FIG. 4. *Electron micrograph of P2 and P4 bacteriophages. The P2 head is 62 nm in diameter, whereas the P4 head has a diameter of 45 nm (100). (Made available to us through the courtesy of R. Calendar.)*

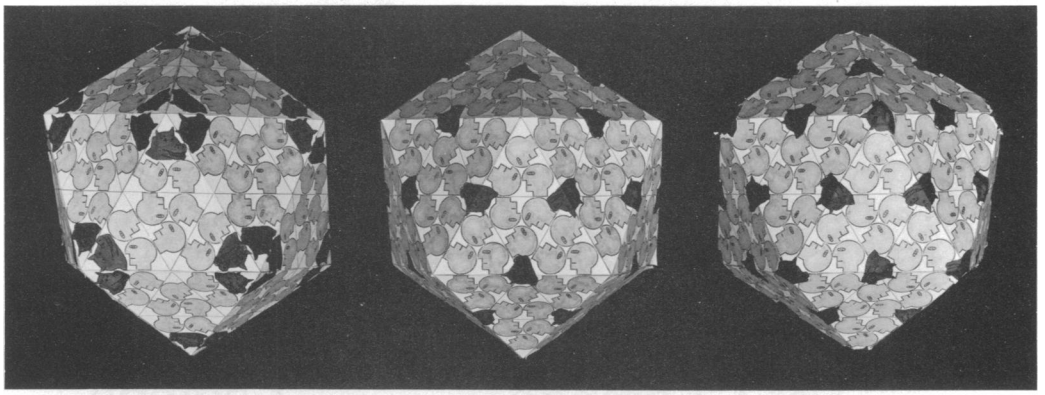

FIG. 5. *A model depicting the possible placements of the putative P4 size-determining protein. The human heads represent the major capsid protein, whereas the hippopotamus heads represent P4 protein 4. Note the different arrangement of p4 at the fivefold and sixfold vertices of this T = 4 icosahedron. (Made available to us through the courtesy of R. Calendar.)*

addition of pD, the pE molecules in the head are thought to rearrange from clusters of hexamers and pentamers to trimers (Fig. 7). A similar rearrangement was suggested by Williams and Richards (115). Hohn et al. (55) presented evidence that the petite heads remained petite in size even after addition of pD and that head enlargement occurs at the time of DNA packaging. They suggest that the actual enlargement might provide the force necessary to "suck in" part of the DNA.

The two cases we have described illustrate the difficulty of making simple analogies even between two phages of similar morphological complexity. In the case of the P2-P4 system, the small head contains an extra structural pro-

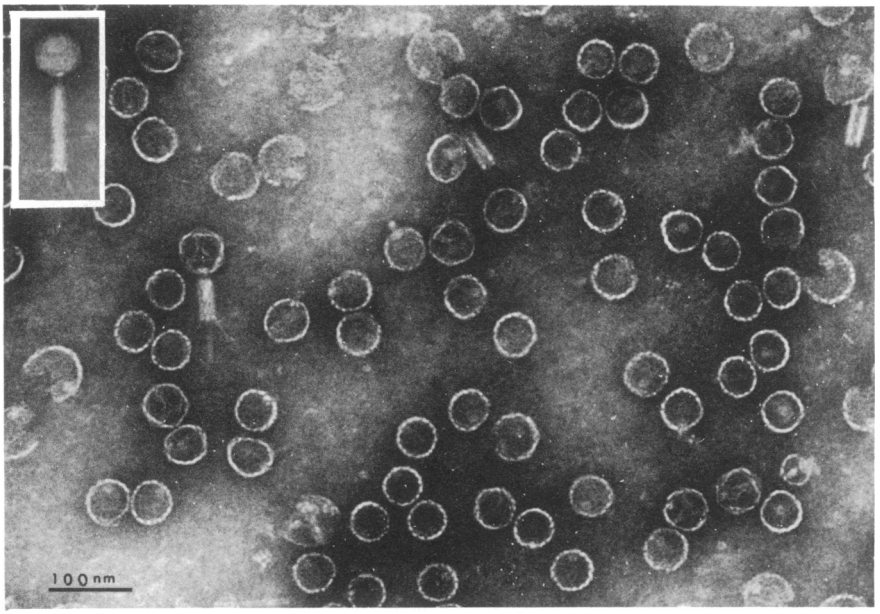

FIG. 6. *Electron micrograph of Mu-1 petite bacteriophage heads. The insert represents a normal Mu-1 virion (91).*

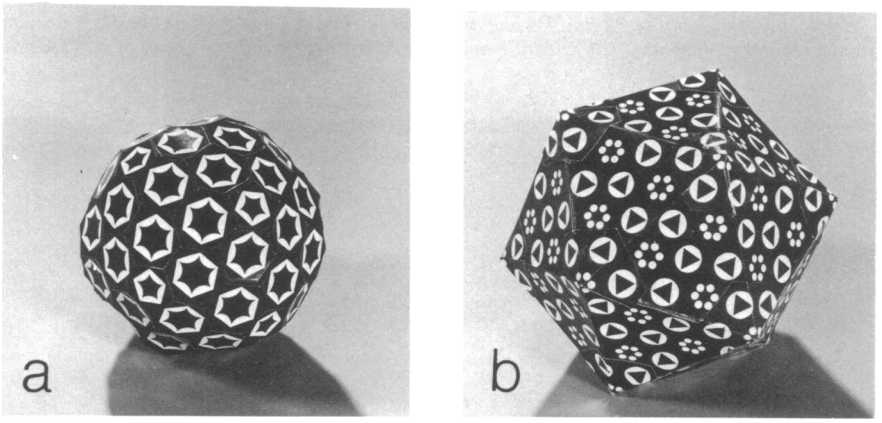

FIG. 7. *Models of petite λ and normal λ capsids photographed in the same orientation. T = 7 levo arrangement. Protein E molecules (circle segments) form clusters of hexamers and pentamers in the petite λ model and trimers in the λ model. Protein D molecules (circles) form clusters of hexamers and pentamers in the λ model. (Kindly supplied to us by A. F. Howatson [60] and reproduced here with the permission of the publisher, Academic Press Inc., New York.)*

tein, whereas in the λ-λ petite system the small head lacks the extra structural proteins. This possible alteration of the structural components by the addition or deletion of other proteins is the primary thrust of the "cumulated strain" model.

T4 HEAD MORPHOGENESIS

Thus far, we have discussed in general terms the problems and concepts involved in the assembly of viruses, without defining some of the steps in the assembly pathways. Casjens and

King (20) heroically reviewed the steps in assembly of a vast variety of viruses: bacteriophages λ, P22, T3, T4, and T5, as well as poliovirus, adenovirus, herpesvirus, etc. What came across very clearly in their discussions was that although each of these viruses has unique properties, striking similarities also exist. For the remainder of our review, we will concentrate on the T-even bacteriophages as models for the assembly of macromolecules, with references to other viruses as required. The T-even bacteriophage embodies most of the features of other viruses in possessing both spherical and helical substructures. We will not discuss T4 tail fiber, sheath, or baseplate assembly, since this has been reviewed elsewhere (10, 20).

Gene Function and Aberrant Structures

The genes involved in the formation of T4 bacteriophage heads can be divided into two groups (40, 82). Group A genes are required for early assembly steps, and the group B genes are involved with the subsequent maturation of the assembled head. The group A genes are comprised of genes 20, 21, 22, 23, 24, 31, 40, and the genes coding for the three head internal proteins. Several of these genes code for proteins that undergo cleavage (36, 58, 70, 77) during head assembly, and a mutation in any one of the group A genes, except the internal protein genes (11, 103), will result in a block of all of the other cleavage reactions. p23, p24, and IPIII are all cleaved to smaller-molecular-weight proteins during assembly (36, 58, 70, 77), whereas p22 is cleaved to acid-soluble polypeptide fragments (36, 58). The cleavage of IPIII and p22 is thought to accompany DNA packaging and in fact may provide the force required for packaging (82). This is similar to the role of the so-called scaffolding protein, p8, in bacteriophage P22 assembly (20).

Infection with a phage containing a mutation in a group A gene generally results in the accumulation of a characteristic aberrant head-related particle in the infected cell (40, 81). Gene 23 codes for the major head protein (41, 67, 101), and infection with an amber mutant in this gene fails to produce head-related structures, whereas temperature-sensitive mutants grown at nonpermissive temperatures result in the formation of deformed heads (41, 81). Certain missense mutations in gene 23 result in the random formation of petite (39) and giant head particles (38). (We will examine the properties of these particles, particularly the giants, in more detail in a later section.) This suggests that p23 is a component part of a complex that ultimately determines the length of the head. Unfortunately, these mutants are not conditionally lethal and this makes difficult their use in studies on size determination. The ordered assembly of p23 into recognizable head structures requires p31 (78) and a host factor (24, 50, 109). p31 interacts with the host factor to prevent the random association of p23 into "lumps" on the cell membrane, and p31 has been termed a solubilization factor (78). The width of the virus particle is probably determined (81) by the "core" (69); the core, as described previously, is a complex comprised of p22 and the internal proteins (103). Without this essential core, p23 is assembled into multilayered polyheads (81) that can separate into individual tubes when isolated (118). A polyhead is a long, tubular head-related structure comprised primarily of p23 in its uncleaved state. (Throughout, symbols for uncleaved gene products will look like this: p23; the cleaved state will be denoted like this: p23*.) Some of the properties of polyheads, core, etc., have been reviewed previously (33, 82), and we will show micrographs of these structures in a later section. Although there is good morphological evidence (81) that the core is responsible for determining the width of the virus head, no direct evidence has been presented that it is involved in length determination.

Defects in the internal protein genes are not lethal, although these proteins are required for maximal efficiency of the assembly of the phage particles (11, 103). The absence of the internal proteins causes the accumulation of some polyheads, and at least one internal protein, IPIII, is required for the formation of multilayered polyheads that arise in gene 22-defective infected cells (81). In addition, some internal proteins are required for the assembly of giant phage particles (32). These results indicate that the core is of primary importance in the establishment of stable intermediates during head formation. Defects in genes 20 or 40 lead to the accumulation of primarily single-layered polyheads (41, 81). Gene 40 is unique in that it is the only group A gene localized in the region of the genome devoted to DNA synthesis (117); amber mutants in gene 40 have not been isolated, although temperature-sensitive mutants do exist. A relationship between the unidentified gene 40 product and DNA synthesis has not been established. It has been postulated that gene 20 (and 40) is required for the formation of the hemispherical caps at the ends of the head structure (81).

Defects in genes 21 or 24 result in the formation of head particles termed "tau-particles" (40, 69, 81). The tau-particle (Fig. 8) is thought to represent a prohead intermediate (79, 82), is

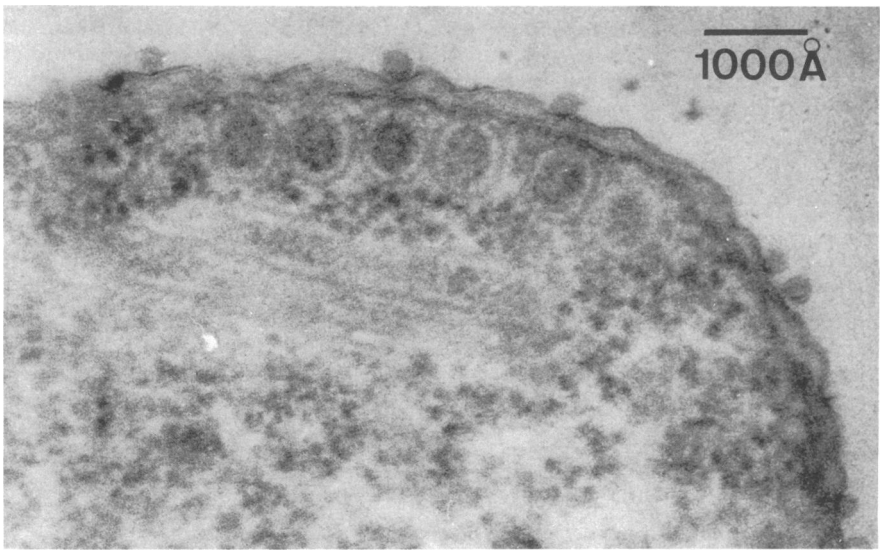

FIG. 8. *Electron micrograph of a thin section of E. coli B infected with a mutant in gene 24 (T4D 24 [amB26]). The phenotype of such an infection is the production of tau-particles and polyheads. This micrograph shows a group of membrane-associated tau-particles, as well as longitudinal sections of polyheads. (Kindly provided by the Information Theory Group, Basel.)*

of uniform size, and is synthesized at the bacterial membrane (1, 104). p24* is a component of the phage particle (77) and certain temperature-sensitive mutants in gene 24 grown at intermediate temperatures give rise to giant phage particles, although the majority of particles consist of normal phage (1). p21 has not yet been identified, although gene 21 is believed to code for a T4-induced protease activity (16, 52, 83, 95). Gene 21 is required for the in vitro cleavage of p22 (95), p23, and the internal protein IPIII (83). Cleavage of p24 in vitro has not been observed.

The group B genes are genes 2, 4, 13, 14, 16, 17, 49, 50, 64, and 65. Several of these genes (genes 2, 50, 64, 65) do affect the cleavage of the head proteins described above (77; R. A. Johnson and U. K. Laemmli, quoted in reference 82). However, only gene 50 affects the cleavage of the structural proteins in an assembled structure. Defects in gene 50 lead to the accumulation of particles in which p24 is not cleaved. Apparently, the majority of the group B mutations can affect the efficiency of head assembly. The findings that p24 is the only protein not observed to undergo cleavage in particles resulting from infection with T4 defective in gene 50 and that p24 is the only protein not observed to undergo cleavage in vitro may indicate that the cleavage of p24 is under a different control than the cleavages of p22, p23, and the head internal proteins. Defects in genes 16 and 17 lead to the accumulation of empty heads (71) that are composed of cleaved proteins (77, 79, 113). It is thought that these genes may be required for attachment of the replicative DNA to the phage prohead prior to DNA packaging (79, 82). Gene 49 defects result in an accumulation of partially filled heads (71, 79, 87), and the replicative DNA pool accumulates as 1,000S units rather than as the normal 200S intermediate (2, 46). It has been implied that p49 is an endonuclease responsible for clipping the DNA protruding from the filled phage head (46).

Prohead Assembly Pathway

Based on electron microscopy evidence, it is clear that distinct head-related structures developmentally precede the mature viral head. When first assembled, the head proteins are in an uncleaved state, and different proteins are cleaved during specific steps of the maturation process. Laemmli et al. (82) summarized the known properties of T4 head assembly in the form of a prohead pathway that is presented, slightly modified, in Fig. 9. It must be emphasized that some critical aspects of this pathway are still tentative; for example, Kellenberger and his collaborators (personal communication) have recently observed that some head precursors contain uncleaved p23 and are filled with DNA. This is not compatible with the scheme in Fig. 9. A provirus scheme such as this has

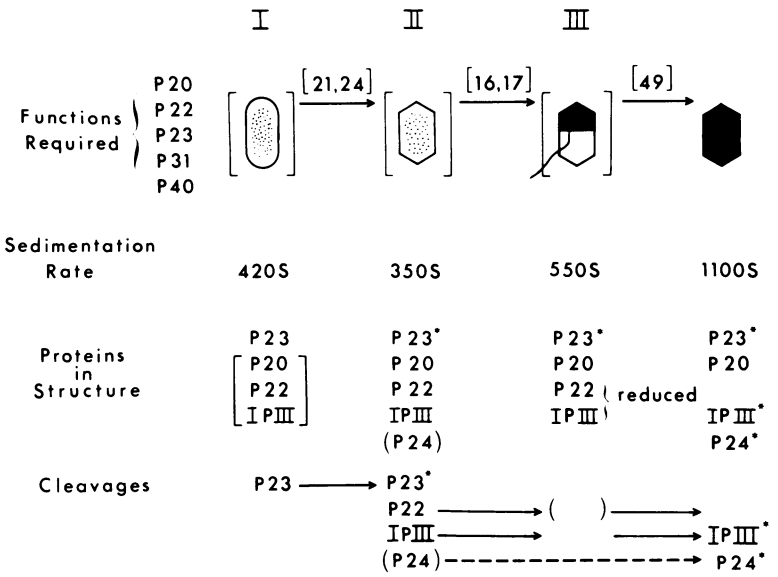

FIG. 9. *Prohead assembly pathway of T-even bacteriophage head assembly. Diagram illustrates the major features of head morphogenesis and was adapted from that of Laemmli et al. (79, 82). Only the group A genes are listed. The brackets denote gene functions which are somewhat speculative, and the dotted line denotes the cleavage reaction of p24, which is still unknown.*

also been generated for λ, P22, poliovirus, and adenovirus (see reference 20). For T4, three proheads are described primarily on the basis of data from pulse-labeling experiments, as well as sedimentation analysis and protein composition studies (79, 82). The earliest prohead that has been reported is termed prohead I. Genes 20, 22, 23, 31, and 40 are required for its formation. As indicated earlier, defects in any of the head genes 20, 21, 22, 23, 24, and 31 inhibit the cleavage of p22, p23, and IPIII, resulting in the accumulation of aberrant head-related structures (9, 70, 77, 80, 86). Morphologically, prohead I has a prolate shape, although the vertices characteristic of T4 phage heads are not yet apparent. It is comprised entirely of uncleaved proteins and is thought to be analogous to a tau-particle in that defects in genes 21 or 24 lead to the formation of similar particles (49, 69, 81). It has been suggested that these particles are essentially polyheads of defined length, since the lattice constructions of the protein subunits that make up the structures are similar in nature (1, 82). It is not clear whether length determination has been achieved at this stage. From the above discussion it would appear that it has, since tau-particles are all of uniform length. However, temperature-sensitive mutants in gene 24 can give rise to giant heads (1), suggesting that prohead I has not achieved a final length. Our recent results suggest that p24 may be involved in length determination, since a decrease in p24 levels leads to giant phage formation (see below). Prohead I apparently is associated with the bacterial membrane (40, 79) and is devoid of DNA (79). Tau-particles (similar to prohead I) formed in cells infected with a temperature-sensitive mutant in gene 24 can be filled with DNA after a shift to the permissive temperature (9), and it has been shown that the majority of the DNA is synthesized after the temperature shift (84). There is strong evidence that empty proheads exist before they are filled with DNA.

Prohead I is converted to prohead II accompanied by the cleavage of p23 to p23*. Concomitantly, there is a change in sedimentation rate from 400S for prohead I to 350S for prohead II (79). This decrease in sedimentation rate is most likely due to a decrease in mass resulting from the cleavage of the major component (p23) from approximately 56,000 to 45,000 daltons (82). As indicated, the action of genes 21 and 24 is believed to be necessary for the conversion of prohead I to II, although this assignment is by no means certain. Prohead II has the same basic composition as prohead I except for the presence of p23* rather than p23. Morphologically, this structure is significantly different in that the vertices are now well defined. DNA is not yet attached to prohead II.

