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Capsid Size and Deoxyribonucleic Acid Length: the Petite Variant of Bacteriophage T4

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A mutant which produces a small-headed ("petite") variant of bacteriophage T4 is described. The mutation (E920g) maps in a new gene (66) between genes 23 and 24. Petite phage particles composed up to 70% of the phage yield. The petite phage was nonviable upon single infection but produced progeny when two or more infected a cell. Its genome was shortened by a random deletion of about 30%, and deoxyribonucleic acid (DNA) extracted from the particles was 0.68 the length of normal T4 DNA. The reduction in DNA length was accompanied by a proportional reduction in head volume. Double mutants between E920g and head-defective mutants in gene 21 produced unusually high frequencies of spherical capsidlike structures (τ-particles).

The occurrence of aberrant forms of mature bacteriophage is a well-documented phenomenon (1, 23). The fact that the aberrant and normal forms have related geometries may reflect the limited morphological possibilities inherent in steps of phage assembly and in interactions between proteins and deoxyribonucleic acid (DNA). The elucidation of these interactions is of obvious interest. Mutants defective in phage structure or assembly have been of great value in analyzing phage morphogenesis (8), and the formal analogies between morphogenetic and metabolic pathways are being developed (5, 6). It was therefore noteworthy when a T4 mutant was isolated which produces many short-headed phage particles similar to those described by Boy de la Tour and Kellenberger (1) and by Moody (23). This variant, also found in normal lysates of $T4^+$ at a very low frequency (13), has the normal tail and attachment structures of T4, but its head is shorter and probably icosahedral. This suggests that the mutant is defective in the function of a gene that is, in some way, responsible for the elongated or "prolate" shape of the normal phage head (14). The experiments presented herein establish the genetic map location of the mutant, describe some of its growth and physical properties, and provide some information on the nature of the mutation responsible for the production of the "petite" bacteriophage T4 variant.

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MATERIALS AND METHODS

Media. The composition of the EHA top and bottom agar used was given by Steinberg and Edgar (31); the composition of M9S was given by Champe and Benzer (3).

Bacterial and phage strains. In most experiments, and for the preparation of stocks, the three *Escherichia coli* strains B, S/6, and CR63 were employed. Methods for the preparation of host cells and indicators were given by Epstein et al. (8). The strains *E. coli* Hfr H(λ), su_I^- (CA244), and su_I^+ (CA267), an isogenic $su_I^\pm$ pair, were the kind gift of S. Brenner.

The T4 mutants used are shown in Fig. 3. Their characteristics and the plating conditions used were described previously (8). The T4 mutant E920g (Geneva) was derived from a mutant E920 (isolated by R. S. Edgar) in a series of backcrosses to wild-type phage. The final derivative was called E920/96/41, shortened to E920g.

The original mutant E920 isolated by Edgar yielded a high frequency of short-headed "petite" phage (65 to 90% of the complete particles in a lysate) when grown on *E. coli* B. Analysis of the backcrosses showed that the original mutant E920 contained, in addition to the mutation causing petite phage production, two minute-plaque mutants and a temperature-sensitive (*ts*) mutant. Neither the *ts* mutant nor the one minute-plaque mutant which was tested produced petite phage. Stocks of mutant E920g for crosses and complementation tests were prepared with *E. coli* CR63 grown in M9S as host. The mutant E920g obtained from the backcrosses can be identified by its minute-plaque type on S/6, either by use of standard plating conditions or, more readily, after long incubation at 37 C on 4- to 5-day-old plates

(Fig. 1). It was difficult to distinguish mutant and wild-type plaques on CR63 under usual plating conditions, but on S/6 the distinction could readily be made (Fig. 1).

Bacteriophage crosses. *E. coli* CR63 cells were grown overnight in H broth at 37 C with aeration, diluted 1:1,000 into H broth, grown at 30 C to ca. 2×10^7/ml, concentrated by centrifugation to 2×10^9/ml, iced, and used within 20 min. Phage stocks were adjusted to a concentration of 4×10^9/ml. Adsorption of phage to bacteria was for 12 min (5 + 7 min) at 30 C in the presence of cyanide to give a total multiplicity of infection of approximately 12. At 5 min after the addition of phage, anti-T4 serum (inactivation rate constant, $k = 3$) was added. Infected cells were diluted ($1:10^4$) 7 min later into H broth at 30 C and were lysed after an additional 97 min. Scoring of total progeny was on CR63; *am*$^+$ recombinants and the E920g parental type were scored on S/6. Recombination frequency was estimated as twice the number of wild-type plaques found divided by the total number of wild type plus E920g on S/6, and as wild type divided by the total on both S/6 and CR63. Each set of crosses included, as a control, the pair of very distant markers E920g and *amN55*.

Complementation tests. For complementation tests, lysates of mixedly infected bacteria were produced as follows. *E. coli* B cells were grown in M9S at 30 C to a concentration of 2×10^7/ml, concentrated, and resuspended in M9S at a concentration of 2×10^9/ml. They were then added to the appropriate suspension of phage so that the final mixture contained 10^{10} phage/ml and 7×10^8 bacteria/ml. Under these conditions, adsorption is very rapid. After 4 min at 30 C, M9S medium at 30 C was added to reduce the infected-cell concentration to 0.7×10^8 to 1×10^8/ml, and aeration was begun; 11 min later, the cells were superinfected at a total multiplicity of 5 phage/bacterium with the same phage mixture used in the primary infection. Infected bacteria were lysed after 120 min by the addition of chloroform. Lysates were prepared for electron microscopy by use of phosphotungstate negative staining. A drop of lysate was placed on the surface of a carbon-coated grid and allowed to remain for about 1 min. The grid was then washed with 2 to 3 drops of water followed by 2 to 3 drops of neutral sodium phosphotungstate. The frequency of petite phage was scored either visually on the microscope screen or by examining micrographs. The scoring was based on shape differences, i.e., short-headed versus normal anisometric particles; thus, if any intermediate-sized, anisometric particles were present, they were scored as normal phage. The ratios of the two phage types, repeatedly determined on different samples of the same lysate, were reproducible to about $\pm 5\%$.

