THE PROTEIN OF THE HEAD

OF

BACTERIOPHAGE LAMBDA

by

GAEL HARLING HUNTLEY

B. Sc., University of British Columbia, 1958
M. Sc., University of British Columbia, 1959
B. Sc., Oxford University, 1962

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Name: Gael Harling Huntley
Degree: Doctor of Philosophy
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Examining Committee

Senior Supervisor

C. L. Kemp

External Supervisor
Head, Dept. of Cell Biology
University of Toronto

L. Siminovitch

Examiner

H. L. Speer

Examiner

J. S. Barlow

Examiner

I. R. Glen

Examiner

W. E. Vidaver

Date Approved: June 24, 1970

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ABSTRACT

In lysates prepared from Escherichia coli lysogenic for normal bacteriophage lambda, two types of head are present: the normal lambda head, and a smaller form, petit lambda. Both head types are totally absent in lysates only when mutations occur in either of two phage genes (E or F).

This work was undertaken to characterize the structural protein of the lambda phage heads and to determine the relationship between the two types of head. Also, an attempt was made to identify the genes responsible for the production of the lambda head protein.

Purified bacteriophage lambda heads have been produced from tail-less mutants by methods involving differential centrifugation and anion exchange chromatography. A sedimentation coefficient of approximately 480 S, and a density of 1.27 g/cm^3 were determined for the empty normal lambda heads. Both these values are very similar to those of the petit lambda particles. Normal and petit lambda heads differ, however, in the surface charge they carry with petit lambda being bound more tightly to the ECTEOLA anion exchange

cellulose indicating that, under the conditions of elution, it carries a lower net positive charge.

Breakdown with alkaline buffers in the presence of sodium dodecyl sulfate or acidic buffers in the presence of urea showed that both normal and petit lambda heads consist of the same major protein monomer. The molecular weight of the protein subunit was determined by disc gel electrophoresis and confirmed by tryptic peptide mapping in conjunction with amino acid analysis. SDS disc gel electrophoresis gave a value of 45,000 and amino acid analysis, 43,000.

A minor protein is present in normal lambda heads and absent in petit lambda. It has a molecular weight of about 14,000 as determined by SDS disc gel electrophoresis and 15,000, by amino acid analysis.

The reagents used to break down the heads indicate that both salt and hydrogen bonds act in maintaining head structure, but of the two, salt bonds are of greater importance.

Preliminary results indicate that gene E codes for the major protein of the bacteriophage lambda head.

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CHAPTER I

THE PROTEIN OF THE BACTERIOPHAGE LAMBDA HEAD

Introduction to the Genetics and Morphology of Bacteriophage Lambda

The temperate bacteriophage lambda can infect certain strains of Escherichia coli initiating a lytic cycle in which phage development terminates in lysis of the host bacterial cell. Alternatively, lambda, being a termperate phage, can enter a lysogenic state and integrate its DNA into that of the host bacterium with the result that virus genome replication is brought under host control. Induction by various agents releases the prophage and the production of free phage ensues. Phage development falls into three stages: (1) the synthesis of deoxyribonucleic acid (DNA), (2) the formation of morphological protein components and their assembly with DNA to produce infectious phage particles, and (3) the production of an enzyme which lyses the bacterial cell freeing the progeny phage particles. The resulting lysate contains whole

infectious phage, separate phage heads and tails, and a non-infectious form, petit lambda (pλ), which resembles normal phage heads but never becomes joined to a tail (Karamata et al., 1962; and Kemp et al., 1968).

This work was undertaken to characterize the structural protein of the lambda phage heads and to determine the relationship between the two types of phage heads, normal and petit. Also, an attempt was made to identify the gene responsible for the production of the lambda head protein.

Studies of mutants of bacteriophage lambda have shown that different sites on the lambda chromosome act in the development and maintenance of lysogeny, and in stages occurring during vegetative multiplication of the virus and lysis of the host cell. The lambda chromosome may be divided into three regions according to the functions they regulate (Skalka, 1966). These regions are a central segment, acting in lysogency, the right arm, controlling the early functions of DNA synthesis, and the left arm, controlling the late functions of morphogenesis (figure 1).