Prohead III is the first prohead to be associ-

ated with the phage DNA, and genes 16 and 17, at the very least, are required to effect the conversion from prohead II (79). The sedimentation rate increases to 550S, probably due to the association of DNA. At this point, the core proteins undergo cleavage: p22 is cleaved to acid-soluble fragments and the internal proteins are cleaved to lower-molecular-weight proteins. It has been suggested that the cleavage of the core proteins is required for the progressive packaging of the DNA. Laemmli et al. (82) have shown that, in vitro, high concentrations of internal peptide fragments, presumably the cleavage products of p22, can lead to the collapse of purified phage DNA. In some respects, the cleavage of p22 and the internal proteins resembles the role of scaffolding protein in P22 bacteriophage DNA packaging. Here, the core protein is displaced by the DNA, freeing the core protein for reuse (20). It has been shown that defects in genes 16 and 17 do not affect the cleavages of the T4 core proteins. This reveals a possible flaw in the prohead assembly pathway and suggests that genes 16 and 17 exert their function after the cleavage of the core but before the packaging of the DNA. This may indicate that DNA packaging involves more than just the cleavage of p22 and the internal proteins. Alternatively, defects in genes 16 and 17 may simply result in an uncoupling of the cleavage events from the packaging event, although some positive force must be required. Yet another unresolved point concerns when p24 is cleaved. p24* is contained in the mature phage head, but it is not known whether prohead II or III contains p24.

Gene 49 function is required for the conversion of prohead III to the final, mature head form. It has been shown that in the absence of gene 49 function, partially filled heads accumulate (71, 79, 87) and that when the p49 activity is temperature sensitive, the partially filled head formed under nonpermissive conditions can be converted to the mature form when shifted to the permissive condition (84). It has been suggested that gene 49 codes for an endonuclease activity that is responsible for cleaving the DNA protruding from the filled head (46). Although the regulation of this event is not clear, it does not appear that the nuclease simply recognizes a full head, since it has been shown that the activity coded for by gene 49 can cleave head-size pieces of DNA in vitro (46). In vivo, it has been demonstrated that in the absence of gene 32 function, head-size pieces of DNA are generated from the concatameric replicative DNA in the absence of any head formation (M. Curtis and B. Alberts, personal communication). On the other hand, it is very unlikely that the cleavage is uniformly at a specific site on the DNA, since shorter than normal (39, 94) and longer than normal (38, 111) lengths of DNA can be packaged into petite and giant heads, respectively. It has also been shown that the T-even phage genome is terminally redundant (90), and this would result in different gene sequences being present at the ends of DNA after packaging. As indicated earlier, in the P2-P4 bacteriophage system, P2 proheads could package the smaller P4 DNA, but P4 proheads could not package any of the larger P2 DNA (53,100). In the case of bacteriophage λ, Kaiser et al. (63) have shown that in vitro only concatameric DNA could be packaged using petite λ heads as head precursor. They postulate that DNA cutting is a necessary part of the DNA packaging reaction.

The functions of the remaining T4 group B genes are required before the assembled head can be attached to the tail structure. The mature head is a prolated structure, approximately 110 nm long (Fig. 10). It contains the cleaved proteins p23*, p24*, IPI*, IPII*, IPIII*, the fragments derived from p22, as well as p20, and several proteins present in minor amounts whose importance remains to be evaluated. How icosahedral heads spontaneously join to tails of helical symmetry is a subject much discussed but for which little concrete information is available (20, 92).

Role of the Host in Assembly

Thus far, we have emphasized that phage assembly proceeds through a series of prohead stages that require cleavage of the structural proteins for continuation of the maturation process. In our zeal to illustrate that each of these steps is under the control of well-defined bacteriophage genes, we neglected the possible effect of the host. Georgopoulos (47) isolated certain mutants of *E. coli* that did not propagate bacteriophage λ. One of these mutants, called *groE*, also failed to support the growth of T4 or T5. Bacteriophage adsorption and DNA injection were normal, but the cleavage of specific structural proteins was blocked. In the case of T4 and λ, it was shown that the cleavage of head proteins was blocked (24, 49). Several workers (24, 49, 50, 109) reported that a modified T4 gene 31 overcame the bacterial lesion. In the case of T5, *groE* mutants inhibited the cleavage (and consequently the assembly) of a tail protein (120). From a different viewpoint, Chao and Speyer (22) reported that, when T4 was grown on a host deficient in endonuclease I and polymerase I, a large number of short-headed (petite) particles were formed. Earlier we discussed the involvement of the host mem-

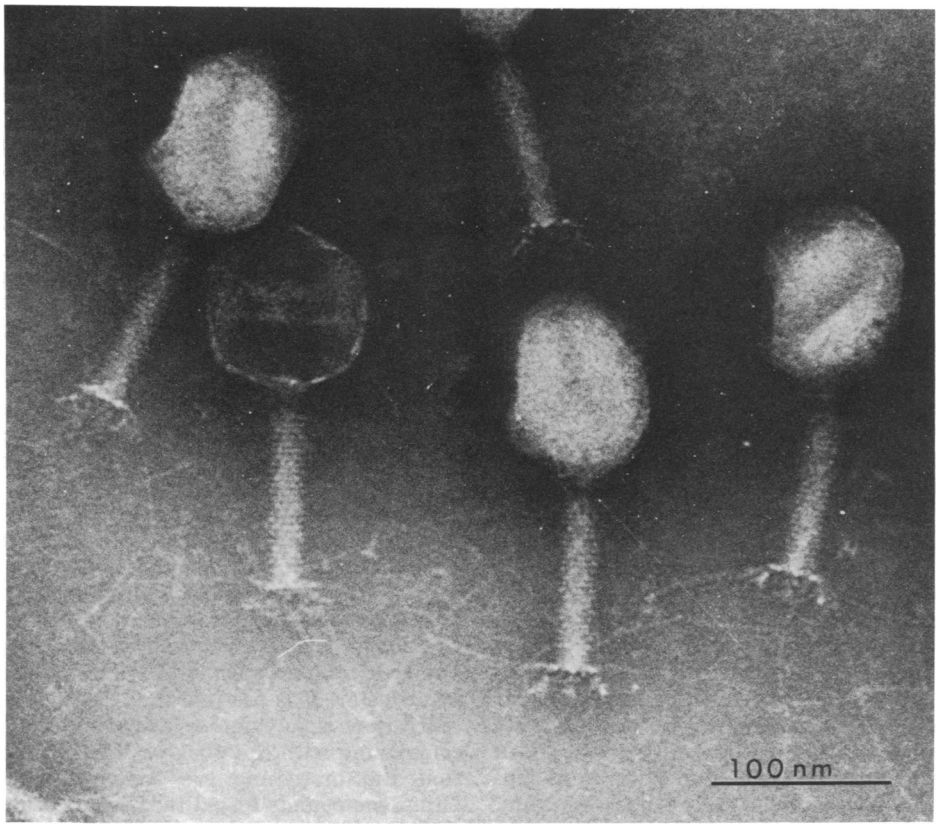

FIG. 10. *Electron micrograph of mature T4 bacteriophage.*

brane with T4 morphogenesis, and this has been used to interpret the role of gene 31 in obviating the effect of the *groE* mutation. Casjens and King (20) relate that L. D. Simon accounts for the possible role of host DNA synthesis in phage morphogenesis with a model in which a small amount of DNA is associated with proheads during assembly and that the bulk of the DNA is pulled in during the later stages of assembly. *groE* could also be a host mutant that affects synthesis of certain phage gene products that are required for cleavage. Gene 31 might then be viewed as a regulatory element able to overcome this effect. More general aspects of the role of the host in assembly have recently been reviewed by Georgopoulos and Eisen (48).

CHEMICAL INDUCTION OF STRUCTURAL ABERRATIONS

Our interest in the assembly of T-even bacteriophage began several years ago when we speculated that the basis for the formation of aberrant substructures must be a direct effect on the protein subunits themselves (29). We reasoned that if the amber mutations could alter the conformational properties of the subunits, then perhaps growth of the wild-type bacteriophage in the presence of amino acid analogues or anti-metabolites could well have the same effect. It had been known for some time that certain anti-metabolites depressed viral yields, but a systematic electron microscopy examination of the resulting lysates had not been attempted. Because of the work described (40) on the bacteriophage amber mutants, this approach was feasible, since the defects that might possibly arise (such as polyheads, small heads, polysheaths, polytail tubes, etc.) would be readily identifiable by virtue of their unique morphology and/or their association with other bacteriophage substructures.

T4 Head-Related Aberrant Substructures

Several amino acid analogues were tested and it became immediately apparent that certain compounds (*p*-fluorophenylalanine, 5-methyl-DL-tryptophan, and DL-7-azatryptophan) greatly

depressed the viral yield but did not increase the amounts of any particular structural aberration. Other compounds, however, significantly increased specific aberrations (25, 29). In Table 1 some of the more interesting compounds are listed which specifically led to the formation of head-related aberrations. L-Canavanine is the only amino acid analogue that induces the formation of polyheads (Fig. 11). These polyheads appear to be of two tubular types, one with closed ends and the other with open ends. Both tubular types contained variable amounts of core material similar to that reported for T4 gene 20 polyheads (69, 82). The significance of the closed polyheads (as well as rare monster phage particles) will be made clear in a later section. The only other compound that specifically gave rise to polyheads was dimethyl sulfoxide (25, 33). This compound is particularly interesting since it had previously been shown that treatment of phage particles with dimethyl sulfoxide results in the separation of heads from tails (30). Perhaps in vivo, dimethyl sulfoxide interferes with the same configuration of subunits as it does in vitro, except that in this instance a prohead continues to elongate, resulting in polyheads. The analogues of L-canavanine, i.e., arginine and homoarginine, neither depress the phage yield nor induce the formation of polyhead substructures. The common failure of arginine and homoarginine may be related to the fact that homoarginine does not act as a repressor in the arginine biosynthetic pathway (96), whereas canavanine does (89). Also, the guanido group of canavanine has a dramatically altered pK relative to either arginine or homoarginine and conceivably has a more dramatic effect when incorporated into polypeptides. Two other types of head aberrations were noted: short-headed and multitailed phage (Fig. 12 and 13). The short-headed phage were induced by the polyamines putrescine and cadaverine (as well as diaminopropane and, to a lesser extent, agmatine) and the histidine analogues triazole-2-alanine (TRA) and thiazolealanine. Polyamines are already present during phage growth (3), so that very high concentrations of polyamines (125 μM) are required to induce small heads. A second effect of the four polyamines that induce short heads is the formation of multitailed phages. It is possible that these two aberrations are functionally related; however, other agents that lead to small head production do not also lead to multitailed phage. Many of the multitailed phage appear to have small heads, but head size is difficult to judge. High concentrations of arginine (4 mg/ml) also lead to the production of small heads, and this may correlate with the production of putrescine from arginine in the host cell during infection (93). It is curious that only the straight-chained aliphatic polyamines lead to these aberrations, and those with secondary amino groups (spermine and spermidine) do not. Both spermidine and putrescine are associated with the DNA within the phage head (3), suggesting that the mere ability to complex with the DNA is not a significant factor. It is possible that these high concentrations of polyamines lead to such complete complexing with the phage DNA that replication and processing are adversely affected. Other compounds tested that could also affect DNA replication and processing, such as proflavin and actinomycin D, also lead to the formation of short heads (29). The histidine analogues TRA and thiazolealanine also give rise to small-headed phage, although TRA is more effective. Our primary reason for listing so many compounds that led to the formation of short-headed phage is to emphasize that the common denominator cannot be simply incorporation into an essential protein. Rather, it must involve some unspecified effect on the

TABLE 1. *Distribution[a] of induced head aberrations*

Compound	Viability	Small heads	Polyhead	Polysheath	Polytail tubes	Multitail
None	1.0	360	10	190	270	4
L-Canavanine	0.005	174	520	230	3,810	1
L-Arginine	1.0	370	10	250	160	2
L-Homoarginine	1.0	370	10	190	320	2
Putrescine	0.12	4,480	10	840	1,110	57
Cadaverine	0.31	3,060	4	300	750	51
Spermine	0.09	310	0	30	60	5
Spermidine	0.02	390	0	10	1,650	1
1,2,4-Triazole-3-alanine	0.04	3,770	130	240	420	2
2-Thiazolealanine	0.12	1,870	190	830	2,070	1

[a] All values for aberrant structures are given per 2,000 normal phage particles. Polystructures are expressed as multiples of the normal length structure. The values boxed indicate significant increases in that aberrant substructure.

assembly pathway. Perhaps these anti-metabolites interfere with assembly by altering the specificity of an enzyme(s) that is required for the production of particular structural elements. Earlier, we discussed the observation (22) that hosts deficient in certain aspects of DNA synthesis gave rise to small-headed phage. It is possible that all these observations are related, but to date we have too little evidence to support further conjecture.

T4 Tail-Related Aberrant Substructures

As is evident in Table 1, head-related aberrations were not the only defects noted. In general, polysheaths (Fig. 12) are not specifically generated by any one compound. Putrescine and thiazole alanine increase the relative amounts of polysheath, but not dramatically. The most significant increase in tail aberrations involved polytubes. In Table 2, we have listed the effects of the proline analogues azetidine-2-carboxylic acid (ACA) and thiazolidine-4-carboxylic acid on the maturation of T4. The amount of polytubes increased about 100 times when phage was grown in the presence of ACA and about 30 times in the presence of thiazolidine carboxylic acid. The decreased effectiveness of the latter may be due to the finding that thiazolidine-4-carboxylic acid has the same steric configuration as proline in terms of affecting the α-helix (112). This may indicate that incorporation or some other direct effect on the protein subunits may be necessary to interfere with the assembly of this helical substructure.

Before conjecturing about the mechanism for the formation of polytubes, it is important to justify identifying such structures as such. First of all, only proline analogues give rise to high quantities of this structure. In fact, growth of the bacteriophage on a proline-requiring host in the presence of the analogue increased the amount another eightfold (29). Second, some phage particles were observed which had tail tubes of almost twice their normal length physically attached to heads (Fig. 14). A more definitive answer would necessarily involve phage protein-specific antibody assays or characterization of the protein subunits contained in purified polytubes, but this work has not been done. In terms of a model, it is unlikely that the length of these structures is determined by a vernier-type interaction between sheath and tube subunits, since we never observed polysheath connected to polytubes. Two possibilities remain. (i) The template model: as in polytails from λ bacteriophage, polytail tubes could arise from the failure of an interacting complex to terminate when the end of a hypothetical "fibrous material" is reached. Unlike λ, for T4 only one structural gene (gene 19) has been implicated to tail tube assembly. (ii) The cumulated strain model: tail tube subunits do assemble in vitro into tubes of varying lengths (99) which are quite similar to the structures described here. Since these tail tube subunits were prepared from purified tubes by chemical treatment, it is possible that some conformational changes in the subunits occurred in the preparation. This could well lead to a different conformational strain generated during in vitro assembly. We noted that the proline analogue more structurally similar to proline gives rise to fewer polytubes than did ACA. Perhaps, in this case, the strain generated was different. Which experiments must be done to distinguish the possibilities is not clear. Perhaps some attempt should be made to study in vitro assembly between tube subunits prepared from polytail tubes and a mixture of cell extracts prepared from bacteria infected with tube-deficient or baseplate-deficient mutants. Curiously, growth of λ bacteriophage in the presence of ACA (Shuve and Howatson, personal communication) leads to the formation of polyheads.

Effect of Anti-metabolites on the Maturation of Specific Gene Products

In ascertaining the mechanism for chemical induction of this array of aberrant substructures, it is necessary to consider this effect on cleavage of the structural proteins. Jacobson and Baltimore (62) reported that L-canavanine and ACA interfere with the proteolytic cleavage of poliovirus structural proteins. Figure 15 represents an autoradiogram prepared from an SDS-polyacrylamide gel of ^{14}C-labeled extracts prepared from a normal T4 phage infection and those prepared in the presence of L-canavanine, TRA, *p*-fluorophenylalanine (PFPA) and ACA. It is clear that canavanine has the most dramatic effect on the maturation of the structural proteins. The cleavages of p22 to acid-soluble fragments, p23 to p23*, p24 to p24*, and IPIII to IPIII*, are inhibited, indicating that canavanine interferes with proper head assembly, as has been evidenced already by the induction of polyheads. The cleavage of *alt* protein (57), which was previously mistaken for p20 (14), is also inhibited (Fig. 16). p12, a baseplate protein, is absent from canavanine-treated extracts. In addition, new protein bands (X and Y) appear slightly above p10 and p18. A third band, Z, is intensified in the presence of canavanine. A fourth band, A, is missing and probably represents an early phage protein, since canavanine-treated extracts labeled early in infection contain greater amounts of band A.

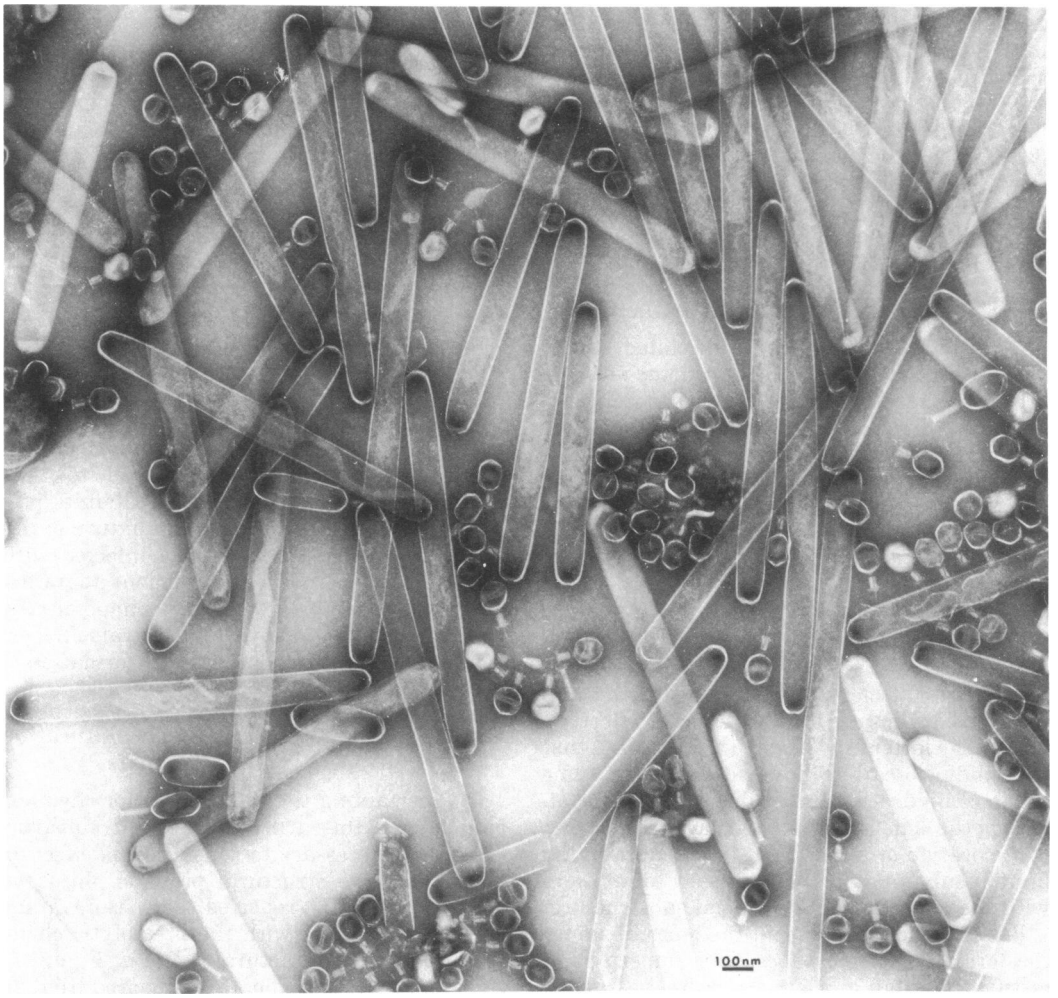

FIG. 11. *Electron micrographs of T4 polyheads induced by canavanine (29). These were chosen to illustrate the open and closed types of tubular polyheads. (a) A field of closed polyheads obtained after purification in a gradient (32) is presented; (b) an area containing primarily open polyheads. Note the "core" material within some of these polyheads.*

Band X has been identified as a precursor of p10, a baseplate protein, and band Y is a product of gene 18, the single structural gene for sheath (71, 73). Further experiments (14) have shown that canavanine effectively inhibits the formation of tails, probably as a result of aberrations induced in p10 and p18. However, before we can conclude that these essential structural proteins of baseplate and sheath undergo cleavage during maturation, we must first show that these reactions occur in the absence of canavanine, which has not yet been possible. Preliminary data suggest that band Z is a precursor to *alt* protein, but conclusive evidence utilizing an amber mutant in the *alt* gene is required. Canavanine clearly inhibits the appearance of p12 (a baseplate protein). Attempts to identify a possible precursor-product relationship have failed (14), so if p12 does not undergo cleavage, this is the only example of canavanine inhibiting the synthesis rather than the cleavage of a gene product. TRA does not noticeably affect any of the known cleavage reactions or any of the additional proteins affected by canavanine. This is not surprising, since the only aberration involved short-headed phage, which are complete virions in every sense except for their genome length (39, 94). (We will discuss the protein composition of these short-headed phage in a later section.)