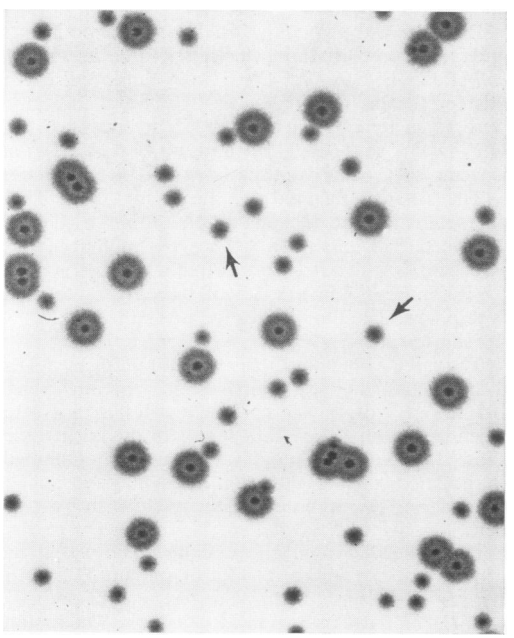

FIG. 1. *Plaque morphology of T4*$^+$ *and T4E920g E. coli S/6; 4-day-old plates. Arrows indicate E920g plaques.*

Purification of petite phage. A lysate of T4 E920g grown on B was filtered through Celite to remove debris, concentrated by centrifugation for 50 min at 30,000 × *g* in a Sorvall SS-24 rotor, and taken up in a small volume of phosphate-NaCl buffer. The concentrated phage suspension was applied to the top of a 5 to 30% sucrose gradient and centrifuged at 20,000 rev/min at 4 C for 20 min in the SW25 rotor of a Spinco model L centrifuge. The more slowly migrating visible band of petite phage was collected and dialyzed against phosphate-NaCl buffer.

DNA content. The lengths of DNA molecules were measured by the surface film adsorption technique of Kleinschmidt and Zahn (19). The DNA was liberated from bacteriophage by osmotic shock from 5 M ammonium acetate or ammonium nitrate as described by Caro (2). Specimens were photographed in a Siemens Elmiskop I electron microscope, and length measurements were made from prints, by use of a map-measuring device.

RESULTS

Mapping and complementation. The primary phenotype of the E920g mutant is the production of the short-headed (petite) T4 phage which are readily identifiable microscopically. However, it was necessary to establish a more rapidly determinable phenotypic correlate for effective further study. The minute-plaque morphology of the E920g mutant on *E. coli* S/6 was established as such a correlate in the following experiment. From a backcross of E920g × T4$^+$, 29 small plaques (see Fig. 1) on S/6 were picked, replated on S/6, and reisolated. From these reisolates, small stocks were grown on *E. coli* CR63, and used to infect *E. coli* B. The latter lysates (plated on S/6 to check homogeneity of plaque type)

were examined in an electron microscope to show the relative yield of normal and petite phage, together with lysates of T4+ and of the initial stock of mutant E920g. The lysates of all 29 isolates, as well as the lysate from the original stock of the E920g mutant, contained high percentages of petite phage under conditions in which the wild type produces 2% or less of the small variant. We conclude from these data that the minute-plaque character, if not identical to that causing production of petite phage, is at least sufficiently linked to the E920g site to serve as a reference marker in crosses. This permits the continuation of genetic analysis by use of plaque morphology.

Ten of the small-plaque isolates were subsequently crossed with one another in five pairs, and the output lysates were checked for the frequency of T4+ recombinants, to test whether more than one site exists which could give rise to the petite phenotype. The results of the presumed self-crosses were as follows: from 8,600 total plaques scored, not more than 0.2% showed the large-plaque morphology (presumptive T4+). As a check on the reliability of identifying plaque morphology, the input stocks were also scored: these showed less than 0.2% large plaques (8,900 plaques counted). These results are compatible with the existence of a single E920g site.

Several backcrosses of the small-plaque isolates to wild type were performed. The input and output E920g allele frequencies were found to be essentially identical, showing that the E920g genotype is not at a selective disadvantage in mixed infections with wild type.

The site of the E920g mutation has been located approximately by two- and three-factor crosses with *am* mutants of the "head" region (genes 20–24). Results are summarized in Table 1 and Fig. 2. They place E920g in the vicinity of the mutant *amB17* (gene 23), between the latter and a mutant, *amN26*, located in gene 24.

When T4 E920g was grown in *E. coli* B, production of petite and normal particles was not synchronous. At the time of normal spontaneous lysis, only 30 to 35% of the complete particles were petite, whereas late lysis of superinfected cells contained on the order of 60% petite particles among the completely assembled phage (Table 2). Such late lysates can, therefore, be used as an abundant source of petite particles. This observation was also reported by G. Mosig (*unpublished data*) and by D. H. Parma (Ph.D. Thesis, Univ. of Washington, Seattle, 1968). For a similar reason—that of optimal sensitivity and petite particle production—the complementation tests described below were also done with lysis-inhibited bacteria, as described in Materials and Methods.

In spite of the extremely "leaky" phenotype of E920g in all hosts tested, a complementation test could be undertaken to determine whether the mutation is included in either gene 23 or 24, or

TABLE 1. *Three-factor crosses of E920g and amber mutants*[a]

am_1-am_2 double mutant[b]	Map distance[c]
B8(20)–B17(23)	4.6, 3.7
B8(20)–N65(24)	1.0, 1.2
B8(20)–N90(21)	6.8, 6.3
B17(23)–N65(24)	1.9, 2.1
B17(23)–N90(21)	3.5, 3.6
N65(24)–N90(21)	1.4, 0.9

[a] The smallest map distances obtained are compatible with E920g being located between genes 23 and 24. The reason for finding a shorter than expected distance with the 24–21 double mutant is not known.

[b] The mutant designation is followed by the gene number in parentheses.

[c] Map distances represent uncorrected values obtained by multiplying the frequency of wild-type recombinants by two (see text).