The left arm of the lambda chromosome contains the genes governing the formation of the main structural components of the phage (figures 1 and 2). The mature lambda phage

Figure 1

The Lambda Genome

References:

Gene R: Campillo-Campbell and Campbell (1965), Campbell and Campillo-Campbell (1963)

Gene S: Harris (1966), Harris et al. (1967), Goldberg and Howe (1969)

Gene Q: Dove (1966), Joyner et al. (1966)

Genes O and P: Harris (1966), Eisen (1967), Skalka et al. (1967)

Genes C_{II} and C_{III}: Isaacs et al. (1965), Bode and Kaiser (1965)

Gene y: Eisen (1967)

Gene x: Radding (1964 a,b), Hogness et al. (1966), Eisen (1967)

Gene C_I: Ptashne (1967 a,b)

Gene N: Protass and Korn (1966), Skalka et al., (1967)

Genes C_{III}, β, Exo and Red, Int. and Att λ: Manly et al. (1969)

Gene Int: Zissler (1967), Gengery and Echols (1967)

b_2 Region: Kellenberger, Zichichi, and Weigle (1961), Jordan (1964), and Campbell (1965)

Left Arm: Harris (1966), Kemp et al. (1968), Mount et al. (1968), Parkinson, (1968), Thomas et al. (1967), Weigle (1966)