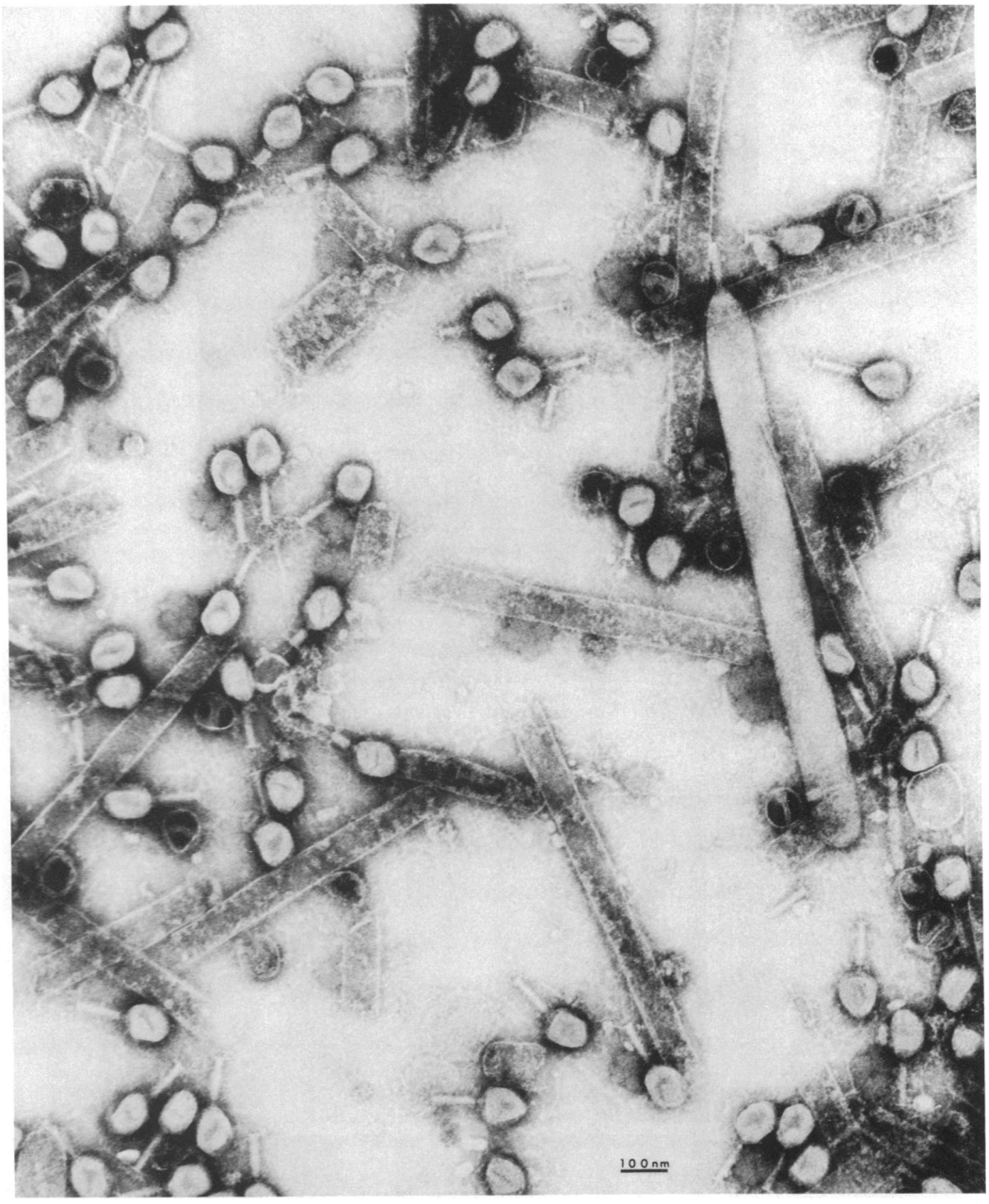

FIG. 11B

PFPA and ACA inhibit the cleavage of p22, p23, and p24, as does canavanine. Very little of the label in any of these three extracts is capable of being chased, indicating an inhibition of proper head assembly. PFPA, like canavanine, depresses the level of the *alt* protein, although ACA does not. Interestingly, ACA, which does not lower the level of the *alt* protein, does not completely inhibit the cleavage of IPIII, as does PFPA and canavanine. ACA does inhibit the cleavage of p23. TRA, PFPA, and ACA do not affect the appearance of p12, nor do they induce the appearance of bands X and Y. It is apparent that it is difficult to reach conclusions solely on the basis of inhibition of cleavage reactions when a defect in any of the genes in group A of

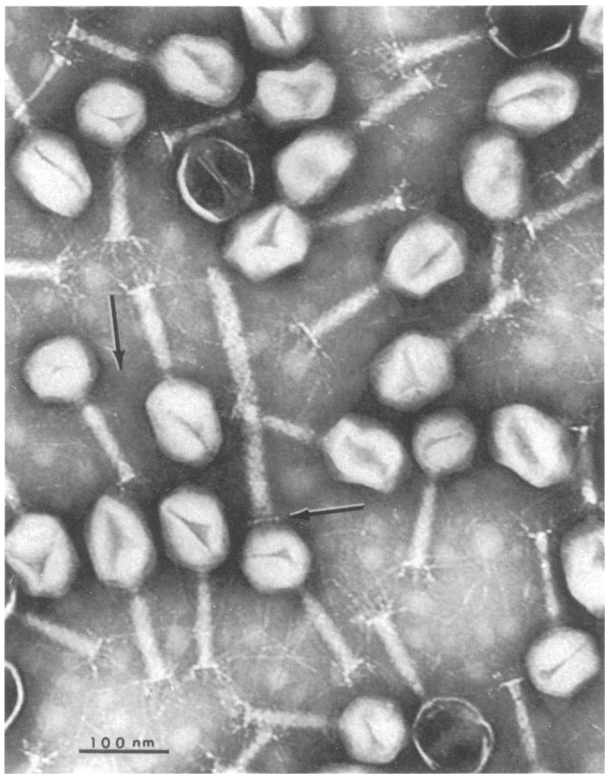

FIG. 12. *Electron micrograph of short-headed (petite) variants of bacteriophage T4. This specimen originated from a lysate prepared in the presence of TRA (29). Note the presence of a polysheath structure with baseplate attached.*

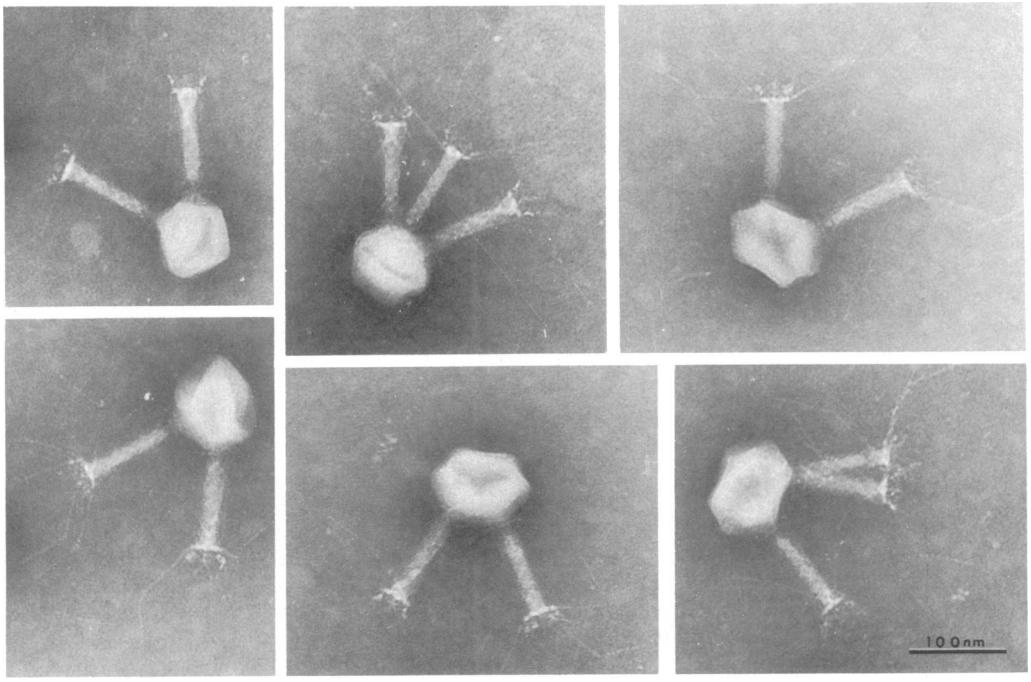

FIG. 13. *Electron micrograph of multitailed T4 prepared from a lysate which had been treated with putrescine. Some heads appear to be of normal size, whereas others appear to be petite.*

TABLE 2. *Distribution[a] of induced tail aberrations*

Compound	Viability	Small heads	Polyheads	Polysheath	Polytail tubes	Multitail
Proline	1.0	280	20	160	80	0
L-Azetidine-2-carboxylic acid	0.001	300	30	100	32,490	0
L-Thiazolidine-4-carboxylic acid	0.14	250	40	50	10,690	0

[a] These values were calculated as indicated in Table 1.

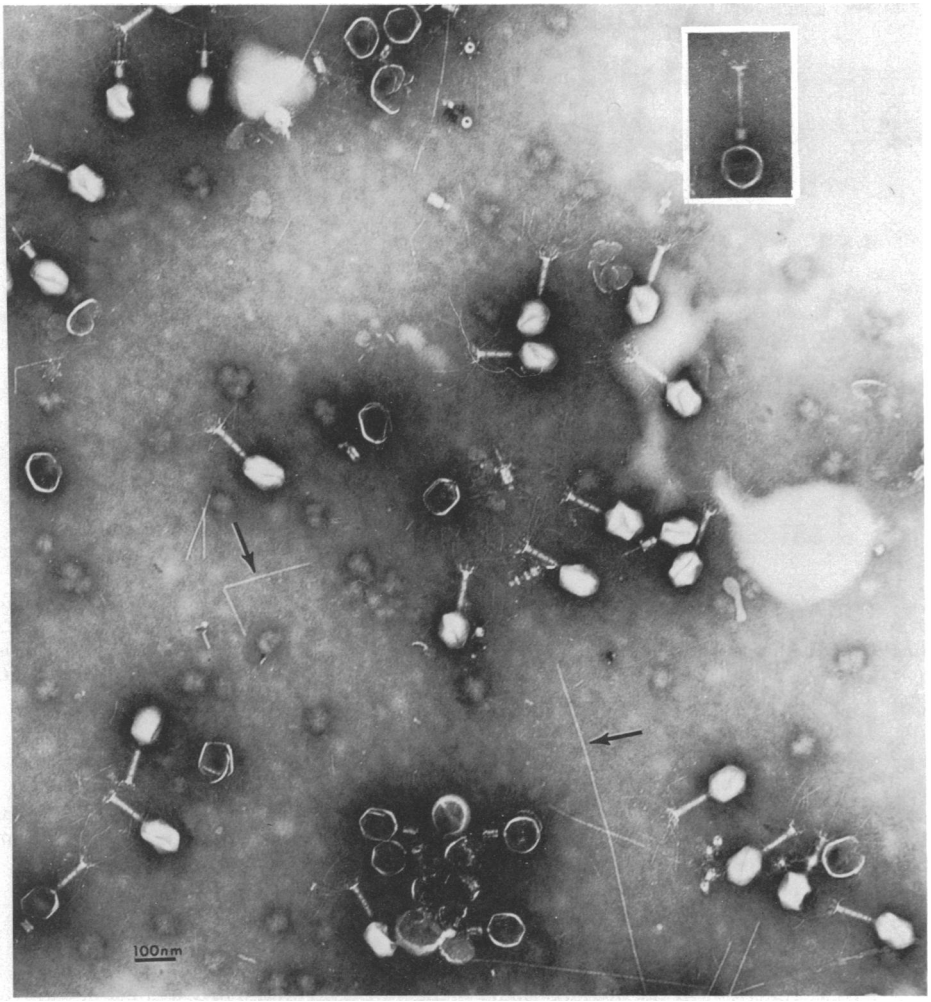

FIG. 14. *Electron micrograph of T4 polytubes induced by ACA. The arrows point to polytubes unattached to other structures. Note the insert, which shows an example of tail tubes about 1.5 times normal length, which are attached to heads at one end and baseplates at the other.*

the head genes will inhibit cleavage in the other proteins. Nevertheless, it is equally clear that different analogues have different spectra of effects on cleavage reactions, with canavanine showing the broadest range.

Cleavage Reactions in Bacteriophage T5

Earlier, we mentioned another example of the effect of an analogue (ACA) on λ bacteriophage assembly. A more interesting effect was demonstrated with canavanine on the growth of T5 (120), the major head protein of which has a molecular weight of 32,000 (119). It is very difficult to demonstrate whether this protein is a product of a cleavage reaction using pulse-chase experiments. In the presence of canavanine, however, T5 polyheads are formed (Fig. 17), and SDS-gel analysis of these structures

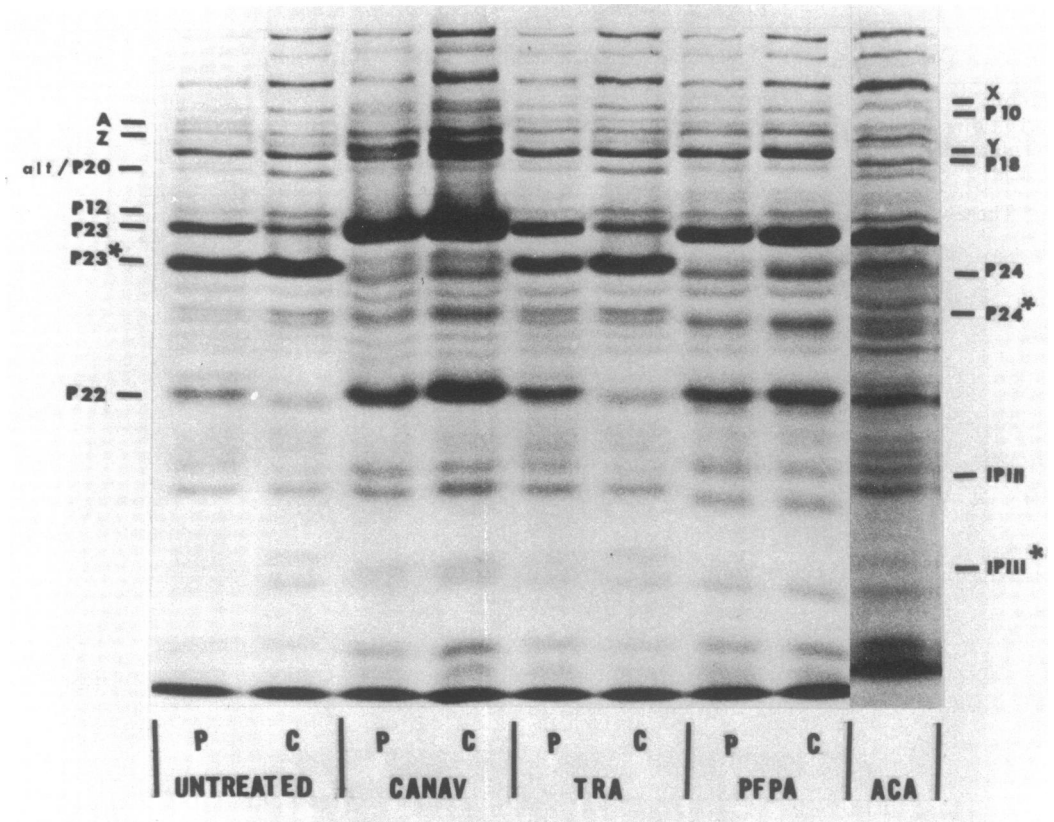

FIG. 15. *Autoradiogram of an SDS-10% polyacrylamide slab gel showing the effects of several amino acid analogues on the processing of T4 proteins. P, Pulse, and C, the chase, in untreated and canavanine (CAN)-, TRA-, PFPA-, and ACA-treated preparations. The numbers and letters identify protein bands contained in this autoradiogram, except the ACA strip, which was analyzed separately. (Reproduced here with the permission of the American Society for Microbiology.)*

(Fig. 18) revealed that the major protein has a molecular weight of 50,000. Based on our experience with T4 cleavage and polyheads, we conclude that the major head protein does undergo cleavage. Earlier, we mentioned that T5 maturation was blocked in a *groE* host and that the specific block involved cleavage of a tail protein. In Fig. 18, band (a) represents this tail protein cleavage reaction. It is obvious that whereas the cleavage of the head protein is inhibited by canavanine, the cleavage of the tail protein is not. Similarly, in the *groE* host, cleavage of the tail protein is inhibited, whereas cleavage of the head protein is not. This indicates that cleavages of the head and tail proteins are regulated separately and that different cleavage enzymes may be involved. This may also be an explanation for the observation that, in T4, canavanine inhibits the cleavage of head and tail proteins, but PFPA and ACA affect the cleavage of primarily head proteins. The understanding of proteolytic cleavage reactions and their role in virus maturation is still in its infancy.