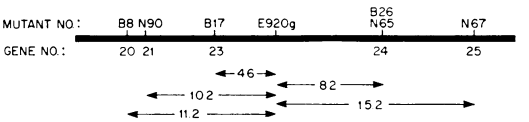

FIG. 2. *Gene map of distances between T4E920g and other amber mutants in the head region of the genome of phage T4. Distances are given as twice the frequency of wild-type recombinants found. For a complete map of T4, see King (18).*

TABLE 2. *Frequency of petite phage in lysates with time after infection*[a]

Time after infection (min)	Per cent petite phage		Total count	
	Expt 1	Expt 2	Expt 1	Expt 2
30	34	36	100	198
45	—	43	—	209
60	51	48	101	171
90	58	57	108	221
130	67	62	107	212

[a] T4 E920g was used to infect *E. coli* B at an input ratio of five phage/bacterium in M9S at 30 C. The culture was superinfected to obtain lysis inhibition and samples were lysed with $CHCl_3$ at the times given. Counts were made by electron microscopy with phosphotungstate negative staining.

in a new gene between them. The host *E. coli* B was mixedly infected with E920g and an *amber* mutant at varying input ratios, but at a constant total multiplicity of infection such that the great majority of infected cells received both parental types. The yield of these mixed infections was then examined in an electron microscope for the relative production of normal and petite particles.

The *amber* mutants produce only fragments of the polypeptide chain specified by the affected gene (29), and we assume that such fragments are inactive. Failure of an *amber* to complement the E920g genotype (and thus identity of the am^+ and $E920g^+$ gene products) would be indicated by a proportion of petite phage in the yield which is insensitive to a change in the input ratios, because, if the *amber* and E920g mutations were in the same gene, the only functional product would be that of E920g, and the output proportion of petite phage would be the same as with E920g alone. Thus, although the total burst size might decrease, the proportion of petite phage should not vary. If the *amber* is in a different gene than E920g, the functional $E920g^+$ product would be present and the frequency of petite phage should decrease with an increase in the *amber* ($E920g^+$) allele frequency.

Results of complementation experiments of E920g with *amB17* (gene 23), *amN26* (gene 24), $T4^+$, and *amB8* are presented in Table 3. All of the input types show complementation in mixed infection. We conclude that the mutant E920g is located in a new gene between genes 23 and 24, which was designated gene 66.

TABLE 3. *Complementation of E920g and amber mutants in E. coli*[a]

Amber mutant (gene)	Per cent E920g mutant in input		Per cent petite phage in output	
	Expt 1	Expt 2	Expt 1	Expt 2
B8(20).....	85	83	55	68
	50	50	45	46
	15	17	23	36
B17(23).....	85	83	20	30
	50	50	3	6
	15	17	0	0
B26(24).....	85	83	46	49
	50	50	20	28
	15	17	3	5
$T4D^+$.....	85	83	50	48
	50	50	30	31
	15	17	9	18
None.....	100	100	59	55

[a] A positive complementation result is indicated by a decrease in the output petite phage frequency as a function of decreasing E920g mutant frequency in the input mixture (see text).

Frequency of petite production. The E920g mutation was initially isolated and recognized because of its very different petite phage production on the suppressor-negative *E. coli* strain B and on the *amber* suppressor strain CR63. However, petite phage production is not sensitive to the amber su_I suppressor of CR63. This was established by examining petite phage production on an isogenic *E. coli* Hfr H (λ) pair, CA244 (su_I^-) and CA267 (su_I^+) (33). Production of petite phage in these two hosts was the same under identical growth conditions. At a late stage of these experiments, we found E920g producing equally large numbers of petite phage on both *E. coli* strains CR63 and B. No reason for this different result could be found upon repeated reexamination of the original phage isolates and bacterial strains, but we have not systematically investigated the effects of different growth conditions. Growth conditions are known to influence the frequency of petite phage produced. For example, higher frequencies were found if cells were grown in M9 medium unsupplemented with amino acids (G. Gujer-Kellenberger, *personal communication*). Studies on spontaneously occurring light particles have shown that their production is very sensitive to the *p*H and ionic composition of the medium (G. Mosig and J. Renshaw-Carnighan, *personal communication*) and to the age of the cells, older cultures yielding a higher frequency of light particles.

The ability of partially purified petite phage to donate am^+ markers in mixed infection with single *am* mutant phage was tested. Mutants at a number of sites distributed over the genome were used (see Fig. 3). For all of these mutants, the marker contribution efficiency was within $\pm 10\%$ of that observed for repeated determinations with the mutant *amB16* in gene 7. These results suggest that the incomplete genomes of petite phage lack parts of the entire T4 genome that are deleted at random in the phage chromosome. The same conclusion was drawn by Parma (Ph.D. Thesis, Univ. of Washington, Seattle, 1968) on the basis of more extensive data.

The DNA content of petite phage may also be estimated from the marker contribution experiment if the proportion of entire and petite particles in the partially purified petite phage preparation is known. Consider N phage of which $N\alpha$ are normal and $N(1 - \alpha)$ are petite. When singly plated on su^- bacteria, these will give $a = N\alpha$ infectious centers (if the infection efficiency is one). When singly infected su^- bacteria are also infected with several *am* phage, the number of infectious centers is

$$C = N[\alpha + f(1 - \alpha)]$$

where f is the fractional genetic length of the

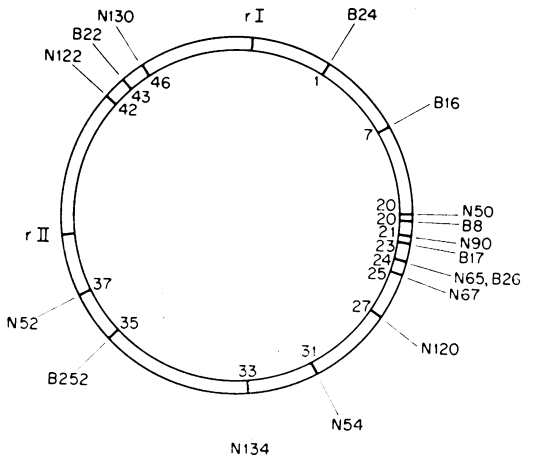

FIG. 3. *Genetic map showing location of mutants used. The number of the amber mutant used is given outside the circle, and the gene number, inside. The genes rI and rII are included as reference markers.*

DNA contained in the petite particles. Consequently

$$f = \frac{c - a}{1 - \alpha} \cdot \frac{\alpha}{a}$$

and α is taken to be the electron-microscopically determined fraction of entire T4 phages in the petite phage preparation.