Figure 1

THE LAMBDA GENOME

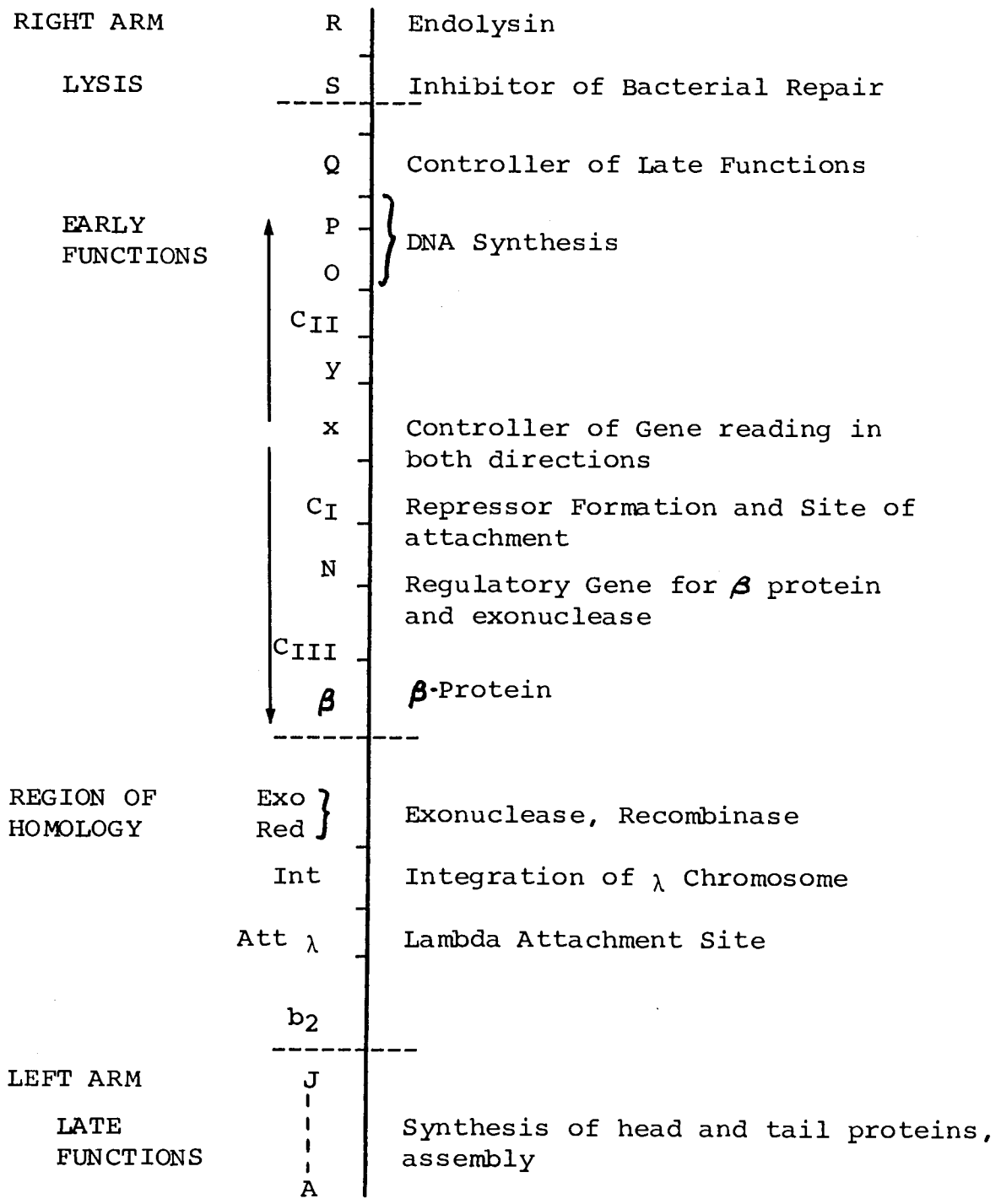


Figure 2

The Left Arm of the Lambda Genome

References:

Harris (1966)

Kemp et al. (1968)

Mount et al. (1968)

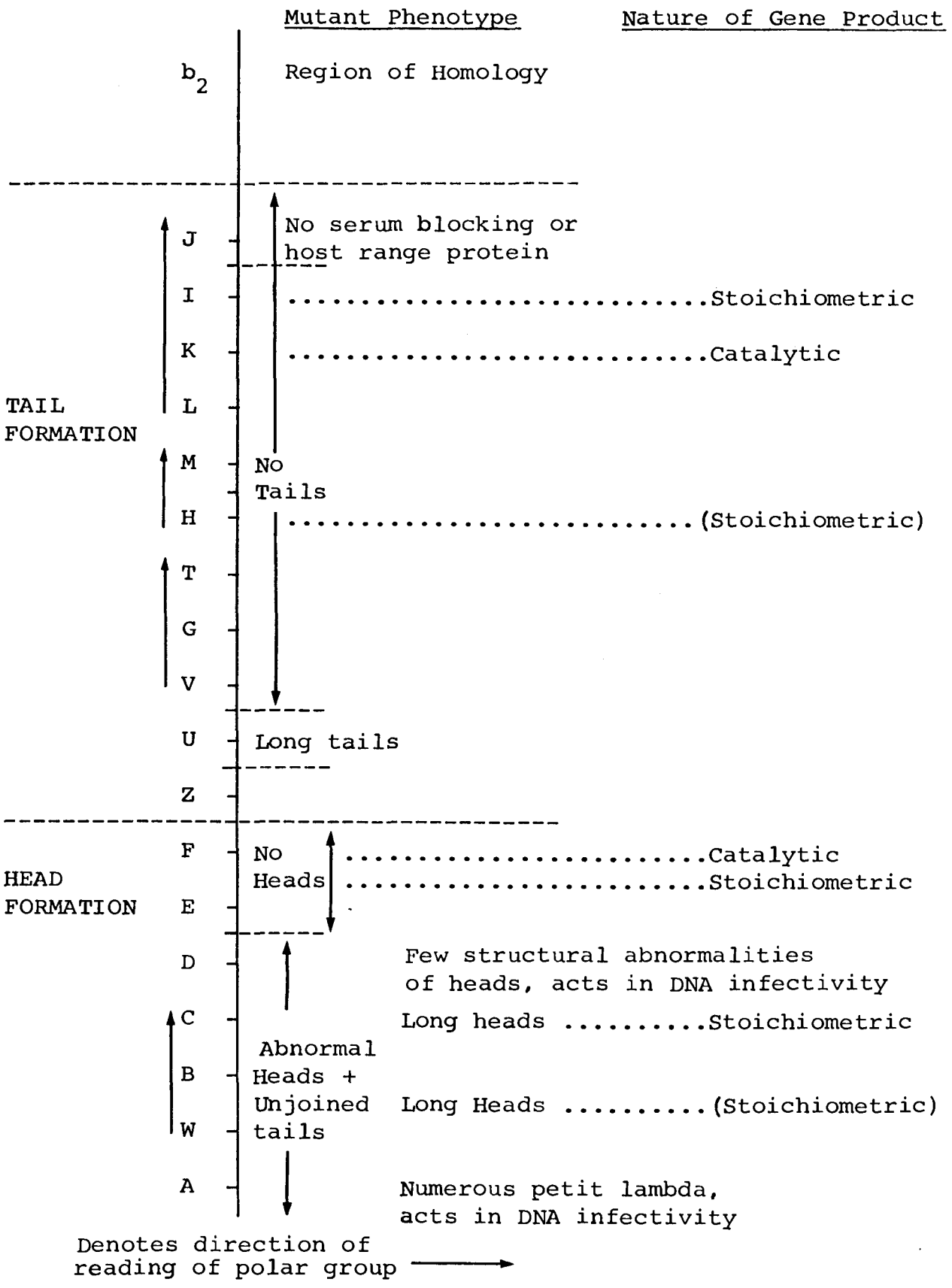
Parkinson (1968)