GIANT BACTERIOPHAGE

One event that is ill-defined in the T4 head assembly pathway is the regulation of head length. Based on the broad effect of canavanine on phage maturation, we explored the possibility that canavanine could be a tool for examining head length control. We first analyzed the macromolecular syntheses that occur during phage maturation in the presence of canavanine. Total RNA synthesis was unaffected, whereas DNA synthesis was inhibited about 70% as compared with the normal phage culture (26). More importantly, total protein synthesis, in spite of a 200-fold reduction in bacteriophage yield, was unaffected (25, 26, 29). We

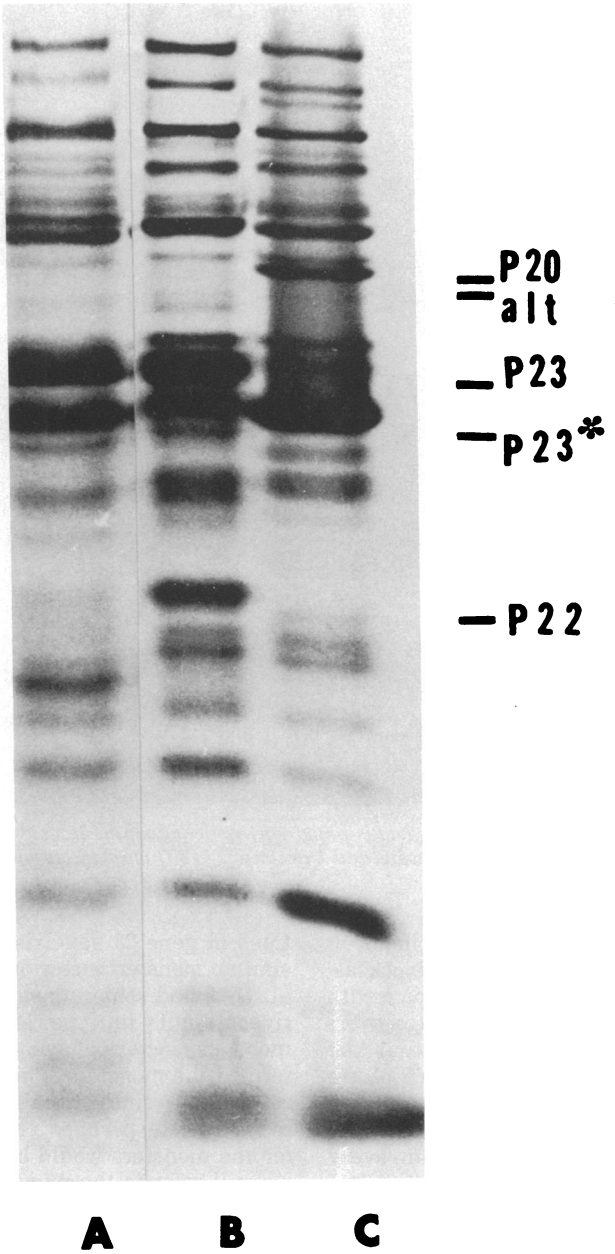

FIG. 16. *Autoradiogram of an SDS-10% polyacrylamide slab gel showing the effect of an inhibition of cleavage on the appearance of the alt protein. Strip A shows the effect of a mutation in gene 21. Both conditions have been demonstrated to inhibit all cleavages of the head proteins. Strip C shows the presence of the alt protein in the intact phage particle. Note that an inhibition of cleavage affected only the appearance of alt and that P20 is clearly present. P22 is missing from strip A, and a portion of the P23 appears as P23* since this canavanine-inhibited extract was used in an in vitro cleavage reaction (16). (Reproduced here with the permission of the American Society for Microbiology.)*

made the tentative assumption at that time that phage proteins were building up in an unassembled state and that, if we could release the block imposed by canavanine, then normal (or perhaps abnormal) phage maturation would commence. The most obvious route was to in-

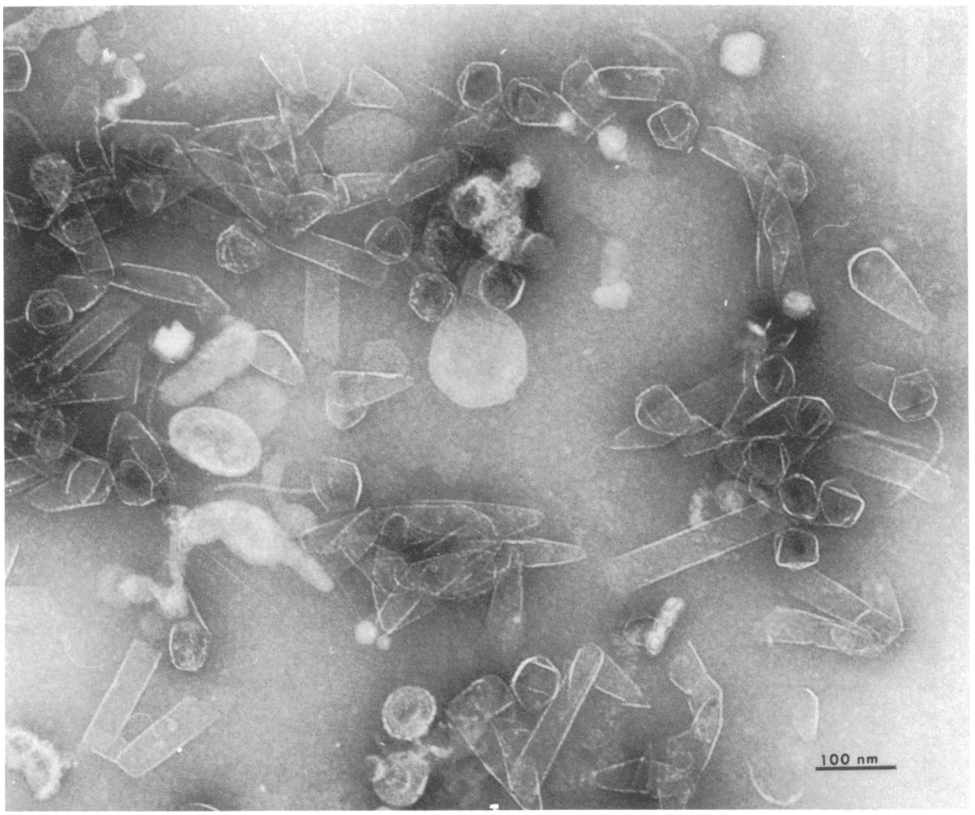

FIG. 17. *Electron micrograph of T5 polyheads prepared from canavanine-treated cells. Note the end-caps in some of the polyheads. (Taken from Zweig and Cummings [120] with the permission of the publisher, Academic Press Inc., London.)*

fect the host with T4, allow early steps in development to occur, add canavanine to block assembly, and then add arginine to compete with the effect of canavanine. Bacteriophage titers were measured, and it was found that the phage yield was restored to about 20% of the untreated phage culture. Examination in the electron microscope revealed that the majority of the phage particles were normal in every respect. However, a small percentage (1 to 3%) of the particles were monsters in that they had enormously long heads, were filled with DNA, and many had one or two tails attached (Fig. 19) (32). These monsters contained as much as 30% of the total assembled phage proteins. Because of their unusual appearance, we termed these monster phage, induced by treatment with canavanine followed by arginine, "lollipops." This choice of name may have been inappropriate. At about the same time we reported on the stimulation of production of these monster phage in a wild-type infection, Doermann et al. (38) reported that certain missense mutations in gene 23 gave rise to the formation of similar monster bacteriophage. Later, Aebi et al. (1) found some unusual temperature-sensitive mutants in gene 24 that also generated monster bacteriophage. Both these groups termed the monsters "giant bacteriophage." In retrospect, since small-headed phage are termed petite, perhaps a more appropriate term for the monsters would be grande. In general, we will refer to the monsters as "giant," since the properties of each type of monster—genetic or induced—have so far been found to be similar, with an occasional lapse into the use of "lollipop."

Rationale for the Use of Giants in Head Length Control Studies

The findings that challenging T4-infected *E. coli* with canavanine followed by a chase with its analogue, arginine, led to the formation of giant bacteriophage (32) opened up several possibilities for the study of head length control. We will present some of these possibilities now,

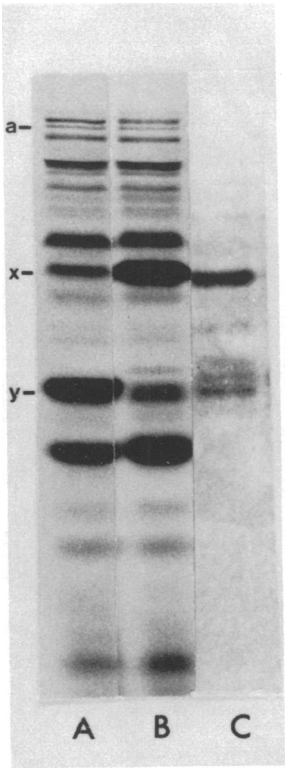

FIG. 18. *Autoradiogram of an SDS-10% polyacrylamide slab gel showing (A) extract of T5-infected E. coli F, pulse-labeled with [^{14}C]amino acids; (B) extract of T5-infected cells treated with canavanine and pulse-labeled with [^{14}C]amino acids; and (C) the structural proteins contained in T5 polyheads stained with Coomassie blue. Note that the major structural protein in polyheads corresponds to band X, the putative precursor to the major structural head protein (band Y) of T5. Note also the tail protein (band a); band b (not labeled) lies just below band a. (Taken from Zweig and Cummings [120] with the permission of the publisher, Academic Press Inc., London.)*

and then in succeeding sections we will discuss the evidence and our conclusions. First of all, the availability of these giants offers the opportunity for comparing the head morphology and composition of these structures with normal T4 heads and polyheads. Kellenberger's group (personal communication) and Ishii and Yanagida (61) examined the surface structure of these giants, as well as giants obtained with mutants in genes 23 and 24, utilizing optical diffraction of high-resolution electron micrographs, and have obtained valuable information about the structure and symmetry relations of T-even bacteriophage heads that could not otherwise have been achieved. Our laboratory has concentrated on the protein composition and length distribution of these structures, and we have been able to consider which genes might be involved in head length determination and at which step(s) of the prohead pathway length is controlled. In addition, the examination of the intracellular events taking place and their sequence is essential for an understanding of the mechanism(s) of action by which canavanine leads to the formation of giant bacteriophage. This includes the investigation of the kinetics of induction of giants, the role of inhibition of proteolytic cleavage, and finally the significance of the canavanine-mediated inhibition of bacteriophage DNA synthesis. It may well be that it is a complex of DNA that provides a scaffold upon which essential size-determining proteins are assembled and that it is the size of the DNA complex that ultimately determines the size of the head. T4 DNA replicates as a concatamer much longer than the mature DNA genome (45), and it might be this characteristic that gives rise to giants. Kaiser et al. (63) showed that packaging of λ DNA into preformed shells requires concatameric DNA. In T5, canavanine by itself inhibits cleavage of head proteins and leads to the formation of polyheads, but it does not inhibit DNA synthesis, and giants are not formed upon addition of arginine, although the phage yield is restored (120). Evidence that the size of the T4 head is involved in DNA maturation was suggested by work with short-headed variants (petite), where it was found that the length of the DNA packaged (39, 94) is related to the length of the head. This latter work was taken as evidence for the "headful" hypothesis (107). The possibility that a prohead might first be constructed and T4 phage DNA packaged later has already been dealt with.

Obviously, a systematic and varied approach is required for the unraveling of the complexities of head length determination. For the next several sections, we will attempt to explore the various possibilities.

Kinetics of Production of Giants by Canavanine

Canavanine acts in some manner to induce the formation of T4 giants, which appear after an arginine chase. Experiments were done to determine the minimum exposure to canavanine necessary to induce these giants as a function of the stage in the viral maturation process (13). Measurements of the amounts and the lengths of the giants were made to determine possible causal relationships. As is shown in

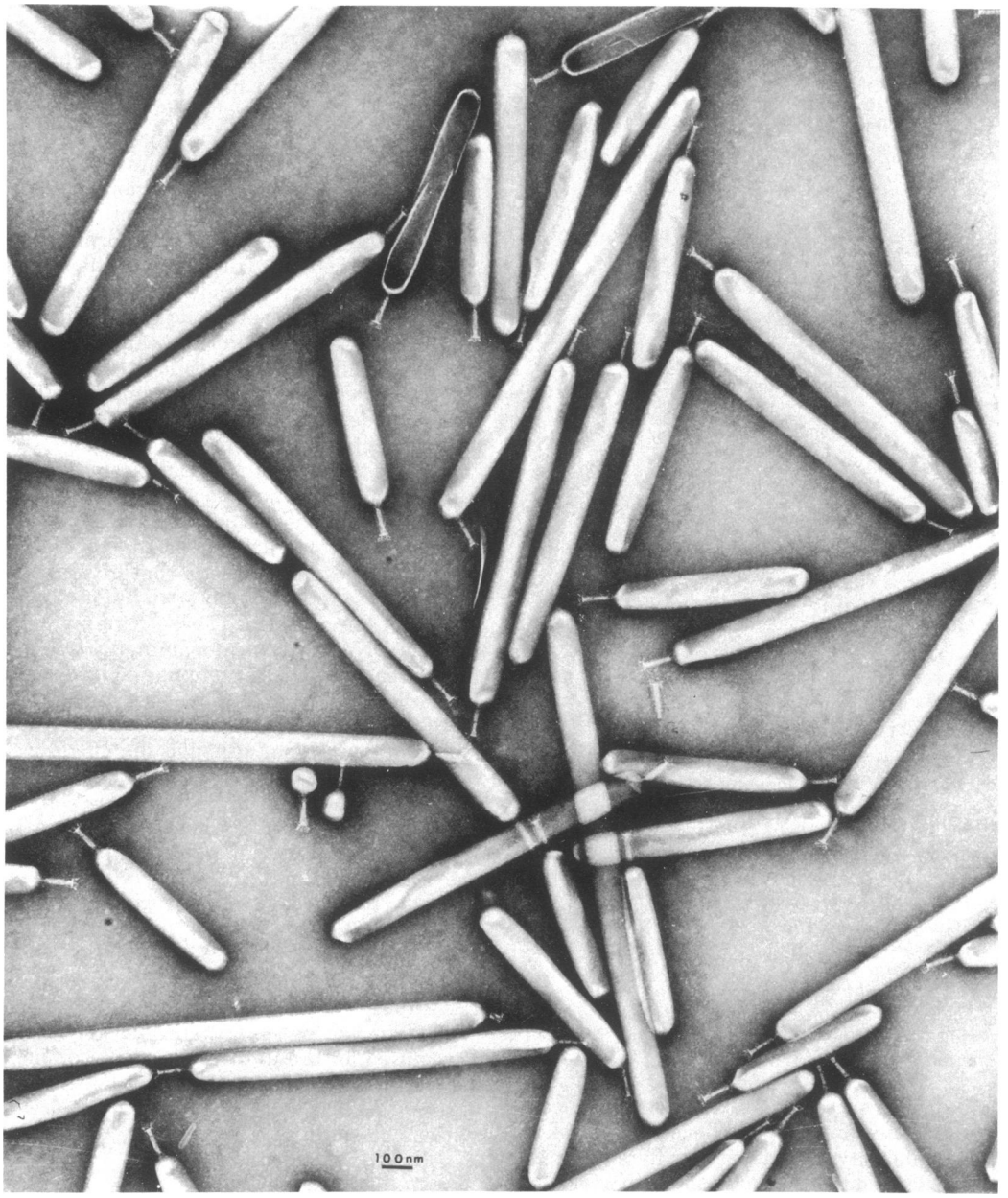

FIG. 19. *Electron micrograph of a purified preparation of T4 giants prepared using canavanine (32). Note that many of the longer giants have tails at both ends.*

Table 3, when canavanine is added at 10 min postinfection (p.i.), a 3-min exposure to canavanine is sufficient for the subsequent formation of giants. However, when canavanine is added at 5 min p.i., 20 to 25 min of exposure is required. The data also showed that the percentage of giants, as well as the average equivalent head length, increases with increasing duration of exposures to canavanine. When canavanine is added at 5 min p.i., the percentage of giants induced always remains low. Although the length distribution of the giants induced by adding canavanine at 5 min p.i. was not measured, the heads are consistently shorter than those where canavanine is added at later times. These data suggest that at early

times in the infection the step involved in the induction of giants, i.e., size determination control, is not yet functional.

At 10 min p.i., a 3-min exposure to canavanine is sufficient to induce giants. What was left unanswered was how much time is required to produce giants after the addition of arginine. We added canavanine at 10 min p.i. and arginine at 25 min p.i. and then sampled the T4-infected culture at various times. Up to 40 min p.i., few, if any, giants were observed and the phage yield was still depressed. Starting at 50 min p.i., about 2.1% of the phage population consisted of giants, and this percentage persisted for the next 2 h of sampling. What changed in this time course was the average length of the giants. At 50 min p.i., i.e., 25 min after the addition of arginine, the average length was about 4 phage head lengths. By 95 min p.i., the average length had increased to about 8 head lengths and remained thus throughout continued incubation (13). These results indicate that shorter giants mature first and longer giants mature last; it is possible that the longer giants are incomplete when the cells are ruptured at earlier times. However, half-completed or broken long giants are not observed in the electron microscope.

TABLE 3. *Characteristics of T4B giants formed after minimal exposures to canavanine*

Time p.i. (min) after addition of:		Giants (%)[b]	Avg head length (phage equivalents)
Canavanine[a]	Arginine[a]		
5	20	<0.1	
5	25	0.5	ND[c]
5	30	0.8	ND
10	12	<0.1	
10	13	0.8	4.3
10	15	1.6	5.9
10	20	2.2	6.9
15	17	<0.1	
15	18	0.8	4.5
15	20	1.1	6.4
15	40	2.3	7.1

[a] Concentrations of canavanine and arginine were 130 μg/ml and 260 μg/ml, respectively. The phage were sampled at 150 min p.i.

[b] Approximately 1,000 phage were counted in each sample.

[c] ND, Not determined.