Measurements of this sort gave a value of about 0.5 for the fractional genetic length. However, if the petite phages were less stable than normal particles, this measure would overestimate the size of the deletion. Also, if α is overestimated owing to the presence of other classes of particles, then f will also be overestimated.

A second measure which is not sensitive to possible differential stabilities of small and normal particles is that of the ratio of single to double marker contribution, i.e., the double am^+ contribution relative to the contribution of one of the single am^+ markers in mixed infections of petite and double *am* mutant phages. For mutant sites separated by distances equal to or larger than that of the deletion, the ratio of double to single marker contribution (D/S) is constant. We may define the D/S ratios for such widely separated markers as related to the size of the partial genome in the following manner: $D/S = (2\beta - 1)/\beta$, where β is the size of the petite genome relative to that of normal particles (neglecting the region of terminal redundancy in normal particles, and the size of the two genes in which the *amber* mutations occur). Several measures with the use of distant sites gave a value of 0.70 for the relative size of the petite genome (Table 4); similar results were found by Parma (Ph.D. Thesis). This value will be larger than the true value for the petite genome if the population of defective phage contains a significant proportion of particles larger than the isometric phage which form the majority of the population of purified petite particles. We estimate from electron microscopy that the fraction of other particle types is small (less than 5 to 10%), although small differences would be difficult to detect. Finally, we note that D/S measurements for markers which are neither closely linked (i.e., closer than the length of one gene) nor linked by distances of the same size order as the deletion can be used to test whether the additivity of the intervals determined is proportional to physical distances.

Mosig (27), using small particles which arise spontaneously in lysates of T4, has also measured the genetic length of the DNA molecule in the small particles, and has discussed the theory of distance measurements in detail. The results which she obtained agree well with those we have found for the petite phage.

DNA content of petite phage. Lengths of a limited number of DNA molecules from both petite and normal T4 phage were measured in an electron microscope with the use of the protein film (2, 19) technique. To improve the precision of the determination, only extended molecules from the petite preparation were measured (see Fig. 4). The lengths of normal T4 molecules were 53 ± 1.5 μm, and those of molecules from preparations of purified petite phage were 36 ± 1 μm, the length ratio of the latter to the former being 0.68.

Dimensions of phage heads. Measurements

TABLE 4. *Genetic determination of the relative size of the E920g genome*

Double mutants used	Ratio of double to single marker contribution[a] (D/S)	Estimate of genome length, β[b]
B16(7)–B252(35)	0.54	0.69
B16(7)–B252(35)	0.55	0.69
B252(35)–N120(27)	0.57	0.70
N120(27)–B22(43)	0.61	0.72
B24(1)–N134(33)	0.62	0.72

[a] Determined by plating of mixedly infected cells before lysis on both permissive and restrictive bacteria. The multiplicity of infection was 3 to 4 double *am* phage/bacterium, and about 0.1 petite phage/bacterium.

[b] The following was used to estimate β: $D/S = \frac{(2\beta - 1)}{\beta}$.