Thomas et al. (1967)

Weigle (1966)

Figure 2

THE LEFT ARM OF THE LAMBDA GENOME



particle is comprised of two parts, the head and the tail (figure 3). All the mutants in the left arm fall into two groups: tail donors in which the DNA does not become infectious and the head morphogenesis is aberrant and (2) head donors in which heads, but no tails are produced (Weigle, 1966; and Parkinson, 1968).

Although the lambda tail is structurally and biochemically simple (Eiserling and Boy de la Tour, 1965; Kemp et al., 1968; Mount 1965; Buchwald and Siminovitch, 1969;), the region governing its morphogenesis is genetically complex (Naha, 1968; Parkinson, 1968; Buchwald and Siminovitch, 1969; and Thomas et al., 1967).

Mutants affecting the production of heads are investigated in this thesis. In lysates of normal lambda lysogens, two types of head are always present; the normal head, which may or may not be attached to a tail, and petit lambda, a smaller form which is generally empty (as determined by penetration of negative stain) and lacks a tail (figure 3). The production of functional phage heads consists of (1) the synthesis of the protein subunits, and (2) the assembly of these into heads with the incorporation of infectious DNA inside them. It has been suggested that the DNA is wound around protein spools or "cores"

Figure 3

Electron Micrograph of Normal Lambda Bacteriophage

The micrograph shows both full and empty normal lambda particles (n) and an empty petit lambda head (p). Magnification 200,000 x. (Photo courtesy of Dr. C.L. Kemp.)