As has been discussed, canavanine affects phage production, DNA synthesis, formation of giant bacteriophage, etc. In the study of each of these induced effects, it is necessary to know whether canavanine exerts a specific effect independently or whether all are expressed simultaneously within the cell. In an attempt to address this problem, we studied the effect of altering the concentration of canavanine on each of these processes. We found (13) that the concentration of canavanine which resulted in the most dramatic response for inhibition of DNA synthesis, reduction of phage yield, or the formation of giants (when arginine was added at 25 min to counteract the canavanine) was between 12 and 20 μg/ml (Fig. 20). It should be noted that, as intimated earlier, the formation

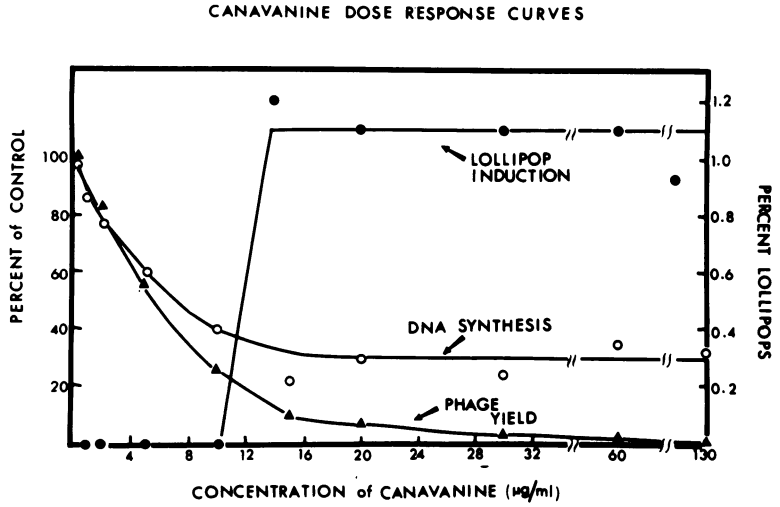

FIG. 20. *Effect of increasing concentrations of canavanine on T4 DNA synthesis, phage yield, and the induction of giants (lollipops [13]). In each case, canavanine was added at 10 min p.i. (and arginine was added at 25 min p.i. to induce giants). (Reproduced here with the permission of the American Society for Microbiology.)*

of giants exhibits an "all or nothing" response. Giants that are induced by low concentrations of canavanine had heads shorter than those induced at the higher concentrations.

It is obvious from this set of experiments that the production of giants by canavanine involves two major steps: the induction step, which requires a minimum exposure of 3 min, and the formation step, which takes about 25 min. The induction step requires a T4 late function to be expressed. It is clear that the longer exposures to canavanine and the longer recovery periods result in longer giant heads. Canavanine appears to be exerting its effect on specific steps in phage morphogenesis.

Compartmentalization of Assembly

So far, we have dealt with the mechanism by which canavanine leads to the formation of giant bacteriophage in general terms. In light of the effect of canavanine on cleavage reactions (14, 120) and the presence of p23 in polyheads (31, 70, 77), it was considered essential to determine the composition of giant bacteriophage. This can be examined in two ways: (i) the overall composition and (ii) the contribution to the completed structure of structural proteins synthesized in the presence of canavanine. Cells were grown and infected with T4B in the absence of canavanine and labeled with [^{14}C]amino acids from 12 to 19 min; T4B infected-cells were also treated with canavanine at 10 min, and arginine was added at 25 min, at which time [^{14}C]amino acids were added, and incubation was continued; finally, T4B-infected cells were treated with canavanine at 10 min, and [^{14}C]amino acids were added from 18 to 25 min, followed by the addition of arginine at 25 min. Casamino Acids, 1.5%, was added to halt incorporation of [^{14}C]amino acids. In each case, normal phage and bacteriophage giants were isolated and purified (32), and the protein components were analyzed on SDS-polyacrylamide gels as before (77, 108). To interpret this experiment, it is necessary to compare the protein bands detected by staining (Fig. 21A–C) with those detected by autoradiography (Fig. 21D–F). When [^{14}C]amino acids are added during the canavanine inhibition periods, only those proteins synthesized in the presence of canavanine should contain label. Each sample applied to the gel contained essentially the same amount of p23*. Several facts emerge from this type of analysis. First, in the experiment where ^{14}C label was not added until after the addition of arginine, the major head protein (p23*) has a molecular weight of 45,000 in both normal phage and giants (32, 38). In giants, the amounts of internal proteins and p24 (see Table 4) are roughly proportional to the amount of p23*, and the amount of tail proteins is diminished, as would be expected in such head-dominated structures. These quantities are tabulated in Table 4. We conclude that giants mature through the same series of cleavage events as do normal phage. Second, normal T4B labeled in the absence of canavanine yield structural proteins that stain in proportion to the amount of [^{14}C]amino acids present (Fig. 21A and D). No difference is observed between the phage proteins labeled after the addition of arginine and those labeled without any exposure to canavanine and arginine. However, normal T4B, purified from a lysate in which proteins were labeled during the period of canavanine inhibition (18 to 25 min) and then allowed to mature after the addition of arginine and 1.5% Casamino Acids, clearly differ with respect to stained and labeled proteins (Fig. 21B and E). Very few of the proteins synthesized in the presence of canavanine are assembled into the normal phage structures. On the other hand, several proteins (p23*, IPIII*, bands 18 and 19) in the giant bacteriophage (Fig. 21C and F) are labeled to a degree similar to that in the untreated phage. Certain structural proteins are reduced relative to p23* in the giant phage sample. The stained gel (Fig. 21C) shows that although the amount of p24* may be reduced, a second band is present in compensating proportions at a position just above p24* [labeled (p24)]. This giant-specific band is below the normal position for p24 as expected, but the possibility exists that it is an aberrant form of p24, i.e., one improperly cleaved. Table 4 lists the contribution of each gene product to the total protein synthesized, under the conditions described. The number of bands and their molecular weights are in good agreement with earlier results (32, 36). Most significant, perhaps, is that very little of the p23* (band 10) synthesized in the presence of canavanine is used in the assembly of phage with normal-sized heads, although the same p23* is selectively incorporated into giants at levels greater than those seen with normal untreated phage. Relative to p23*, labeled IPIII* is assembled into the untreated phage and giants to a similar degree. IPIII* is assembled into the treated phage to a greater extent than most proteins. The labeled band 9 protein is assembled into the untreated and treated phage to approximately the same extent but it is not detected in the giants (band 9 protein is the *wac* protein [113], and it is located at the head-tail junction). Proteins represented by bands 13 and 19 also appear to be selectively

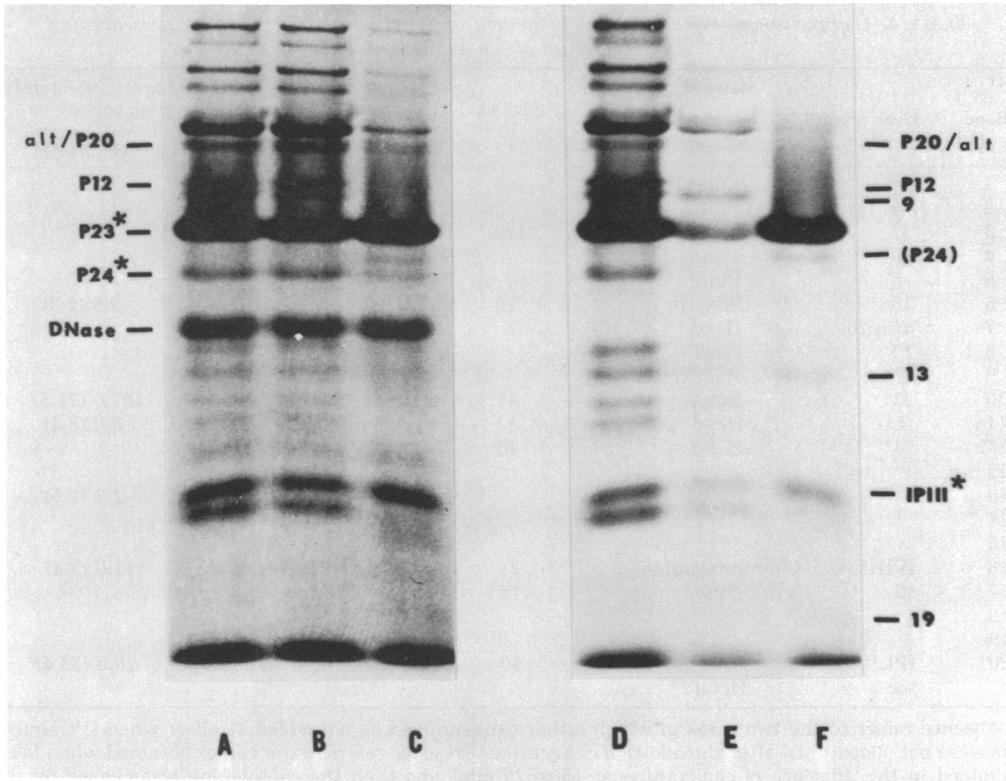

FIG. 21. *Photograph of a stained SDS-10% polyacrylamide slab gel (A, B, and C) and an autoradiogram of the identical gel (D, E, and F) showing the effect of canavanine on the utilization of T4 proteins in phage assembly (14). (A) and (D) Untreated, purified phage; (B) and (E) phage obtained from a lysate treated with canavanine and arginine where the [^{14}C]amino acids were present during the inhibitory canavanine period; (C) and (F) giants prepared from the same lysate as the phage in (B) and (E). The bands in the stained gel represent the entire protein complement, and the bands in the autoradiogram represent only the proteins synthesized in the presence of canavanine. Deoxyribonuclease was added to digest the phage DNA. (Reproduced here with the permission of the American Society for Microbiology.).*

assembled into giants, implying that they too are distributed throughout the phage head. It should be recalled that p12 and the *alt* proteins are missing from canavanine-treated extracts. Here, p12 and *alt* (bands 7 and 8) were synthesized and assembled into the treated phage and giants only after the arginine chase. From these data, it is not possible to determine whether the p20 made during the exposure to canavanine is utilized in the construction of giants. A p20 band is not detectable, but this may simply mean that p20 is located only at the apices of the phage head and as a result it would be diminished relative to p23* in the giants.

Polyacrylamide gel electrophoresis cannot be used to ascertain whether p22 made during the exposure to canavanine is used in the assembly of the giants as opposed to normal heads, since p22 is cleaved to acid-soluble fragments. To examine the fate of the p22, an acid-soluble extract of the phage and giant particles was prepared (16, 52), and the extracts were applied to Sephadex G-50 columns to isolate the fragments. The rationale behind this procedure is similar to that described above. It is necessary to examine the relative specific activities of fragments in each of three samples: the normal, untreated phage; the phage isolated from the canavanine-treated culture; and the giants from the canavanine-treated culture. In each instance, the same relative amount of head protein was used for the extraction of the soluble peptides. The results (Fig. 22) show that p22 made during the exposure to canavanine is preferentially used in the assembly of giants. Very little of the p22 made during the exposure to canavanine is used in the assembly of phage of normal head length.

Thus, we see that there are at least five

TABLE 4. *Comparison of the maturation of structural proteins synthesized in the presence and absence of canavanine*

Band	Gene product	Structure	Mol wt ($\times 10^{-3}$)	Absence,[a] % total protein[b]		Presence,[a] areas[c] of autoradiogram peaks	
				Phage	Giants	Phage	Giants (%)
1	34	Fiber	155	3.5	0.4	1.0	
2	7	Plate		1.3			
3	37	Fiber	120	3.1	0.3	1.5	
4	10	Plate		1.5		1.5	
5	6	Plate		2.0		3.5	
6	18	Sheath	70	11.5	2.6	5.3	3.5 (2.3)[c]
7	*alt*/p20	Head		3.9	0.3		
8	12	Plate		1.8			
9	Wac	Whisker		2.2		5.8	
10	23*	Head	45	36.0	52.5	17.8	107.0 (71.5)[c]
11a	(24)	Head					5.0 (3.3)[c]
11b	24*	Head	40	7.1	11.8	2.0	
12				1.5			
13				1.5		0.7	2.2 (1.5)[c]
14				0.9		0.6	
15				1.0			
16	IPIII*	Internal	21	3.8	4.2	5.0	11.0 (7.4)[c]
17	19	Tube	18	4.6	0.6	4.8	
18							
19							
20[d]	IPI,II	Internal	10	12.8	27.3	11.0	20.0 (13.4)[c]
	soc[e]	Head					

[a] Absence refers to the two cases in which either canavanine was not added at all or when [^{14}C]amino acids were not added until after the addition of arginine. Presence refers to the values obtained when label was added in the presence of canavanine at 15 to 25 min, and then the canavanine was chased by the addition of arginine.

[b] Percentage composition was averaged from values obtained in the two untreated cases described in footnote *a*.

[c] The actual peak areas from tracings of the autoradiograms illustrated in Fig. 22E and F are listed rather than percentages. Bracketed percentages are for giants only and are listed for comparison.

[d] Band 20 represents all those proteins that ran with the front in a 10% SDS-polyacrylamide gel electrophoresis.

[e] *soc* refers to the 10,000-dalton nonessential structural protein discovered by Forrest and Cummings (43) and Larcom et al. (85) and named structural outer capsid protein by Ishii and Yanagida (61).

proteins that are selectively utilized in the assembly of giant bacteriophage, as contrasted with normal phage that mature in the same milieu. These are the major head protein (p23*), band 11a protein (possibly of gene 24 origin), bands 13 and 19 proteins, and p22. IPIII* made in the presence of canavanine is also assembled into giants with near normal efficiency and into normal phage with greater than normal efficiency. Proteins with molecular weights approximately equal to those represented by bands 11a and 13 are present in tau particles isolated from cells infected with a T4 ts21$^-$ phage (80). These results clearly indicate that all these proteins exist in some compartmentalized state, probably as a head precursor that eventually matures to form the giant head. Heads of normal length presumably originate from a prohead pathway initiated after the arginine chase.

It is natural to ask: if the proteins are compartmentalized, what of the DNA? The identical experiments described above were repeated under conditions where only the DNA synthesized in the presence of canavanine was labeled. The phage and giants were purified and analyzed, and the results are shown in Table 5, including a comparison of results, with [^{14}C]amino acid-labeled particles. Whereas the number of counts per minute per microgram of protein of treated and untreated phage differed by a factor of 5, the number of counts per minute per microgram of DNA was the same for untreated and treated phage, as well as for giants. These data illustrate that there is no compartmentalization of the phage DNA during the exposure to canavanine.

Properties of Giants

Multiplicity of reactivation. The simplest way of testing whether giant bacteriophage are infectious is to plate them with sensitive bacte-

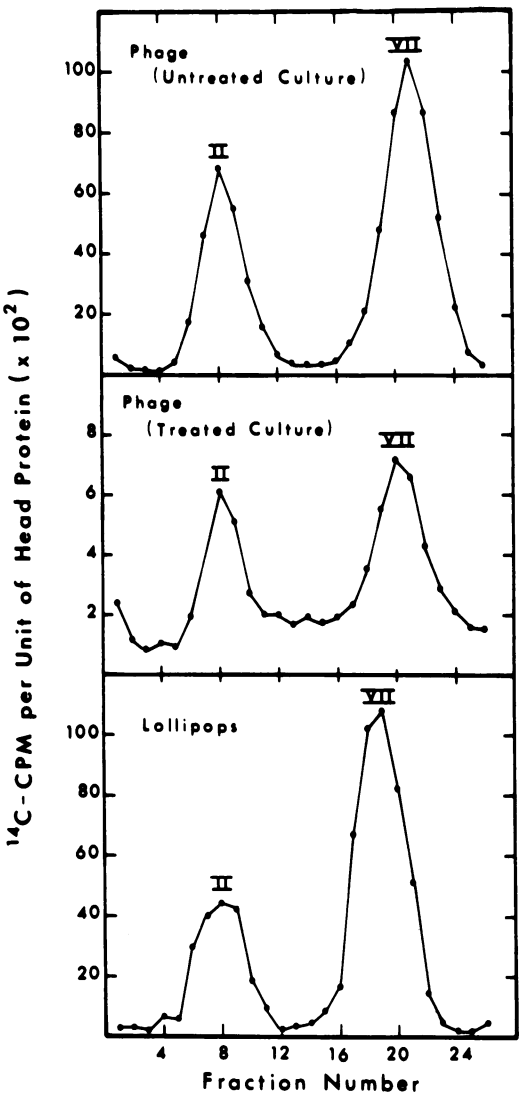

FIG. 22. *Effect of canavanine on the utilization of the precursor (presumably p22) to the acid-soluble internal peptides of T4 (16). The canavanine conditions and labeling regime were the same as in Fig. 21. In each case, an acid-soluble extract from the same number of phage equivalents was applied to a Sephadex G-50 column. II and VII refer to the nomenclature described previously (52). Note the change in the ordinate scale for the middle panel. (Reproduced here with the permission of the American Society for Microbiology.)*

ria. This was done (32, 38), and the results clearly indicate that these monsters are viable. A more elegant method for testing for viability as well as determining the presence of multiple copies of phage DNA was devised by Doermann et al. (38) using the giant phage derived from a missense mutation in gene 23. They hypothesized that, if giants are viable, they might be able to inject more than one copy of phage DNA. These multiple copies should then be able to complement one another. This is akin to multiplicity reactivation achieved when several ultraviolet-inactivated normal phage rescued one another. Giant phages should therefore exhibit high levels of resistance to ultraviolet. This expectation was borne out by the data in Fig. 23, where it can be seen that giants are considerably more resistant to ultraviolet than normal phage and that the relative resistance is diminished when normal phage is mixed in at increasingly greater proportions. Doermann et al. (38) concluded that giants are infectious and are capable of injecting multiple copies of phage DNA. The presence of multiple copies of phage DNA also implies that giants would have a greater density in CsCl gradients than normal phage. This was confirmed using both canavanine-induced giants (32) and gene 23 mutant giants (38): it was found that giants of T2L, T4D, T4B, and T6 were, on average, about 0.036 g/cm^3 more dense than normal phage. Considering the possible size of the DNA contained in some of these giants, it is possible that some bacterial DNA may have been packaged. This possibility was excluded when Cummings et al. (32) measured the density of glucosylated native and denatured DNA from T2L and T4B giants in Cs_2SO_4 gradients (34) and were able to detect only phage-specific DNA.

Genome length and packaging. Much has been discussed about the role of concatameric DNA in bacteriophage packaging. With the information that giants contain the equivalent of several copies of phage DNA, it was natural to inquire whether this DNA was one continuous piece or multiple copies. Doermann et al. (38) measured the length of DNA obtained from giants of 4 head lengths and did indeed find DNA lengths three to four times longer than normal mature DNA. However, only two of the nine molecules measured had this length, and the others were one to two or two to three times normal-length DNA. We too (unpublished data) measured the lengths of some giant DNA in the electron microscope and found several molecules four times normal length; however, most were shorter than this but as long or longer than normal mature DNA. We attributed this paradox to the difficulties of measuring lengths of such long DNA (220 μm for DNA four times the normal length) in the electron microscope. Therefore, we turned to the viscoelastic method developed by Kavenoff et al. (65, 66). This method is especially advantageous, since accuracy is increased with increasing DNA length, handling of the DNA is minimal,

TABLE 5. *Total incorporation of proteins and DNA synthesized in the presence of canavanine into phage particles*[a]

Determination	Phage (untreated culture)[b]	Phage (treated culture)[b]	Giants
Protein (μg)	28.4 (16.0)	46.0 (17.5)	24.5 (11.5)
^{14}C counts/min	60,212 (93,869)	19,585 (35,021)	44,100 (68,735)
Counts/min per μg of protein (normalized)	1.0 (1.0)	0.20 (0.17)	0.85 (1.10)
DNA (μg)	11.5	10.4	9.8
^{3}H counts/min	20,978	20,735	16,315
Counts/min per μg of DNA (normalized)	1.0	1.07	0.91

[a] Proteins or DNA were labeled only during the exposure to canavanine, as described in the text. The phage and giants were purified, and the total counts per minute per microgram of protein or DNA were determined. The Lowry protein assay was used to determine total protein (88), and the diphenylamine reaction was used to quantitate relative amounts of DNA (102).