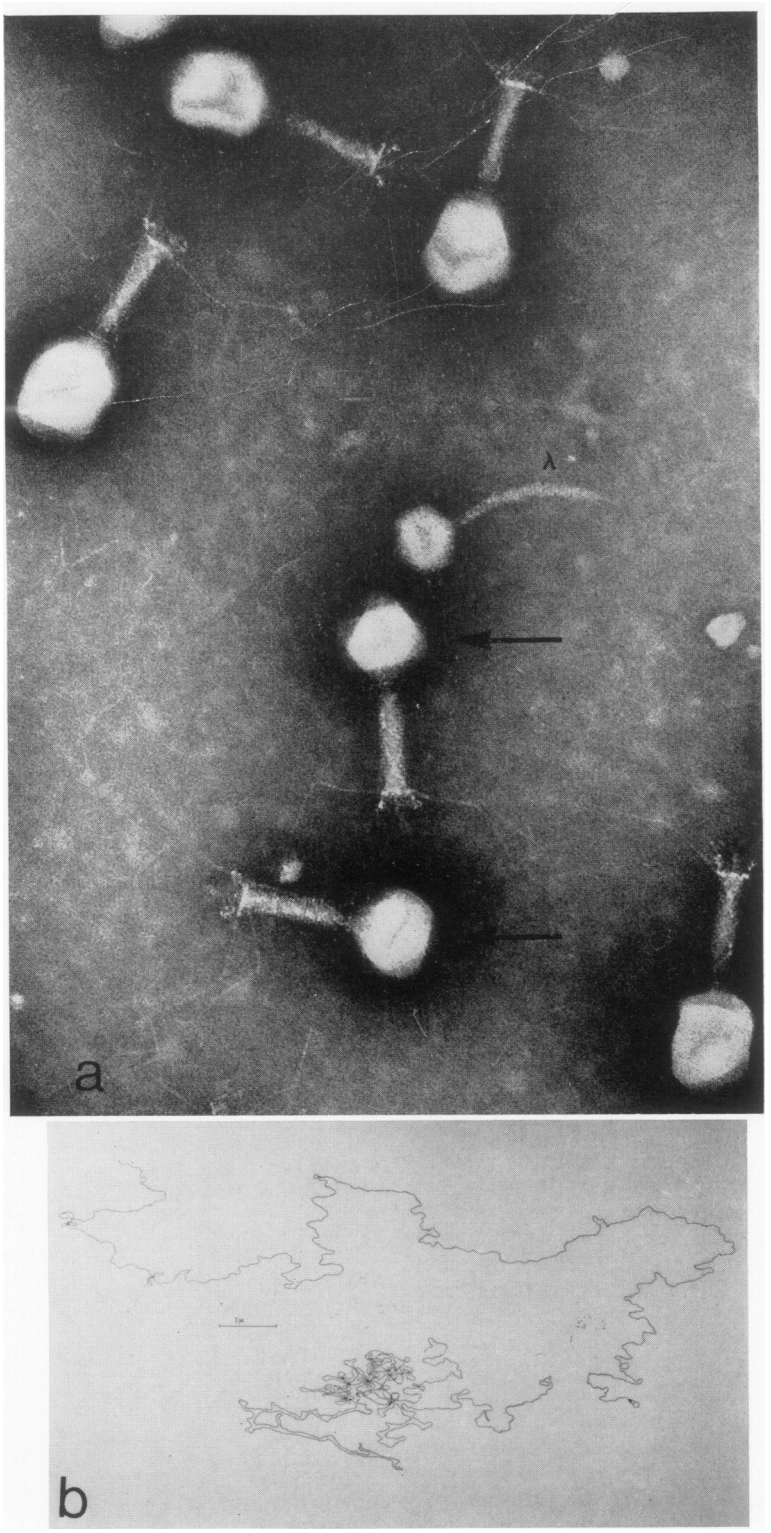

FIG. 4. *a. Electron micrograph of T4D, petite phage (arrows) and phage λ. b. Tracings of two photographs of molecules of T4 DNA. The upper, extended molecule is 36 μm long, from a preparation of purified petite particles, and is typical of the seven molecules measured. The lower molecule of T4D⁺ DNA is 54 μm long.*

were made of micrographs of petite and normal T4 prepared by negative staining in neutral sodium phosphotungstate. The micrographs were calibrated by comparison with micrographs of a cross-ruled diffraction grating (Fullam), and between 100 and 200 particles were measured for each type. The mean results were as follows: normal T4, 80 × 110 nm; petite T4, 80 × 90 nm. Since a consideration of the volumes of the two particles is so greatly dependent on an accurate knowledge of the particle dimensions, we have listed a comparison of a few of the published values of the sizes of T-even phages obtained by different methods of preparation for electron microscopy (Table 5). It is obvious that the range of linear dimensions is great, resulting in volume differences of nearly a factor of two between the smallest and largest values. It is difficult to determine which values represent most closely the true absolute dimensions. Some methods, such as freeze-drying and thin-sectioning, minimize distortion due to surface tension flattening, but introduce unknown shrinkage artifacts; the degree of flattening in negatively stained preparations is related to the thickness of stain, which is quite variable for large particles such as phage heads. Aside from the problem of preparation methods, magnification calibration procedures vary greatly in different laboratories, and there are few examples of comparative studies in which different methods were used in the same laboratory. To this end, we have made a series of measurements on the dimensions of phage λ, which has an isometric, probably icosahedral, capsid. Figure 5 shows the dimensions obtained by a variety of methods. It is clear that there are significant differences, depending upon the method chosen and, for one method, upon the stain thickness (compare "positive" and negative stain values). The smallest values were obtained with thin sectioning and uranyl acetate positive staining; negative staining and metal shadowing gave larger dimensions. The range of values for a given method was about ±10% from the mean value, and the means for different methods varied by nearly the same amount. Thus, volume differences between icosahedral viruses would have to approach 30% to be just detectable by electron microscopy, and independent estimates of the volumes are necessary (see Discussion).

Since it is also important to estimate the thickness of the protein shell surrounding the condensed DNA, measurements were made on micrographs of sections of a mixed embedding of λ and T4. Only those particles which showed a clearly visible tail of normal length were measured, to assure that the section was as nearly parallel to the particle axis as possible. The values were 5.0 nm for T4 (31 particles sectioned) and 4.0 nm for λ (42 particles sectioned).

Properties of some E920g-am double mutants. Double mutants of E920g and *amber* mutants B8 (gene 20), N90 (gene 21), N98 (gene 22), B17 (gene 23), and N54 (gene 31) were prepared as follows. *E. coli* CR63 cells were mixedly infected with E920g and the desired *amber* mutant. The lysates were plated on CR63; plaques were picked and spot-tested on S/6 and CR63. Those failing to plate on S/6 were examined for plaque morphology on *E. coli* CA267 (Su_I^+), and the minute plaques were picked. Multicycle stocks of these isolates were grown on CR63 and examined microscopically for petite phage. As a check, all presumed double mutants were back-crossed to $T4^+$, and the lysates were plated on S/6 to show that plaques typical of E920g could be recovered; lysates prepared from these plaques were then checked microscopically to confirm the presence of petite phage.

The double mutants were grown for a single cycle on *E. coli* B by infecting cells at 2 × 10^8 per ml at 37 C in H broth with an input ratio of five phage per bacterium, followed at 8 min by superinfection at the same multiplicity. Samples

TABLE 5. *Comparison of phage head dimensions*

Phage	Head size (nm)		Conditions[a] and reference
	Width	Length	
T2	80 ± 2	119 ± 4	PT negative stain, fixed, *p*H 7 (4)
T2	65	95	Frozen-dried, metal-shadowed (35)
T4	70 ± 3.5	—	Sections (9)
T4	78 ± 4.0	—	Air-dried, metal-shadowed (9)
T4	80 ± 1.5	—	PT negative stain (9)
λ	54	—	PT negative stain (13)
λ	55 ± 2.5	—	UA negative stain (12)
λ	65	—	UA negative stain (7)

[a] PT = phosphotungstate; UA = uranyl acetate.