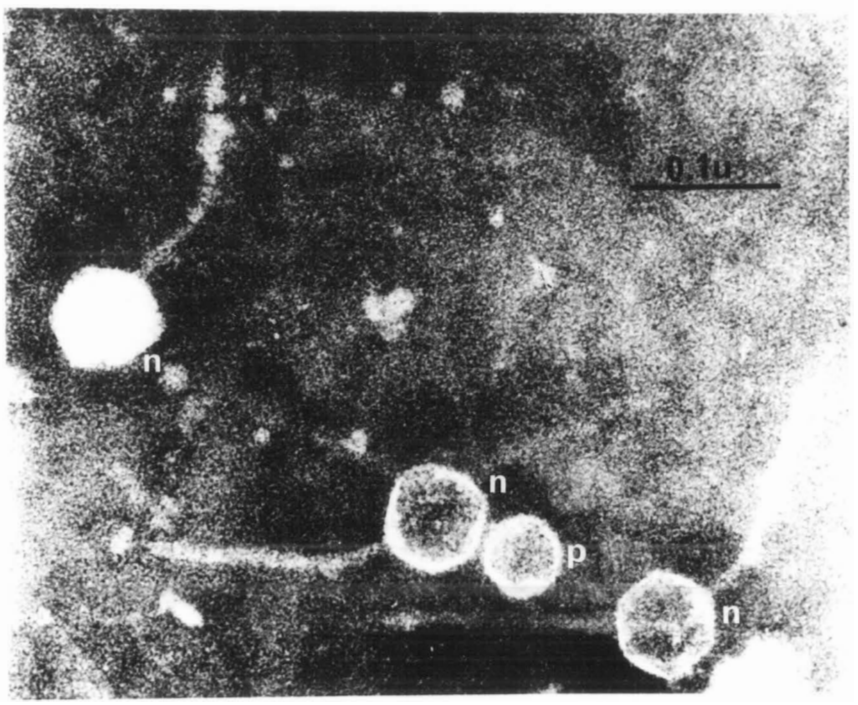


FIGURE 3

detected by the electron microscope in normal lambda heads (Kaiser, 1966). The formation of the petit lambda heads also involves the synthesis of protein subunits but these have a different packing arrangement in the final structure (Kemp et al., 1968) and DNA is never present within them (Karamata et al., 1962).

Studies of the genes governing lambda head development suggest a clustering of functions with genes A to D controlling the assembly of protein subunits and E and F the synthesis of the structural protein of the head (Mount, 1965; Harris, 1966). Lysates of lysogens of mutants in the first group of genes produce both heads and tails. However, these are not joined to form mature phage particles and the heads show varying degrees of aberrance. Mutants in gene A produce numerous petit lambda particles, mutants in gene B produce large numbers of aggregated heads and tubular heads, mutants in gene C produce large numbers of tubular heads, while mutants in gene D produce heads with only slight structural deviations from the normal (Kemp et al., 1968). Polarity occurs among the genes in this region, specifically in the series W-B-C (Parkinson, 1968) (Figure 2).

The major morphological components of the head are

controlled by genes E and F. Temperature sensitive and suppressor sensitive (sus) mutants in E produce no heads (Mount, 1965; Kemp et al., 1968). Also sus mutants in F appear to produce no heads, although the mutants available in this region show a high reversion rate, so no definite conclusion can be made. The number of major protein components in the head based on this genetic evidence cannot exceed two. Thomas et al. (1967), however, attributed a catalytic function to gene F, indicating that it acted somehow as a controller of head synthesis, and a stoichiometric function to gene E, which would suggest that it controlled the head structural protein.

Chemical studies on whole lambda ghost proteins have indicated the presence of a major morphological component of molecular weight 47,000-55,000 which, on the basis of its prevalence in whole phage, has been ascribed to the head (Dyson and van Holde, 1967; and Villarejo et al., 1967).

Statement of the Problem

The preceding literature survey has indicated some interesting features of bacteriophage lambda morphogenesis. In lysates of wild-type lambda lysogens, two kinds of head are

always present; normal and petit. In lysates of lambda lysogens mutant in late functions, both head forms are absent only when gene E, or possibly gene F, are defective.

What is the relationship between the normal and petit lambda head types? Are these two head forms composed of different protein subunits, or are they both formed from the same protein subunit packed differently to give the different overall structure seen in normal and petit lambda? If purified normal and petit lambda heads can be degraded to the same major protein, it becomes evident that they are built from the same protein subunit. Purification of normal and petit lambda heads free from one another is necessary in order to determine with certainty the number and nature of the proteins present in each entity. A single major protein in both head types would point to the involvement of only one gene coding for the structural protein of both kinds of head.

The experiments embodied in this thesis therefore are aimed at answering the following three questions:

(1) What is the relationship between the normal and petit lambda head types with respect to their protein subunits?

(2) How many major proteins are present in each type of lambda head, and what are their physical and chemical parameters?

(3) Which gene determines the major protein of the head?

CHAPTER II

PREPARATION AND PURIFICATION OF BACTERIOPHAGE LAMBDA HEADS

Introduction

In the preparation of the purified normal and petit heads of bacteriophage lambda, both head types had to be prepared free from contamination by one another, phage tails, and other phage and bacteria specified material present in a lysate. Two major methods were available. The first method was to purify infectious phage from a lysate of a normal lysogen, to separate heads and tails by chemical means, and then to isolate and purify each entity. Brenner et al., (1959) applied this procedure successfully to T-even phages. The second method was to purify phage heads, prepared from a lysate of cells of a mutant lysogen incapable of producing morphologically normal tails, and thus circumvent the problems arising in purifying a mixture of components. Some attempts to produce purified

lambda phage components by the first method were reported by Villarejo et al. (1967) who indicated that they had difficulty disintegrating the phage into morphologically detectable heads and tails. Dyson and van Holde (1967) likewise could not separate identifiable phage structures after chemical treatment of the phage.