[b] The untreated culture was not treated with canavanine. The treated culture was treated with canavanine from 10 to 25 min p.i. The giants and phage from the treated culture were isolated from the same lysate.

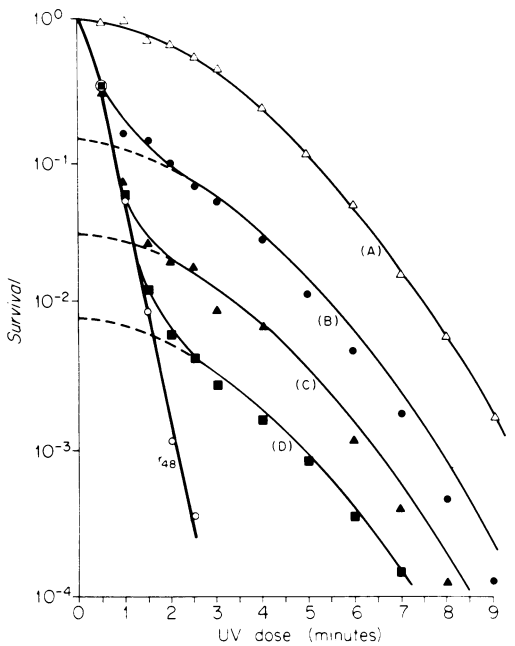

FIG. 23. *Survival of giant phages as a function of ultraviolet (UV) dose. A stock of pure giants (about 4 average head lengths) was purified and mixed with suspensions of normal-length phage carrying the mutation r48. (A) Pure giants, (B) 15% giants mixed with 85% r48, (C) 3.3% giants with 96.7% r48, and (D) 0.8% giants with 99.2% r48 (38). (Reproduced here with the permission of the American Society for Microbiology.)*

and information is obtained concerning the heterogeneity of the DNA. We (111) measured the size of the DNA from giants with heads of 4, 8, and 13 head lengths for both native and denatured molecules. The results, including the calculated proportions of full-size molecules, half-size molecules, and one-sixth-size molecules, are listed in Table 6. It is apparent that the longest giants contain DNA of the greatest molecular weight (1.4×10^9), and there is a fair agreement in relative size with the shorter giants. In addition, the molecular weight of denatured DNA from the longest giants is almost exactly half that of the native, indicating that the DNA is one continuous piece without single-strand interruptions (or internal regions of RNA that would be hydrolyzed by the NaOH denaturation). What is equally striking, however, is that only 6% of the molecules, for both native and denatured DNA, have this long DNA and that about 60% are only one-sixth this size. The origin of this smaller than giant (but still larger than normal) DNA is not known. Several attempts (111) were made to isolate the giants in different ways and all gave this result. We speculated that the length of these giants would give more flexibility to the structure and perhaps provide some shearing force on the packaged DNA. Alternatively, the concatameric DNA described by Frankel (45) could be packaged in this manner, with only a few complete concatamers being packaged. Most of the time, however, some processing of these complete concatamers must occur during packaging. In aberrant structures such as these, no information is available about the efficiency and accuracy of gene 49 product in processing the DNA. Earlier, we mentioned the finding of Curtis and Alberts (personal communication) that head size pieces of DNA could be generated in the absence of head formation. Perhaps, during the packaging of giants, inad-

TABLE 6. *Molecular weight of giant DNA*

Phage DNA	Mol wt $\times 10^{-8}$	Mol wt[a] (%)	Mol wt/2 (%)	Mol wt/6 (%)
Native				
13 × T2	14 ± 4	6 ± 2	31 ± 9	59 ± 18
8 × T4	10 ± 0.3			
4 × T4	8 ± 0.7			
T2	1.08			
Denatured				
13 × T2	6.9 ± 1	6 ± 1	40 ± 5	55 ± 5

[a] The data in these columns refer to the percentage of DNA molecules with molecular weight (mol wt), that with molecular weight mol wt/2, etc., obtained from giants of 13 times normal head length, 8 times, 4 times, and normal T2 head length.

vertent cuts are made, but the packaging continues and a few giant DNAs escape this cutting. In any case, the data do support the "headful" hypothesis of Streisinger et al. (107): the longer the head, the longer the DNA.

Surface morphology. Many icosahedral viruses have been characterized with regard to T number, capsomer arrangements, etc., based on their characteristic appearance in the electron microscope. With regard to studies on the T-even bacteriophage, the surface always appeared to be smooth, without dominant capsomers, and Williams and Fraser (114) suggested that the head probably had the structure of a bipyramidal hexagon. Moody (92) speculated that the T4 head is icosahedral but the evidence, although detailed, was somewhat inferential. Two newer techniques have provided substantial evidence that T-even heads are indeed icosahedral. Bayer and Remsen (7) showed that the surface morphology could be more readily visualized using freeze-etching techniques, and Branton and Klug (18) recently used this procedure to great advantage. They reported that the head of T2 consists of two fivefold-symmetry end-caps with $T = 13$ icosahedral symmetry separated by a band of 20 capsomers, which corresponds to one turn of a flat helix. On this basis, the short-headed variants, petites, would lack the 20-capsomer band. Longer variants would include additional turns of the flat helical band. Branton and Klug (18) also discussed the similarity between the number of capsomers their model would predict and the number found experimentally. They cited unpublished evidence of Wagner and Laemmli that there are about 860 copies of p23* within the phage head. According to the model (18), there are 900 structure units made up of 840 (140 × 6) at hexamer positions and 60 (12 × 5) at pentamer positions. They concluded that p23* must account for all the hexamer positions. T4 heads were also found to contain about 40 copies of p20, which could account for the pentamer proteins in the end-caps by themselves or in combination with p23*. Mutations in gene 20 do lead to polyheads, so it is likely that this protein is utilized in the construction of the two fivefold-symmetry end-caps.

The second technique was one introduced for T4 phage by Finch et al. (42). This involved optical filtering of diffraction patterns obtained from high-resolution electron micrographs. They found that T4 polyheads consist of helical tubes with a surface lattice having rotational sixfold symmetry (this symmetry will become clear in Fig. 25 and 26). They emphasized that the theory of Caspar and Klug (21) concerning icosahedral viruses predicts that this is the only surface lattice that can be folded into a closed structure while quasi-equivalent bonding between the structure units is preserved. More recently, Aebi et al. (1), Steven et al. (105), and Ishii and Yanagida (61) have used this technique to study the surface morphology of coarse polyheads, giant phage obtained using mutants in genes 23 and 24, giant phage prepared using canavanine, and reassembled sheets. These structures offer the real advantage of providing a greater surface for analysis as well as presenting a variety of p23 assemblies for comparison. Kellenberger's group examined several structures, some of which are illustrated in Fig. 24: giants obtained from a ts mutant in gene 24 (105), a missense mutant in gene 23 (1, 38), giants of T2L and T4D produced by canavanine treatment (32), and sheets of T2 prepared in vitro from disassociated T2 heads (98). The first observation made was that the lattice constants calculated from optical diffraction patterns of these assemblies are different (1, 105). Coarse polyheads yield a lattice constant of 11.2 μm, all giants give 12.4 μm, and T2 sheets show 10.2 μm. Clearly, different assemblies of p23 (or p23*) yield different morphological interactions. This is demonstrated more directly in Fig. 25 and 26. Three types of polyheads are possible: the coarse polyhead containing p23, with a lattice constant of 11.2 μm; type A polyheads containing p23*, with capsomers containing six protomers forming an "empty" hexamer; and type B polyheads containing p23* with capsomers containing six protomers surrounding a central mass (6 + 1) (105; U. Aebi and A. Steven, personal communication). Several conclusions can be drawn from such optical diffraction studies. First and foremost, the diffraction spots originate from the proteins in the head shell. Second, the diffraction patterns of coarse polyheads containing p23 differ from those of type A or B containing p23*. Third, T4 giants obtained from either missense mutants in gene 23, a ts mutant in gene 24, or those produced by

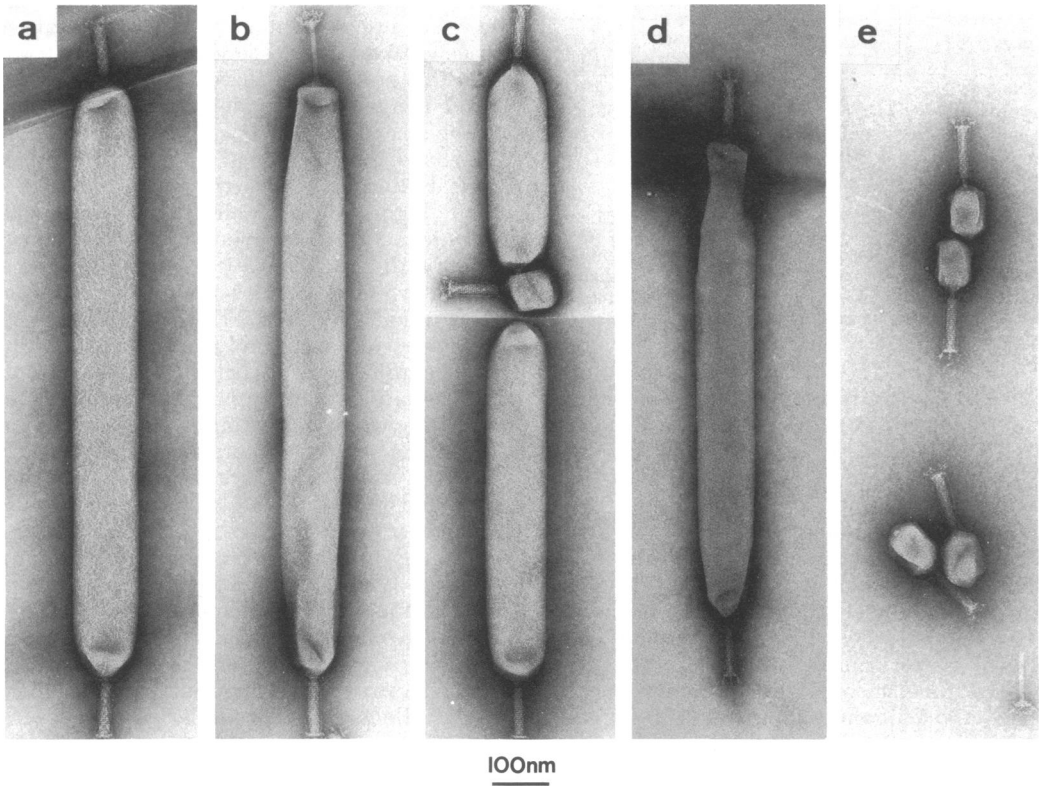

FIG. 24. *Electron micrographs of various T-even phage giants. All preparations were negatively stained with 1% uranyl acetate. (a) T4D giant produced under conditions semipermissive for gene 24. The temperature-sensitive mutant 24 (tsB86) was grown at the intermediate temperature of 35 C. (b) T4D giant produced by point mutations in gene 23 [T4D 23 pt (19-80)]. (c) T4D giant (lollipop) and wild-type phage grown in the presence of canavanine. (d) T2L, canavanine-induced giant. (e) T2L phage particles. (Kindly provided by the Information Theory Group, Basel.)*

treatment of T4D$^+$ with canavanine all give identical patterns, indicating that all three giants have an identical structure. Sheets of T2 head protein resemble type A polyheads in some respects but clearly yield yet another structure. This is not surprising since these sheets do not represent closed structures and most likely contain only p23*; Cummings et al. (31) showed that assembly of dissociated T2 (or T4) heads could exclude proteins other than p23*. Finally, giants obtained from treatment of T2L with canavanine yield a diffraction pattern quite similar to that seen with type A polyheads and different from patterns of giants obtained from T4 mutants in gene 24, gene 23, or canavanine-induced T4 giants. Earlier, it had been shown (43) that T2L heads differed from all other T-even heads in that they lack a 10,000-dalton structural protein. This protein accounts for about 10% of the total head protein, so its absence in T2L is considered significant. Steven et al. (105) attributed the different diffraction pattern of T2L giants to the absence of this protein. Cummings et al. (31) presented evidence that this protein is also absent from T4 polyheads, so it is not surprising that the diffraction patterns of T2L giants and polyheads are similar. Ishii and Yanagida (61) also found that the diffraction patterns of T2L giants differ from those of T4 giants and performed a set of truly elegant experiments. They were able to isolate mutants of T4D which lacked this nonessential protein, which they termed *soc* (Structural Outer Capsid protein), and, in addition, were able to purify large amounts of the protein from T4D *soc*$^+$ phage. They were able to show that the *soc* protein associates with *soc*$^-$ phage in vitro. Treatment of *soc*$^-$ giants with *soc* protein results in a striking change in the diffraction pattern. The patterns from these "reassociated" particles resemble quite closely the patterns obtained from either T4 or T2H giants. Ishii and Yanagida (61) concluded from their studies that the surface lattice of T4 (and

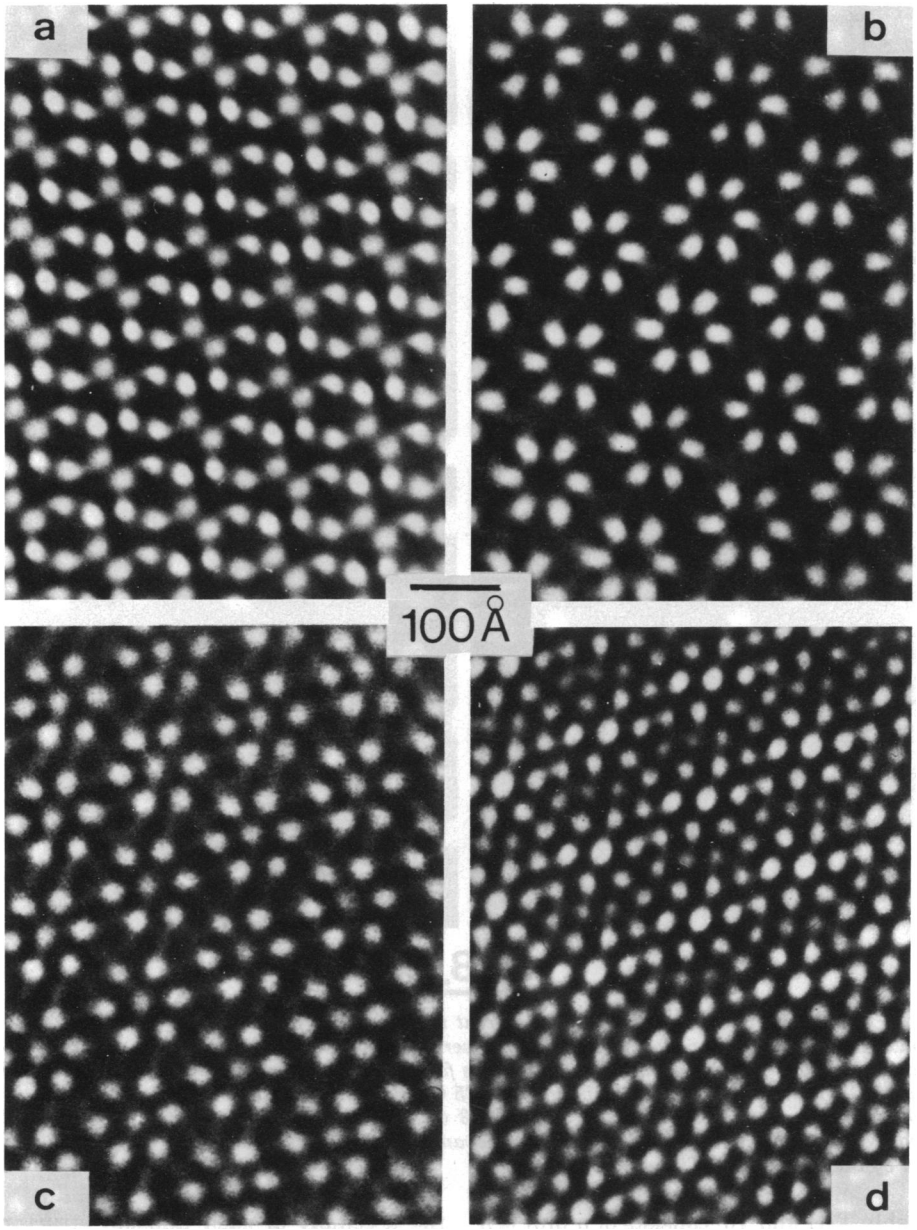

FIG. 25. *Surface lattice structures of different types of T4D "polyheads" imaged by optical filtration of electron micrographs. All preparations were negatively stained with 2% sodium phosphotungstate. (a) "Coarse" polyheads composed of p23. These particles correspond to the surface structure (except with respect to folding the hexagonal lattice) of tau-particles. (b) Type A polyheads and (c) type B polyheads (105) have a 13.0-nm lattice constant and contain p23*. These are in vitro transformation products of initially coarse polyheads, resulting from cleavage of p23 and cooperative lattice transformations. (d) Giant-type polyheads (105) also have a 13.0-nm lattice constant and contain p23*. In addition, they contain soc protein (61). The surface lattice of these particles is indistinguishable from that of giant (and presumably, also wild-type) T4 capsids. (Kindly provided by the Information Theory Group, Basel.)*

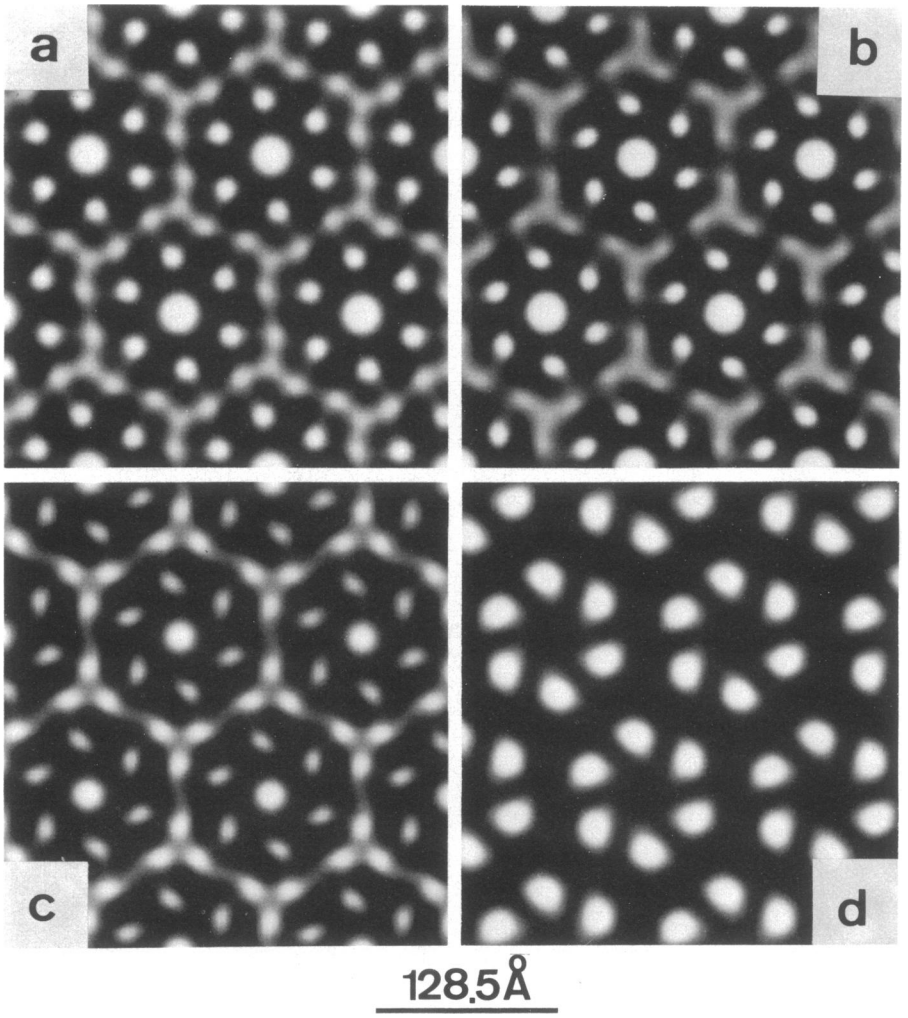

FIG. 26. *Surface lattice structures of four different types of giant phage capsids, obtained by computer filtration of electron micrographs. The preparations were negatively stained with 1% uranyl acetate. (a) T4D giants produced by growth conditions semipermissive for gene 24; the temperature-sensitive 24 (tsB86) was grown at 35 C, intermediate between the permissive (25 C) and nonpermissive (41 C) extremes (105). (b) T4D giant phage produced by point mutation in gene 23 (38). (c) T4D and (d) T2L giant phage (lollipops) produced by growing phage in the presence of canavanine, subsequently chased by arginine (32). (Kindly provided by the Information Theory Group, Basel.)*

presumably T2H and T6) consists of a unit cell of near hexagonal symmetry containing six p23*, six *soc* proteins, and possibly one 40,000-dalton protein, forming a (6 + 1) + bridges morphological unit. It is still not clear what role is played by this nonessential *soc* protein in assembly. Additional in vitro experiments would be most interesting. For example, how would *soc* affect the surface morphology of coarse polyheads that contain p23? How would it affect T2 sheets? This sort of in vitro assembly should provide a great deal of insight into the assembly of such unusual structures, as well as provide information on T4 phage assembly.