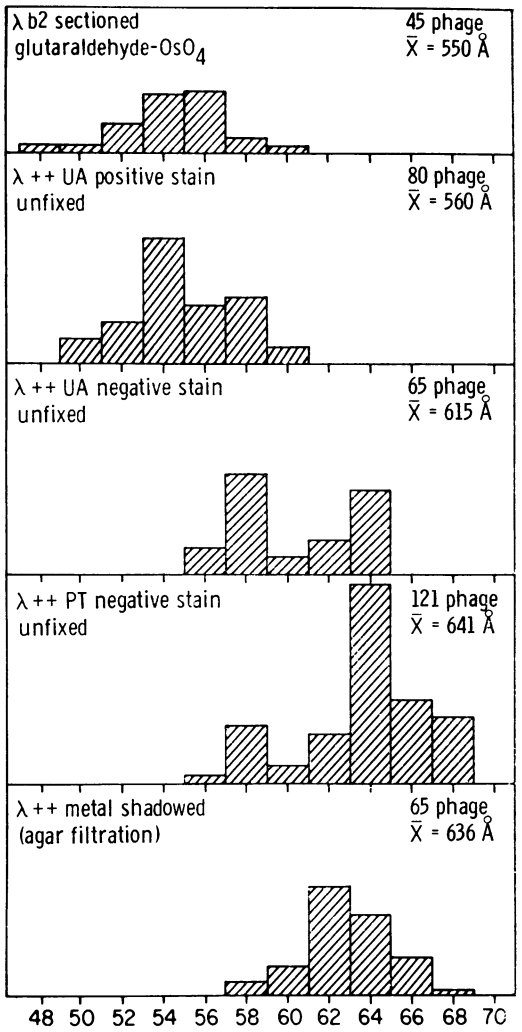

FIG. 5. *Comparison of distributions of measured diameters of phage λ prepared by different methods.*

were prepared for electron microscopy at 70 min by use of the in situ partial lysis method described by Kellenberger et al. (17), which permits the observation of the contents of individual infected bacteria.

Individual cells infected with E920g alone had a burst size of 100 to 300 particles/cell, and usually contained from 30 to 70% petite phage. All cells examined contained both normal and petite phage; thus, the petite phage seen in mass lysates do not arise from a small fraction of the infected cells which produces a large yield containing only petite phage. All cells were also seen to contain a few polyheads and τ-particles (see below).

E920g(66)-amB8(20). Restrictive bacteria infected with gene 20 mutants alone produce tubular structures called polyheads (9, 14). Examination of single infected cells has shown that they are produced in bundles oriented parallel to the cell's long axis, which contain about 20 polyheads per cell at 70 min after infection (20). Multilayered polyheads consisting of several concentric cylinders are also produced (17).

We have found that restrictive cells infected with a double mutant in genes 20 and 66 produced about 20 polyheads/cell, with about 15 to 20% of these present as multilayered cylinders of three to six concentric layers. The same cells produced up to 20 phage particles per cell and 80% of these were petite. The polyheads appear indistinguishable from those produced by gene 20 mutant-infected cells, although, unlike normal polyheads, they do not appear to survive complete lysis of the cell, since chloroform-induced mass lysates of the infected bacteria contain few if any polyheads. By contrast, single-cell studies made on cells infected with the gene 20 mutant alone showed about 30 polyheads/cell, and only 3% of these were multilayered. The cells contained up to 40 phage particles per cell, and 20 to 30% of these were petite phage. The reason for such a large number of phage particles produced under restrictive conditions is probably related to the fact that the mutant *amB8* is somewhat "leaky" (8).

E920g(66)-amN90(21). Cells infected with *amN90* in gene 21 produce abnormal headlike particles which lack DNA and which are smaller than normal heads (8, 15). These structures (now called τ-particles) were shown to exist in two forms: the majority were anisometric, similar to the prolate shape of normal T4, whereas a minority were roughly spherical.

Our results with restrictive cells infected with the double mutant E920g-*amN90* showed that many τ-particles were produced, but that the majority were isometric (spherical). Measurements made on several hundred particles from 10 cells showed that about 80% of the τ-particles were isometric, whereas only 20% were prolate (Fig. 6 and 7). All cells examined also contained a few polyheads and whole phage.

E920g(66)-amN98(22). An *amber* mutant in gene 22 grown under restrictive conditions was shown to produce a high frequency of multilayered polyheads (15). The double mutant in genes 66 and 22 produced an equally high frequency of these multilayered concentric tubes, and the total amount of polyhead material appeared to be the same as in the control lysates of cells infected with the gene 22 mutant alone.

E920g(66)-amB17(23) and E920g(66)-amN54 (31). No heads or head-related structures are formed in restrictive cells infected with *amber*