Mutants of bacteriophage lambda are available in which synthesis of phage structural components is suppressed (Campbell, 1961; Weigle, 1966). Such a mutant, deficient in tail formation, which produced heads only was utilized in the preparation of the purified heads. However, in order to study the protein of which the normal lambda head is composed, it was still necessary to remove the petit lambda from the mixture. Separation of protein and nucleic acids of the T-even phages had been achieved by the anion exchangers, diethylaminoethyl cellulose (DEAE-cellulose) and epichlorhydrin triethanolamine cellulose (ECTEOLA-cellulose) (Creaser and Taussig, 1957; and Taussig and Creaser, 1957). The use of these cellulose derivatives was applied to the separation of the normal and petit lambda heads.

Materials

Bacterial and Phage Strains: The bacterial and phage strains used are listed in Table I.

Media: Growth medium containing per liter of distilled water, 1.0 g NH_4Cl, 1.5 g KCl, 2.4 g $MgSO_4.7H_2O$, 1.2 g trizma base, 5.0 ml 80% lactic acid, 1.6 ml glycerol, and 1 ml of a 0.011 g/l solution of $CaCl_2$. This mixture was autoclaved and then 20 ml of a 50 mg/ml solution of sterile casamino acids (Difco), 0.5 ml of a 5 mg/ml solution of vitamin B_1, thiamin, and 5.0 ml of sterile M/30 K_2HPO_4 were added. The mixture was brought to pH 6.8-7.2 by the addition of 10 N KOH under aseptic conditions. This medium was derived from H medium (Hershey et al., 1951).

Nutrient Broth: 8g Difco bacto nutrient broth and 5 g NaCl were added to one liter of distilled water. Nutrient agar: Nutrient broth containing 15 g Difco bacto-agar per liter. Top agar for plates: Nutrient broth containing 6 g Difco bacto-agar per liter.

Buffers: Tris-Magnesium: 0.01 M trizma base brought to pH 7.0 with HCl, and made 0.01 M with respect to Mg SO_4. Sodium Phosphate Buffer: 0.01 M NaH_2PO_4: Na_2HPO_4, pH 7.0. Chromatographic Buffers: Starter Buffer: 0.01 M Sodium

TABLE I

BACTERIAL AND PHAGE STRAINS

Strain	Genotype	Source	Comment
Bacteria:			
RC600	pm^+	C.R. Fuerst	The standard E. coli K 12 strain used as an indicator for λ (Appleyard, 1954).
W3350	pm^-	C.R. Fuerst	E. coli W3350 used as a non-permissive host for sus mutants of λ (Campbell, 1961).
Y 10	Lac^+, pm^+, λ^+	C.R. Fuerst	A derivative of E. coli K 12 lysogenic for λ^+, induced by UV and used as a source of λ^+ (Mount et al., 1968).
Bacteriophage:			
λ sus M87		C.R. Fuerst	A suppressor-senstivie mutant in gene M deficient in tail synthesis used as a source of normal and petit lambda heads (Campbell, 1961).
t 61		C.R. Fuerst	A defective mutant in gene E deficient in head production, used in the preparation of tails (Mount et al., 1968).

phosphate buffer, pH 7.0, containing 0.01 M $MgSO_4$. End buffer: Starter buffer made 0.5 M with respect to NaCl.

Cellulose Ion Exchangers: ECTEOLA cellulose (Sigma Chemical Co.) was generated before using by washing 15 min with 1 M NaOH, 15 min with 0.3 M NaH_2PO_4, and then 2-3 times with starter buffer until the pH and molarity had stabilized. DEAE cellulose (Sigma Chemical Co.) was generated before using by washing 15 min with 0.5 M NaOH, 15 min with 0.3 M NaH_2PO_4, and then 2-3 times with starter buffer.