Mixed-Infection Experiments

It has been demonstrated that giants produced by canavanine are identical in all measured respects to giants generated randomly by mutation in genes 23 or 24. Further, proteins synthesized in the presence of canavanine are preferentially utilized in the assembly of giant phage precursors, which contain at least six

proteins, including the major head protein p23 and the core proteins (p22 and internal proteins). It has also been shown (16) that gene 21-dependent proteolytic activity from canavanine-treated extracts is markedly inhibited, whereas the substrate proteins retain a high susceptibility for cleavage. Presumably, the 21-dependent proteolytic activity is inactive in the canavanine-treated giant complex; after the addition of arginine, a new component of the cleavage complex is synthesized and added to the prohead of the giant. Cleavage then occurs and the giant head matures. However, these results have not identified the specific canavanine-induced giant induction step. Aebi et al. (1) have suggested that pairwise interactions of different gene products are the basis for the regulation of assembly. Certainly, one would expect tht p23 and p24 would be involved in such interactions, since mutations in these genes are the only two known instances in which giants are produced in the absence of canavanine. It is conceivable, therefore, that the canavanine induction event is the result of a specific aberrant association between two or more head proteins such that length determination is not achieved. To investigate this possibility, we took advantage of the fact that each T-even phage (T2, T4, or T6) results in a characteristic size distribution of lollipop giants (32). The size distributions for T6 and T4D (or B) are shown in Fig. 27. The average head length for a T6 giant is 4 to 6 head length equivalents, whereas the average head length for a T4 giant is 10 to 14 head lengths, although a small proportion of the T4 giants do fall in the shorter range.

To investigate possible protein-protein interactions involved in head length determination, we determined whether one of these size classes would predominate if T4 and T6 phage were used to simultaneously infect a culture. Each phage was added at a multiplicity of infection of 5; at 10 min p.i., canavanine (130 μg/ml) was added and, at 25 min p.i., arginine (150 μg/ml) was added. The resulting lysate was examined in the electron microscope, and greater than 90% of the giants were in the 3 to 6 phage head length class. That is, the T6 giant-size class clearly predominates. Several control studies were done to substantiate this result. First, we demonstrated that even though the T6 giant predominates, T4 proteins are expressed. T6 proteins differ from T4 proteins in two noticeable respects: (i) the tail fiber proteins differ (32) and (ii) T6 lacks IPIII* (59, 106). Examination of [^{14}C]amino acid-labeled lysates of the mixed infection revealed normal amounts of IPIII* as well as the T4 fiber proteins. Second, we had to ensure that T6 was not excluding the T4 ge-

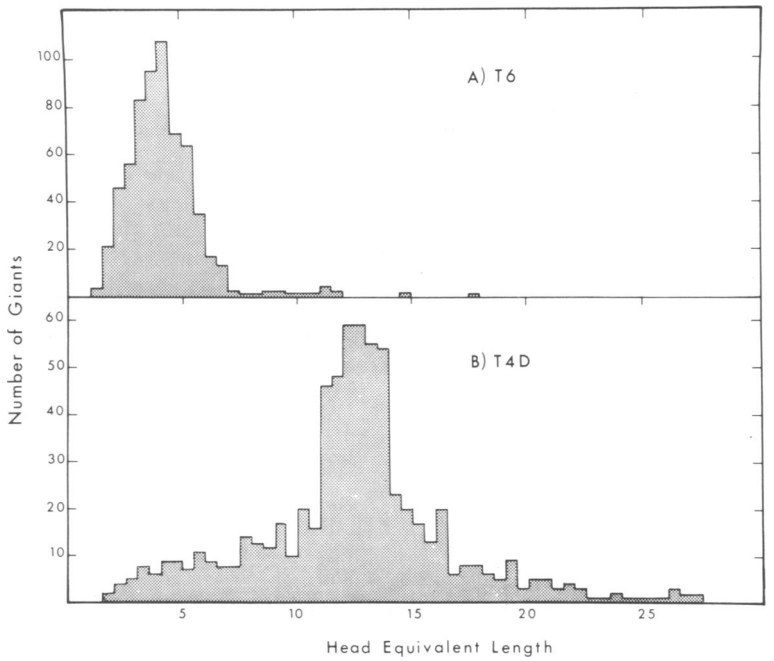

FIG. 27. *Size distribution of giants of T4D and T6 prepared after treatment of infected cultures with canavanine followed by arginine.*

nome. A culture was infected with T4 at zero time and, at 0, 0.5, 1.0, 1.5, and 3 min later, T6 was added. It is not until 1.5 min after infection with T4 that the T4 giant is apparent and, even at 3 min, more than half the giants are T6-like. Finally, a dose-response experiment indicates that not until 1.6 times more T4 than T6 is used for the infection does the T6 domination diminish. This strongly suggests that the control under examination is of a stoichiometric nature, as opposed to a strictly catalytic role.

It was now logical to manipulate this mixed-infection system with crosses involving mutants. The experiments were limited by the fact that very few T6 head mutants are available [T6(20)$^-$ and T6(23)$^-$], but nevertheless the results did lead to meaningful conclusions. Several sets of crosses were performed. In all cases the nonpermissive host *E. coli* B was used, and a multiplicity of infection of 5 for each phage was carefully controlled. First, we crossed T6$^+$ with amber mutants in genes 20, 21, 22, 23, 24, and 31 (the known T4 head genes). Canavanine was added, followed by arginine as before, the resulting lysates were examined, and the size distribution was determined. When T6 is crossed at equal multiplicity with a T4 mutant in gene 20, normal numbers of viable phage are produced but, surprisingly, no giants are found. Mutations in genes 21, 22, 24, and 31 all resulted in the T6-like giant (Fig. 28). However, when T6 is crossed with T4 defective in gene 23, the T4-like giant is obtained. This result was unexpected since the defect in gene 23 would effectively eliminate T4-like phage heads. The majority of the particles would be primarily type T6, with minor T4 proteins incorporated into the structure. In some manner, the T6 control is alleviated by the presence of defective p23 from T4 or the absence of p23. One interpretation of this result is that by removing normal T4 p23, essentially one-half of the outer structural proteins are deleted, which could allow greater availability of "size-determining" minor components that interact with p23. This would be analogous to increasing the input multiplicity of T4 relative to T6, which would then result in the T4 giant. If this were so, a second mutation in the gene 23 mutant might restore the length control factor to its proper

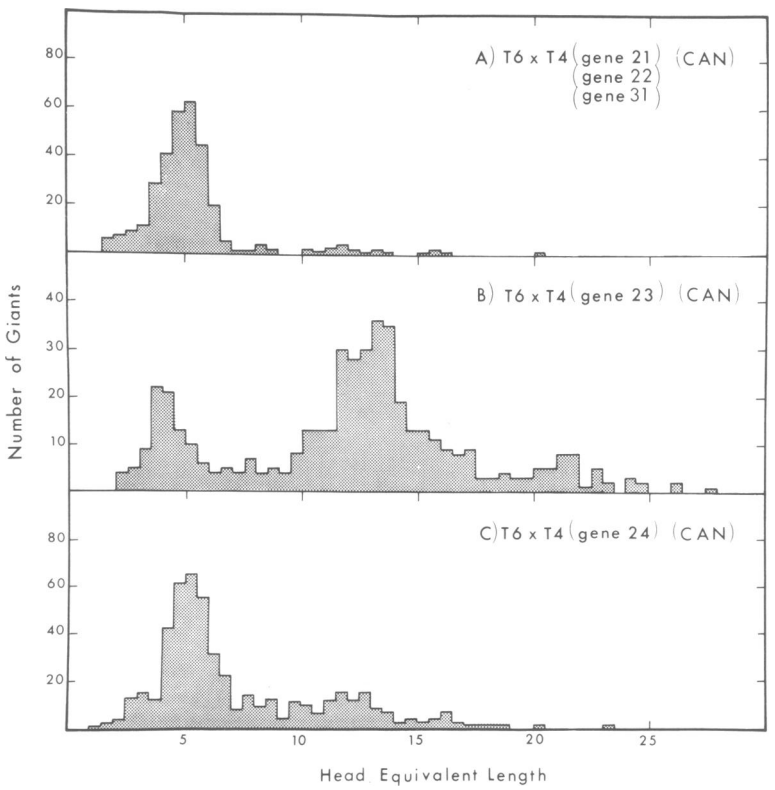

FIG. 28. *Size distribution of giants obtained after canavanine-arginine treatment of cultures infected with (A) T6$^+$ and T4 (21)$^-$, (22)$^-$, or (31)$^-$; (B) T6$^+$ and T4 (23)$^-$; and (C) T6$^+$ and T4 (24)$^-$. Note that T6-like giants are produced except for the T6$^+$ and T4 (23)$^-$ infections.*

concentration. Mutants in genes 20 and 23, 21 and 23, 22 and 23, and 24 and 23 were constructed and used to infect simultaneously with T6. All such crosses yield the T4-like giants; i.e., none of the additional mutations releases the effect of the gene 23 mutation. Either critical concentrations of p23 and size-determining proteins were not involved or the experiment did not work as anticipated. Many factors could be involved that cannot be controlled in vivo. Curiously, the T6 × T4 (20, 23)⁻ did yield T4 giants, even though T6 × T4 (20)⁻ produced no giants.

Second, we determined whether canavanine was necessary to produce giants in mixed infections. For this we used combinations of as many of the pairs as seemed reasonable and performed the experiment as before but omitted canavanine. Three major facts (Table 7) emerge. (i) Only those crosses which include T4(24)⁻ as one of the pairs result in the formation of giants. Even when a wild-type phage is one of the pairs, only mixed infection with T4(24)⁻ produces giants. (ii) All of the giants produced are T4-like (Fig. 29) [this is in contrast to the results obtained in the T6 × T4(24)⁻ cross in the presence of canavanine, which produced T6-like giants]. (iii) Not all T4(24)⁻

TABLE 7. *Effect of mixed infection on formation and length of giants*

Cross[a]	Giants (lollipops)		Small head (%)[b]	Multitails (%)[b]
	(%)[b]	Type[c]		
T4(24)⁻ × T4⁺	0.01	T4	4.0	0.14
× T4(23)⁻	0.053	T4	0.50	0.07
× T4(23)⁻ + CAN	0.18	T4	0	0
× T4(31)⁻	0.005	T4	0.18	0
× T4(31)⁻ + CAN	0.18	T4	1.0	0
× T4(21)⁻, (20)⁻ or (22)⁻	0		4.4	0.22
× T4(21)⁻ + CAN	0.16	T4	6.4	0
× T6⁺	0.027	T4	3.7	0
× T6⁺ + CAN	+[d]	T6	ND[e]	ND
× T6(20)⁻	0.010	T4	0.12	0.37
× T6(23)⁻	0.026	T4	0	0
× T6(23)⁻ + CAN	0.071	T6	0	0
T4(23)⁻ × T4⁺	0		0	0
× T4(21)⁻, (20)⁻ or (22)⁻	0		0.28	0
× T4(21)⁻ + CAN	0.13	T6	0	0
× T4(31)⁻	0		0.3	0
× T4(31)⁻ + CAN	0.14	T4	0	0
× T6⁺	0		ND	ND
× T6⁺ + CAN	+[d]	T4	ND	ND
× T6(20)⁻	0		0	0
T4(31)⁻ × T4(21)⁻ + CAN	0.12	T4/T6	3.7	0
× T6⁺ + CAN	+[d]	T6	ND	ND
T6(23)⁻ × T4⁺ + CAN	+[d]	T6	ND	ND
× T4(20)⁻	0		0	0
× T4(31)⁻	0		0	0
× T4(31)⁻ + CAN	0.11	T6/T4	0	0
× T6(20)⁻	0		2.7	0
× T6(20)⁻ + CAN	0.13	T6	0	0

[a] The nomenclature used here indicates that T4(24)⁻ is an amber mutant of T4D which is defective in gene 24, and so on. Cross indicates that *E. coli* B was infected simultaneously with both phages at a multiplicity of infection of 5:1.

[b] A minimum of 30,000 normal phage was scored to calculate these percentages. For very low percentages, duplicate experiments agreed within a factor of 2 to 3; as many as 900,000 phage were estimated for some determinations. The yield of viable phage was about 20 to 50% of wild type in the absence of canavanine. Canavanine-arginine depressed the yields another fivefold. The percentage of giants produced in a wild-type infection with canavanine-arginine was about 1.5% for T4D (32). This may be compared with the values listed here.

[c] Type of giant phage refers to the head length observed: T6, predominantly 4 to 6 head lengths; T4, predominantly 10 to 14 head lengths; T6/T4, a diphasic distribution of the two with slightly more T6-like; T4/T6, a diphasic distribution of the two with slightly more T4-like. CAN, Canavanine.

[d] Giants were produced, but the percentage was not determined.

[e] ND, Not determined.

crosses produce giants. Crosses with T4(20)$^-$, (21)$^-$, and (22)$^-$ failed, whereas crosses with T4(23)$^-$ or (31)$^-$ and any of the T6 stocks were successful. The result with T4(31)$^-$ is thought to be, in principle, not much different from the result with (23)$^-$ mutants, since p31 affects the solubility of p23 (78).

Third, additional crosses in the presence of canavanine were performed. All of them produce giants but the size distributions differ. (i) All of the T4(24)$^-$ crosses with T4 mutants yield T4-like giants; as before, T4(24)$^-$ crossed with T6 produces T6 giants. (ii) T6 stocks crossed with any stock but T4(23)$^-$ yield T6-like giants. (iii) The cross T4(23)$^-$ × T4(21)$^-$ yields a diphasic distribution (Fig. 30). T4(21)$^-$ × T4(24)$^-$

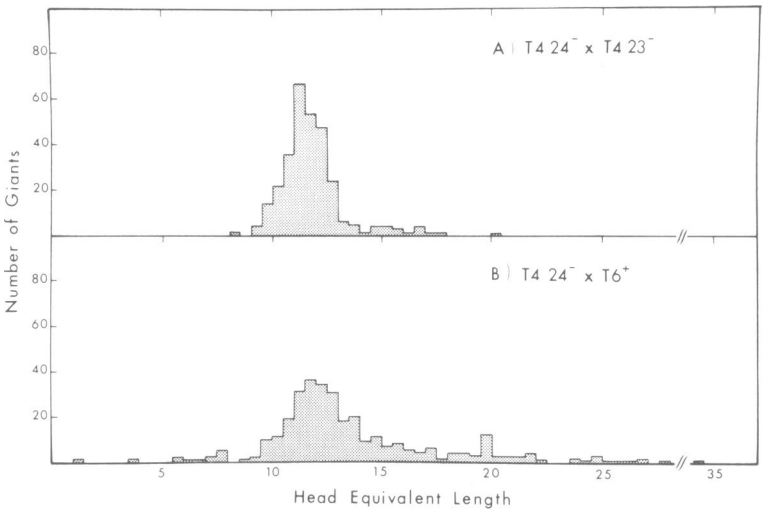

FIG. 29. *Size distribution of giants obtained in the absence of canavanine of some of the mixed infections listed in Table 7. (A) T4(24)$^-$ and T4(23)$^-$ and (B) T4(24)$^-$ and T6$^+$. Note the difference in distribution of (B) with panel C of Fig. 28.*

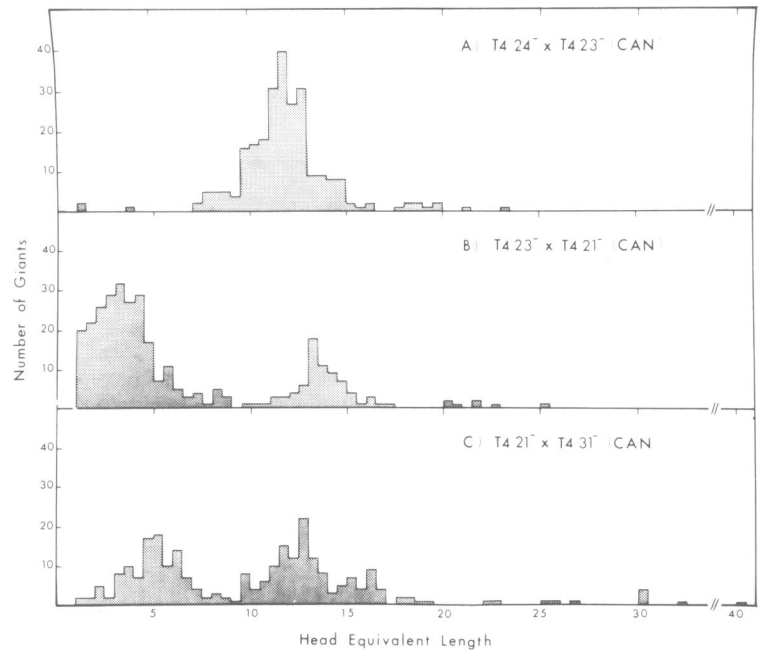

FIG. 30. *Size distribution of giants obtained after canavanine-arginine treatment of some of the mixed infections listed in Table 7. (A) T4(24)$^-$ and T4(23)$^-$; (B) T4(23)$^-$ and T4(21)$^-$; and (C) T4(21)$^-$ and T4(31)$^-$.*

produces T4-like giants, as already indicated.