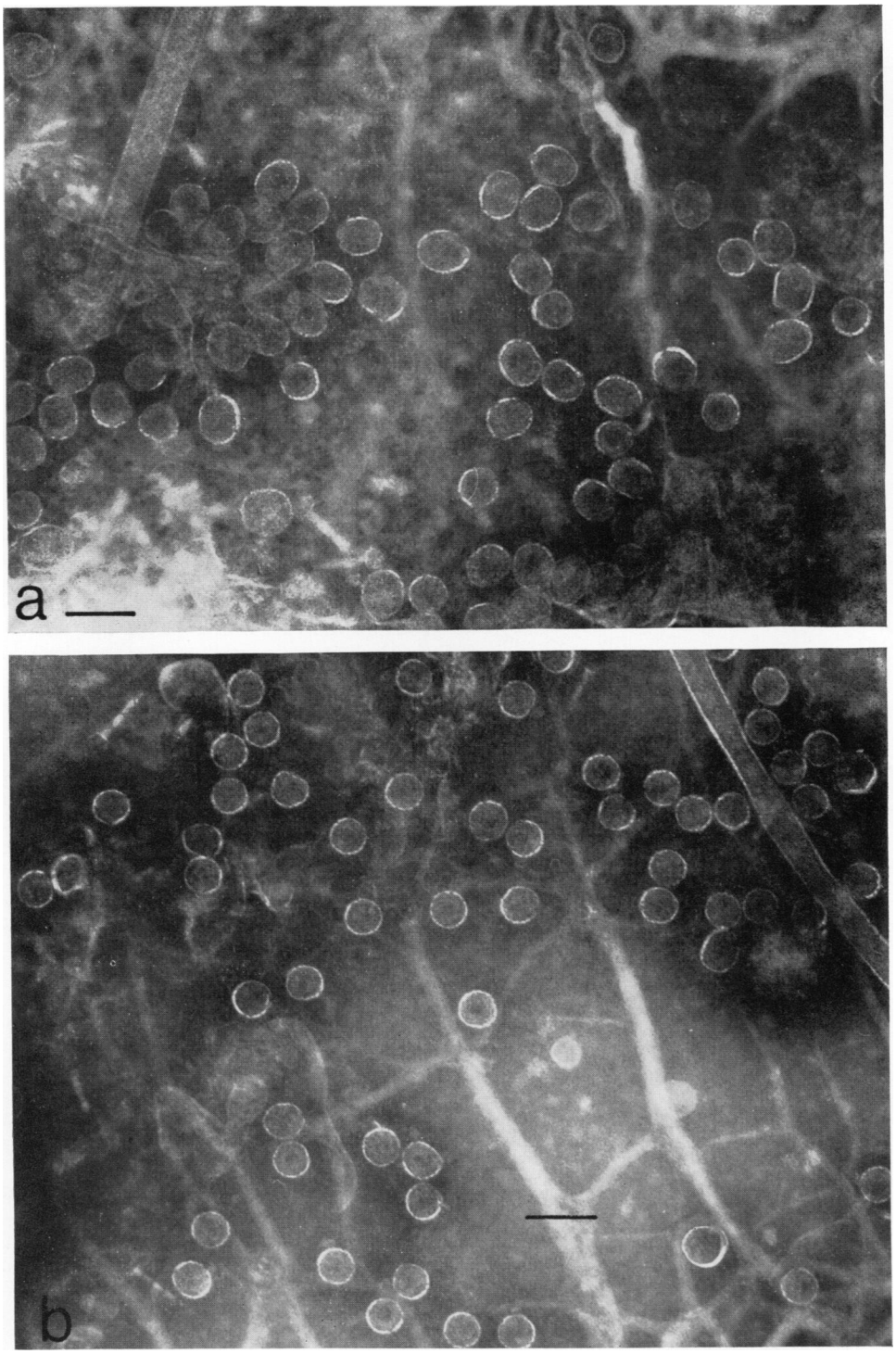

FIG. 6. *Electron micrographs of infected individual cells of E. coli B stained with neutral sodium phosphotungstate. (a) Cell infected with mutant N90(21). Both prolate and isometric τ-particles are visible, with an excess of prolate particles. (b) Cell infected with the double mutant E920g(66)-N90(21). The large majority of τ-particles are isometric. The bar in both micrographs represents 100 nm.*

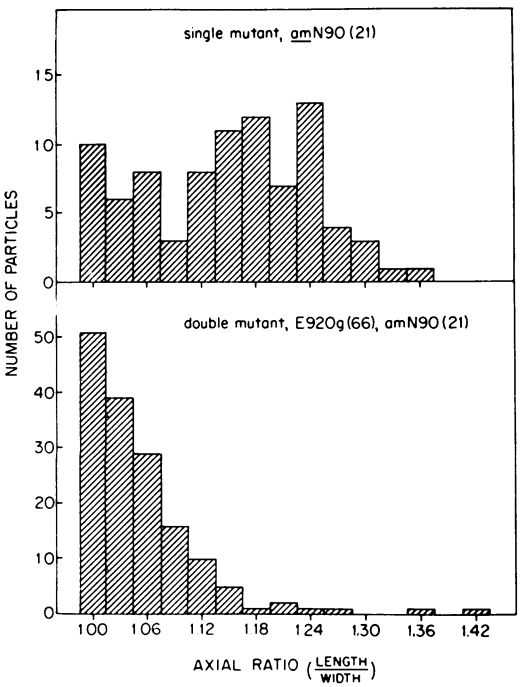

FIG. 7. *Histogram showing the axial ratio (length/width) of τ-particles from restrictive cells infected with N90(21), upper, and E920g(66)-N90(21), lower. In the cells chosen here for measurement, the frequency of isometric τ-particles is higher than normally found.*

mutants in genes 23 or 31 (14, 15). Such cells either lack the major protein composing the head (gene 23 mutants) or produce it in an insoluble form (gene 31 mutants). Restrictive cells infected with double mutants in genes 66 and either 23 or 31 produced neither complete phage particles nor head-related structures.

Based on the double mutant studies reported above, it appears that the functions of genes 20, 21, 22, 23, and 31 are required for the production of normal amounts of petite phage particles.

DISCUSSION

The E920g mutation, which leads to abnormally high production of nonviable T4 particles with short heads, belongs to the cluster of related functions involved in nucleic acid and protein condensation of the phage head. Thus, there are now eight genes known to affect capsid formation (20, 21, 22, 23, 66, 24, 31, and 40), of which six exist in a single cluster. The mutation is located between gene 23, which controls the synthesis of the major subunit of the capsid, and gene 24, which was recently shown by Leibo and Mazu (21) to affect the sensitivity of the capsid to osmotic shock. Growth experiments on different hosts showed that the mutant E920g is not an *amber*, and it is not known whether the phenotype is due to a missing or to a modified gene product. Physiological factors such as the length of time after infection and the composition of the growth medium affect the frequency of petite phage production, as has been observed for polyheads, polysheath, and τ-particles (14). Electron microscopy of infected single cells has shown that the production of petite phage is not due to an inhomogeneous host-cell population.

The amount of DNA in the short head is 0.68 that of the DNA contained in the normal head, and genetic estimates of the length of the petite genome agree well with this value. Mosig (25, 26) described spontaneously occurring particles of lower density than normal T4 ("light particles") which were nonviable upon single infection and which contained 0.67 as much DNA as normal T4, measured by sedimentation through sucrose gradients. Recent experiments by Renshaw-Carnighan and by Mosig (27) showed that these light particles also have short heads, and it is probable that the petite phage and the "light particles" are structurally equivalent.

The buoyant density in CsCl need not be the same, however, since both the particles studied by Renshaw-Carnighan and Mosig (*personal communication*) and those studied by Parma (Ph.D. Thesis, Univ. of Washington, 1968) were selected for osmotic shock resistance, controlled by the adjacent gene 24, whereas the petite particles used in our study have normal sensitivity to osmotic shock.