Methods

Preparation of Phage Mutant Heads

Five ml of an overnight culture of *E. coli* W3350(λ *sus* M87) in the growth medium were added to 500 ml of prewarmed growth medium in 2800 ml Fernbach flasks. The cultures were grown at 37°C in a water bath with vigorous shaking for about 4 hours. The optical density at 650 nm on the Coleman Junior spectrophotometer was then between 0.2 and 0.3 units, corresponding to 4-8 x 10^8 cells/ml. The cultures (as 125 ml aliquots) were irradiated while being shaken in a pyrex dish. The dose received from the General Electric germicidal ultraviolet lamp (peak output at 254 nm) was approximately 350 ergs/mm^2.

Induction of the lysogenic cells, as determined by the number of input cells appearing as infectious centers, was generally greater than 95% under these conditions. Each 500 ml culture was then added to 1500-2000 ml of prewarmed growth medium in 3 l Ehrlenmeyer flasks. These were grown with vigorous aeration by compressed air for 100 min, or until frothing indicated that lysis had begun. Chloroform, 50 ml per flask, was added to complete lysis and sterilize the cultures.

The concentration of input bacteria, infectious centers after U.V. treatment, and of wild-type phage in the lysates was monitored by standard techniques (Adams, 1959). Aliquots of 0.1-0.2 ml of appropriate dilutions of induced bacteria or of phage were adsorbed to 0.2 ml of stationary phase cells of the permissive strain RC600 at 37°C for 15 min. Pour plates were then prepared by overlaying nutrient broth plates with 2.5 ml of top agar. After incubation overnight at 37°C, the plaques were counted.

Preliminary Purification and Concentration

The crude lysate was purified and concentrated to about 1/80 of its original volume by two cycles of low and high speed

centrifugation. The lysate was first centrifuged at a g max of 10,400 for 20 min to remove bacterial debris (GSA head, Sorvall RC2B). The supernatant was then centrifuged 2 hours at a g max of 147,000 (35,000 rpm, A147 rotor, International B60 ultracentrifuge). Each pellet was resuspended in 5 ml of tris-magnesium buffer and left overnight. A further cycle of low and high speed centrifugation was performed with each pellet being resuspended finally in 5 ml of 0.01M sodium phosphate buffer, pH 7.0. Titers of wild-type phage during the centrifugation steps are shown in Table II.

In some of the samples used in the sedimentation analyses, each pellet was resuspended in 5 ml of 0.01M sodium phosphate buffer, 0.01M $MgSO_4$, rather than tris-magnesium after the first high speed centrifugation.

Comparative counts were made of the normal and petit lambda heads in 3 typical sus M87 lysates. There were 966 normal lambda heads and 2384 petit lambda heads over 10 plates.

Separation, Final Purification and Concentration of Phage Structures

The normal and petit lambda heads were separated on an ECTEOLA cellulose column. Generated ECTEOLA cellulose was

TABLE II

TITERS OF LYSATES OF λ+ (FROM Y 10) AND INDUCED W3350 (λ SUS M87) ON THE PERMISSIVE HOST, RC600, AT VARIOUS STAGES OF CONCENTRATION

Crude Lysate p.f.u./ml.	Lysate after one high speed centrifugation p.f.u./ml.	Lysate after two high speed centrifugations p.f.u./ml.
Phage λ^+		
1.19×10^{10}	0.9×10^{11}	1×10^{12}
λ Sus M 87		
8.3×10^{4}	1.6×10^{5}	
2.7×10^{4}	1.2×10^{5}	
1.93×10^{4}	1.3×10^{5}	
1.4×10^{4}	1.1×10^{5}	
1.3×10^{3}	5.0×10^{3}	2.6×10^{4}
8.1×10^{4}	5.0×10^{5}	2.2×10^{6}
2.7×10^{4}	1.3×10^{5}	6.6×10^{5}