All of these results are tabulated in Table 7. The results we listed above are emphasized by the placement of boxes around the more significant data. In addition, small heads are also tabulated, and no significant effect on their production is noted in any of the crosses. Some crosses lead to an increase of multitailed, normal-headed phage. Of the seven crosses that yield multitailed phage, in six of these, T4(24)$^-$ is one of the mutants in the cross; the single exception is T4(23)$^-$ × T4(22)$^-$, in which 0.1% of all the phage was multitailed. This might suggest that p24 is associated with the fivefold symmetry end-caps. However, the amounts of p24* in giants make it likely that p24* is distributed throughout the head shell and not just at the end-caps. It may be significant that, when canavanine was present, none of the mixed infections produced multitailed phage.

Experiments with so many crosses can lead to a rather bewildering array of data. These are complicated experiments to interpret since we have no way of knowing what other maturation factors are affected by the cross. The phage yields are reduced when two amber mutants are utilized, and, although giants are readily detected, their levels are below that observed with wild-type phage grown in the presence of canavanine. Nevertheless, two conclusions emerge. In the absence of canavanine, lesser amounts of p24 may be required for the formation of giants. When the giant-size length is subject to dominance, the exception occurs when defective p23 is present. Beyond any doubt, we have demonstrated that giants can be generated in the absence of canavanine. In many ways these crosses can be thought to mimic the effect of the missense mutants in gene 23 (38) and the unusual temperature-dependent gene 24 mutants (1). Here too, giants are produced in a poorly understood manner. Aebi et al. (1) interpret their results as indicating that p23 is a necessary but not sufficient factor in form determination and that some additional gene product is required. The mere fact that not all missense mutants of gene 23 or all temperatures lead to the production of giants in gene 24 ts mutants suggests that some critical interactions are involved about which we know little. Aebi et al. (1) also point out that similar paradoxes exist for the production of polyheads, since defects in two seemingly different classes of proteins, i.e., core proteins (p22, IPIII) or minor capsid proteins (p20, p24), both produce polyheads, i.e., defective assembly of p23. The apparent existence of such unknown sets of gene product interactions makes further interpretation of the crosses described unwarranted.

CONCLUDING REMARKS

Possible Mechanism(s) by Which Canavanine Produces Giants

When a T-even bacteriophage-infected cell is exposed to canavanine and then chased with arginine, a giant bacteriophage particle which we termed a "lollipop" is formed (32). The appearance of these giants requires at least two major steps (13). The first step, the induction step, involves late phage functions, and a 3-min exposure to canavanine is sufficient to initiate the steps that lead to giants. The second step, the formation step, requires arginine, and this is the step which sets into motion the normal phage maturation machinery and allows the actual production of both normal phage and giants. Two general interpretations are possible: (i) canavanine may directly prevent that specific function which controls head length, and thus canavanine results in continued head elongation; or (ii) canavanine may result in a faulty interaction between specific gene products, which makes head length determination inoperative, while at the same time a separate function is blocked in such a way that phage maturation cannot occur.

Our work indicates that canavanine results in a compartmentalization of proteins such that the proteins synthesized in the presence of canavanine can later be preferentially utilized in giant head assembly. On the other hand, the DNA synthesized in the presence of canavanine can be packaged into giant heads and phage of normal head length with equal efficiencies. This requires that a prohead be formed in the presence of canavanine that later matures to the giant head upon restoration of normal function and packaging of the DNA. This giant head precursor has not been identified. However, it is clear that it must be comprised of uncleaved proteins, since canavanine prevents proteolytic cleavage in vivo and the gene 21-dependent proteolytic activity is severely depressed in canavanine-treated extracts (16). This prohead probably consists of at least p23, p22, the internal proteins, and three unidentified proteins, one of which may be an aberrant form of the gene 24 protein. Occasional structures are seen in the electron microscope which could correspond to these prohead structures (our observations; R. Bijlenga, personal communication). The structures appear to resemble giant particles, except that they are devoid of DNA and appear to contain a protein core which, by its mottled morphology, presumably is comprised of p22 and the uncleaved internal proteins. These particles may well be analogous to the tau-particles seen in (21)$^-$ and (24)$^-$ lysates. One approach to identifying these struc-

tures may be to compare their optical diffraction patterns with the other p23 assemblies (R. Paulson, personal communication). The most predominant head form seen in lysates treated with canavanine are polyheads (29; Fig. 11). The polyheads seem to contain core material, and end-caps are occasionally present. Following the addition of arginine, the polyheads appear to decrease in number, but this is difficult to quantitate.

The mechanism by which canavanine induces giants apparently involves the overriding of the normal length control regulation. In the giant system, at least two characteristic control steps are apparent. Canavanine allows the accumulation of a prohead which apparently increases in length with increasing duration of exposure to canavanine, subject to a maximum length. This implies the loss of a particular function. The maximum attainable head length is phage specific. As is evidenced by the mixed-infection experiments with T4 and T6 in the presence of canavanine, the shorter giants are dominant. This may define a second positive control step in giant head length determination (i.e., something must be added to stop lengthening rather than something being depleted). Alternatively, it could mean that the gene product interactions are altered such that the "cumulated" strain exerted is different and the head structure closes at an earlier state of lengthening. Each of these events could represent a separate control step in normal assembly, or perhaps each of the controls represents a facet of the same identical control which operates during normal assembly.

As discussed, canavanine is necessary for the induction of giants, but arginine is required for their formation. The fact that arginine is required for the appearance of giants indicates that canavanine inactivates a function(s) required to continue maturation through the prohead pathway. We have somewhat casually dismissed the canavanine-induced inhibition of DNA synthesis as a primary event for several reasons. Although synthesis is inhibited, approximately 30% of the normal level of DNA is still synthesized, and this DNA can be used to fill both giant and phage heads (14, 26). One possibility that was not discussed is that since T4 DNA synthesis is dependent on the presence of polyamines (37), canavanine may act by interfering with the polyamine levels in the infected cell. We found that canavanine did inhibit the synthesis of polyamines, but the data indicated that the inhibition of polyamine synthesis was not responsible for the L-canavanine-mediated inhibition of DNA synthesis, nor did it seem to be involved in the induction of giants (15). Basically, canavanine has two gross effects on T4 phage maturation. It inhibits DNA synthesis and prevents proteolytic cleavage. The question is: are both these two effects required for the production of giants? In T5, canavanine inhibits cleavage and leads to the formation of polyheads, but does not inhibit DNA synthesis and does not lead to the production of giants. Moreover, canavanine inhibits T4 DNA synthesis and is incorporated into a protein which is then inhibitory to DNA synthesis (13). In a normal infection, chloramphenicol does not inhibit T4 DNA synthesis at times when canavanine does. This indicates perhaps that new protein synthesis is not required to maintain DNA synthesis at these times and suggests that the proteins in the DNA replication complex may be reusable, but new proteins are constantly being added. The incorporation of a particular canavanyl protein could inactivate the complex and inhibition of protein synthesis by chloramphenicol could prevent its replacement by a functional protein during the chase with arginine. Although the effect on DNA synthesis might not be the primary cause of the production of giants, it cannot be ignored totally. It is important to recall that in the dose-response experiment, giant production parallels the degree of inhibition of DNA synthesis. One could argue that the primary mechanism for the effect of canavanine involves the inhibition of proteolytic cleavage. Interfering with any of the group A genes interferes with the proteolytic cleavage of the other gene products and leads to the accumulation of aberrant head forms, as does canavanine. However, other amino acid analogues interfered with head protein cleavage (ACA and PFPA) without necessarily causing head aberrations. Clearly, cleavage is at the heart of the striking effect of canavanine on head assembly, but we still do not comprehend the complete mechanism.

Size Determination of T4 Heads

In the Introduction, we discussed the possible models (68) by which size determination could be controlled and offered specific examples. For structures such as giant phage and normal phage, probably the two most attractive models are the vernier and the "cumulated" strain models. The template model seems to us to be inappropriate. In the examples offered, we used the P2-P4 bacteriophage system in which the larger P2 head lacked a protein present in the smaller P4 head (53, 100). The T4 phage also have petite variants, so the possibility existed that perhaps petite T4 would have a unique

protein that could be "size determining" in the sense used for P2-P4. This is not the case, however. Doermann et al. (38) examined normal T4D and petite phage proteins on 10% SDS-polyacrylamide gels, and we examined them on 13% gels. In each case no difference in the protein components of normal T4 and petite phage was noted. It is possible that the "strain" on the capsomers in each of the two T4 heads is different. We noted some years ago (31) that lower-molecular-weight proteins in heads of petite phage were more accessible to in vitro I^{125} labeling than were these proteins in normal heads. These observations are certainly not definitive in aiding in the understanding of the mechanism of size determination in T-even bacteriophage.

The key to defining the length control mechanism may be found in the length distributions of the giants. The lengths of the giants are rarely found to be in a continuous distribution. Instead, peaks appear at 4- to 6-head length intervals. This suggests that length determination is dependent on a signal that is received only periodically. Normally, this signal could exert its effect when the flat helical band (18) of proteins is assembled, preparing the prohead for the assembly of the fivefold symmetry end-cap. If for some reason (for example, in missense mutants of gene 23 [39] or in the presence of certain analogues) the signal is given prematurely, then one end-cap is assembled directly on the opposing end-cap. When, for a different reason (as in missense mutants of gene 23 [38], ts mutants of gene 24 [1], or in the presence of canavanine), the signal is not given properly, then it would not occur for another 5 head lengths and then afterwards at these same precise intervals. This repeat phenomenon suggests a helix or combination of helices within the head structure with their own characteristic repeat units coming into register at precise distances. Others have shown that the outer coat (primarily p23) and the inner core (p22, IPIII) are arranged in helical fashion, one inside the other, in polyheads (1, 41, 82). An illustration of the helical structure of the core of polyheads is presented in Fig. 31. In giant formation, the incorporation of canavanine into critical peptide regions of p23, p24, or into the components of the core might be expected to result in changes of the pitch of the flat helices. Internal proteins might be good candidates since they have been shown to contain significant amounts of arginine (106), whereas p22 contains relatively small amounts of arginine (95); in addition, the production of giants by canavanine appears to require some internal proteins (32), even though normal head assembly occurs in their absence. Relatively short durations of exposure to canavanine would then result in a decreased continual perturbation of the phasing and would be expected to generate the shorter giants. Maximum lengths of giants could well be controlled by the dimensions of the cell (32, 38).

This model states that optimal perturbation by canavanine on the phasing of the concentric helices would generate a signal for elongation termination every 4 to 6 head lengths. The question then becomes: why do T6 giants (and some of the missense T4 gene 23 giants [38]) characteristically fall into the 4- to 6-head length category, whereas the T4 giants primarily lie in the 10- to 13-head length region? This suggests that the head length determination signal which apparently occurs at specific head length intervals is not sufficient to ensure termination. A "termination protein" may well read the signal and proceed to initiate capping of the prohead or otherwise terminate elongation. In the T6 system, the event is apparently more efficient; this may reflect minor differences in amino acid composition of the structural proteins, which could lead to different conformational interactions. We have seen that, in certain crosses of amber mutants in T4 [$(21)^-$ × $(23)^-$], short giants do accumulate in the presence of canavanine. Perhaps here the concentrations of essential proteins are such that termination is more efficient. Normally, perhaps, termination may be more sloppy and a few signals are obeyed but, in most, the structure elongates until phasing is again achieved. Here, too, it could be the very removal of the structural proteins by virtue of their assembly into elongating proheads which affects critical concentrations such that when phasing next occurs, the signal is obeyed.

From this discussion, it is not immediately apparent why the mixed infections with T6 and T4$(23)^-$ resulted in a T4-like giant distribution, nor is it apparent why the T6 × T4$(20)^-$ infection failed to yield giants when the T6 × T4 $(20,23)^-$ infection did. These results may reflect basic incompatibilities between certain structural proteins from T4 and T6 in the prohead structures. In the T6 × T4$(23)^-$ infection, the absence of T4-derived p23 (or the presence of defective p23) may have allowed T4 components to compete with T6 components and interfere with the proper termination signal or efficiency of termination. No such component has been identified. The T6 × T4$(20,23)^-$ result supports this view. It may well be essential to have specific defective proteins present in the milieu

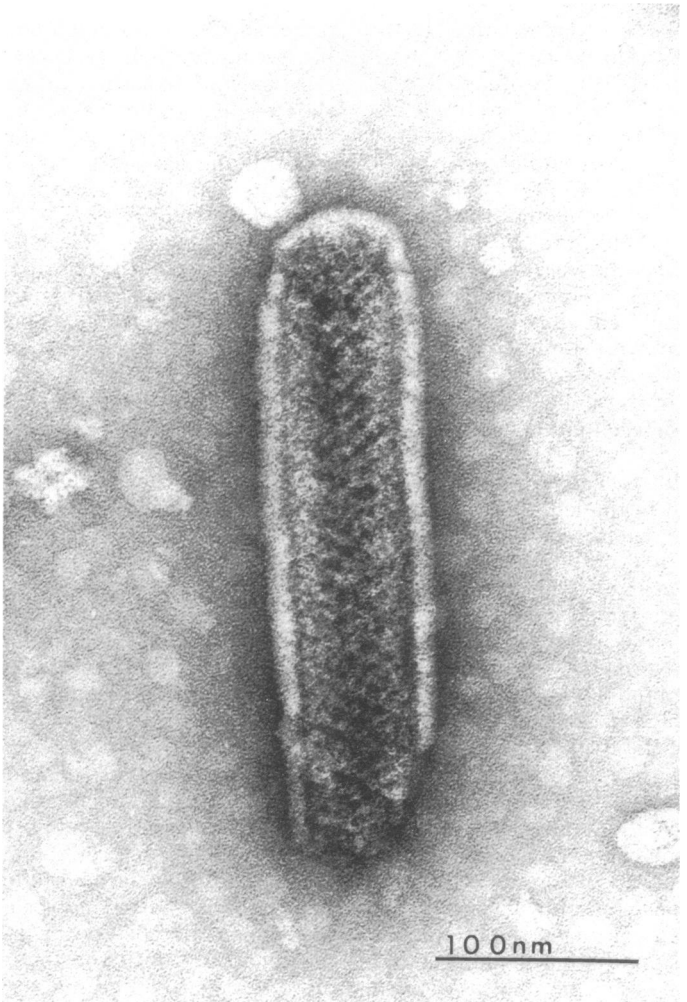

FIG. 31. *Electron micrograph of a T4D polyhead obtained from a culture that had been inhibited by canavanine. Note the helical-like arrangement of the "core" material. (Kindly provided by R. Paulson and U. Laemmli.)*

simply to act as modifiers in the essential interactions. We showed that only crosses involving T4(24)$^-$ mutants could give rise to T4-like giants in the absence of canavanine, even when crossed with T6. Yet, the same mixed infection in the presence of canavanine, which would give rise to "defective" proteins, produced T6-like giants. At this stage, we must emphasize that we used the terms "T4-like" and "T6- like" giants only to distinguish the two size classes. Clearly, T4 alone gives rise to T6-like giants; witness the T4(21)$^-$ × T4(23)$^-$ in the presence of canavanine.

The model as presented draws support from the work with amber mutations. Defects in gene 22 block core formation, and multilayered polyheads result (81). This indicates that p22 is required for form determination, and an interaction between the core and p23 is established. The internal proteins which comprise part of the core are not required for normal phage formation (11, 103), but at least one of the three internal proteins is required for giant formation (32). This implies that the core plays a major role in stabilizing the prohead intermediate and establishes a further relationship between the length of the prohead and the importance of the core. We have shown that in the crosses T4(24)$^-$ is essential when canavanine is absent and T4(23)$^-$ affects the control of giant head length. Several missense mutants in gene 23 have been shown to result in a low frequency

of giant phage formation of varying size distributions (38) as well as petite phage formation (39). Temperature-sensitive mutations in gene 24 have also been shown to result in giant phage formation (1). It would certainly be of interest to produce double mutants of these gene 23 and gene 24 mutants with internal protein mutants (11) to ascertain whether giants would be produced. The possible role of p23 in giant formation has been discussed. The gene 24 product may be involved in the termination step. Our results show that if p24 synthesized in the presence of canavanine is utilized at all, it is probably an aberrant form. The p24* in the giants is apparently synthesized in the postcanavanine period, i.e., after the addition of arginine. Other workers have suggested that p24* is located near the end(s) of the phage head (1). If p24 is not directly involved in the capping process, it may well have the capacity to interfere with it. Our finding that crosses with T4(24)$^-$ in the absence of canavanine lead to the formation of multitailed phages may reflect a role of p24 in the end-capping process. It is important to emphasize (1) that the (24)$^-$ polyheads are the only polyheads whose production is synchronous with that of tau-particles. Since tau-particles are thought to be an early stage in the prohead pathway (9, 80), this again implicates p24 in the termination step. This role may be one of controlling the addition of p23, similar to the case decribed for pU, which regulates the addition of the major tail protein pV in bacteriophage λ (64).

Perspectives

As emphasized early in this review, the bacteriophage is a choice system for studying assembly of macromolecules. We have seen that λ, P2-P4, T4, etc., provide exquisite examples of the complexities of assembly. At the same time, we have demonstrated the power of manipulation of particular gene products either genetically or chemically in understanding the principles involved. For the future, probably the most significant advances in unraveling T4 head assembly will come by studying in vitro assembly. The studies by Kellenberger's group and Ishii and Yanagida have demonstrated that different assemblies of the same structural proteins give rise to different bonding configurations. The recent experiments of Ishii and Yanagida (61) on the addition of *soc* proteins to *soc*$^-$ giant phage illustrate the force of this approach, and we are sure that more studies at this level will be the most fruitful in getting at the gene product interactions required for particular assemblies.

ACKNOWLEDGMENTS

We want to express our appreciation to the many colleagues who provided original illustrations. Special thanks go to members of the Information Theory Group, Department of Microbiology, Biozentrum der Universitat, Basel (U. Aebi, R. Bijlenga, J. van den Broek, E. Couture, B. ten Heggeler, E. Kellenberger, M. K. Showe, P. R. Smith, A. C. Steven, and H. Wunderli), for their generosity with their published and unpublished work on surface lattice analyses of polyheads and giant bacteriophage. We also thank V. A. Chapman and S. S. DeLong for their expert assistance with the electron microscopy. This review could not have been done without the tireless efforts of K. DePew who typed so many drafts. This work was supported in part by grant GB 38447 from the National Science Foundation.

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