The gene sequences of the "light particles" are cyclically permuted over the genome (26), similar to the normal genome of T4 (22), and recent experiments by Parma showed that the E920g genome is also cyclically permuted.

Light, nonviable particles containing intermediate-sized DNA molecules were also found by Mosig (25) with fractional lengths of 0.77 and 0.9 that of whole T4 molecules. Preliminary measurements cited in Mosig (27) and observations by G. Gujer-Kellenberger and E. Boy de la Tour (*personal communication*) suggest that these particles have head lengths intermediate between petite phage and normal T4. If this is true, then the intermediate-density particles have shorter capsids containing DNA packed much as in normal heads, rather than normal-size capsids containing less DNA. It thus appears that head shape and volume on the one hand, and DNA length, on the other, are entirely interdependent properties of bacteriophage T4, similar to that observed for phage P1 (11; D. H. Walker, Jr., Ph.D. Thesis, Univ. of Pennsylvania, Philadelphia, 1966). This is in contrast to phage λ, in which different amounts of DNA

are contained within the same capsid volume, since the density difference observed between variants of λ containing different lengths of DNA can be completely explained by assuming that the mass of phage protein remains constant (11).

From genetic evidence, Streisinger and his collaborators have shown that the genome of T4 is cyclically permuted (30, 32), and Thomas has given physical evidence for permuted DNA molecules (34). To explain this observation, Streisinger et al. proposed that, during phage maturation, genomes are selected at random from vegetative DNA molecules which may be considerably larger than those from complete virus particles (10). Thus, the device for measuring or cutting viral DNA (or both) could be the appropriately sized phage capsid, and need not be some intrinsic property of the nucleic acid.

Electron microscope evidence has been cited (18) suggesting that capsids lacking DNA are produced in restrictive cells infected with *amber* mutants in genes 16, 17, and 49. Recent experiments by R. Luftig, W. B. Wood, and R. Okinaka (J. Mol. Biol., *in press*) have shown that a temperature-sensitive mutant in gene 49 also produces capsidlike structures which appear to contain less than the normal amount of DNA at restrictive temperatures, but which serve as precursors of viable phage after a shift to permissive conditions. They also constructed a double mutant E920g(66)-*ts*(49), and found that both normal and petite capsidlike structures containing little or no DNA were formed at restrictive temperature. These results suggest that the shape-determining properties of the head reside in the proteins composing it and do not depend on the presence of a preformed DNA condensate.

It would be of great interest to know whether the reduction in head volume between normal and petite phage is strictly proportional to the concomitant reduction of the volume of DNA between the two particles. This problem is complex, since it is very difficult to obtain sufficiently accurate measurements of the absolute particle dimensions, as mentioned earlier.

Because of these difficulties, it is also worthwhile to estimate the relative amounts of DNA and protein in the two particles by a method independent of electron microscopy. The molecular weight of T-even phages determined by sedimentation and diffusion is approximately 215×10^6 (4), and the molecular weight of the DNA from chemical measurements is 130×10^6 (28). If we assume that head length but not diameter is reduced in the petite phage, then the density of the petite particle must be less than that of wild type. One can use the known difference in DNA content between the two particles to predict the density difference, in order to see whether there is a direct relationship between the reduction in head volume and the reduction in DNA content. Let us assume the following values for the buoyant densities, ρ, in CsCl to T4, protein, and DNA: $\rho_{T4} = 1.510$ (24), $\rho_P = 1.291$ (14), and $\rho_{DNA} = 1.693$ (28). If the volumes are additive in making the phage out of DNA and protein, then

$$V_{T4} = M_{T4}\frac{f_P}{\rho_P} + \frac{f_D}{\rho_D}$$

where V is volume, M is mass, f_P is the fraction of protein in T4, and f_D is the fraction of DNA in T4. Since, then

$$\frac{1}{\rho_{T4}} = f_P\frac{1}{\rho_P} + f_D\frac{1}{\rho_D}$$

the observed value of $1/\rho_{T4}$ corresponds to $f_P = 0.392$ [a similar relationship was derived for a study of the small P1 particles (11)]. This agrees well with the fraction obtained by subtracting the weight of DNA from the particle weight ($215 - 130/215 = 0.395$). In the above argument, we assume (i) that all space other than protein, DNA, and their solvent shells is freely accessible to both solvent components and (ii) that the solvent shells of protein and DNA are independent, i.e., non-interpenetrating.

Using the above relationship, it is possible to calculate the proportion of protein which must be removed from the normal phage along with the DNA to make the density difference of petite and normal phage equal to the density difference found by Mosig (24), i.e., 0.026.

The change from wild-type to petite phage involves the removal of a protein shell 5 nm thick and 80 nm in diameter along with the DNA contained in that volume. If this shell is uniformly filled with DNA at its dry two-component partial specific volume, then we find that 0.224 g of protein is removed per g of DNA. If this shell contains 30% of the DNA, then the expected density shift on going from wild type to petite phage should correspond to a Δf_P of $+0.062$, or a density difference of -0.025. This is, in fact, the value found by Mosig (24) for the density difference between the two types. This result suggests that the mode of DNA packaging is not grossly changed in the petite particle, and that there is a proportional reduction in both DNA length and head volume in the petite phage.

It should be mentioned, however, that if there were a small DNA-free region near the center of the particle (12), it would have a negligible effect on the measured density.

Kellenberger, in speculating on the possible role of the gene 66 product in phage morphogenesis, has proposed that it could act on an

organizing structure ("core") necessary for the elongated form of the phage head. Our experiments with double mutants of E920g with *ambers* in genes 20 and 21, although consistent with such a proposal, do not help to establish it. We find that polyheads are produced in cells infected with the 20–66 double mutant, but that the frequency of multilayered structures is increased, suggesting that some product necessary for normal polyhead production may be limiting. More striking is the finding that cells infected with the 21–66 double mutant produce many fewer elongated τ-particles than the 21 mutant alone, which indicates that the factor necessary for elongation of these particles may also be provided by the product of gene 66.

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