Phage λ+ was used initially because increases in infectivity could be monitored easily. No simple method existed for quantitatively examining the concentration of phage heads on purification of sus M87, although the titers of wild type phage present would give an indication of the amount of phage heads present in the solutions if it is assumed that there is no loss of infectivity during the concentration procedures.

packed as a slurry in a 2.5 x 45 cm column. The samples, generally 100-110 ml of concentrated lysate, were added and washed into the column with 50-100 ml of the starter buffer (0.01M sodium phosphate buffer pH 7.0). The phage structures were then eluted with a continuous gradient of 0-0.5 M NaCl in starter buffer, 150 ml of each solution. The effluent was monitored as it passed through a flow cell and the optical density at 280 nm recorded prior to collection of 5 ml fractions in a fraction collector. The salt concentration of the fractions was estimated by measuring the refractive index of the solutions in a refractometer and comparing with a standard curve.

The tubes from each peak of the ECTEOLA column were either desalted directly on Sephadex or pooled and concentrated by centrifuging 2 hours at a g max of 147,000. The pellets were resuspended in 1/10 the original volume of sodium phosphate starter buffer. The protein concentration was then 300-500 μg/ml measured by the Lowry technique (Lowry et al., 1951) or by ratio of the absorbance at 280 and 260 nm (Layne, 1957).

The purified phage entities were desalted in 50 ml aliquots on Sephadex G-50 (2.5 x 45 cm column), eluting with distilled

water. Chloroform was added to sterilize the desalted phage entities and they were stored at 4°C. Appropriate aliquots were taken and lyophilized for later experiments.

DEAE Cellulose Column

The concentrated normal and petit lambda heads were also applied to a DEAE Cellulose column. The gradient in this case was 0 -1.0M NaCl in the sodium phosphate starter buffer.

Separation of Other Phage Entities by the ECTEOLA Cellulose Column

Samples of concentrated whole infectious phage (λ+ from Y 10) and phage tails (from T 61) were also applied to ECTEOLA cellulose columns and eluted with a 0 - 0.5 M NaCl in the sodium phosphate starter buffer. The infectious phage were assayed for by conventional techniques (Adams, 1959), and the presence of tails was monitored by the electron microscope.

Density Gradient Centrifugation of Lambda Heads

Fractions containing heads from the ECTEOLA columns were pelleted and resuspended in tris-magnesium buffer. To 8 ml of this solution were added 4 g of cesium chloride. The samples were centrifuged 20 hr at a g max of 300,000 (53,000 rpm in the

SB405 swinging bucket rotor of the International B60 ultracentrifuge. After centrifugation, the position of the visible band in each tube was measured and compared to the meniscus of the solution. The bottom of the tube was pierced and 0.3 ml fractions were collected. The concentration of the cesium chloride was monitored by measuring the refractive index of a drop of each fraction in the refractometer. The three fractions lying in a position corresponding to the visible band were pooled, diluted to 45 ml with 0.01 M sodium phosphate buffer, pH 7.0, and sedimented for 2 hr at a g max of 105,000. The resulting pellets were resuspended in 1 ml phosphate buffer and examined in the electron microscope.

The density of cesium chloride solutions of different concentrations was determined gravimetrically at 20°C and the refractive index measured at 20°C to construct a standard curve relating the two values.

Results

ECTEOLA Cellulose Columns

Yields of Phage from the Column

Prior to using the ECTEOLA columns routinely, they were standardized using infectious wild type phage (λ+) and assaying

for peaks of infectivity of the phage which were eluted by the salt gradient. Phage yields varied according to the treatment of the lysate prior to loading it on the column. Crude lysates, lysates dialyzed into starter buffer, or given one high speed centrifugation and resuspended in starter buffer were applied to various columns. Complete recovery occured only when the growth medium was removed from the lysate either by dialysis into starter buffer or by high speed centrifugation followed by resuspension of the phage pellet in starter buffer. The amino acids and other nutrients in the growth medium, if they were not removed prior to loading the column, appeared to hinder the attachment of the phage to the ion exchange cellulose. A peak fraction containing phage eluted from the column before the gradient was begun if the column was overloaded. An amount of material equivalent to 10 ml of the lysate concentrated by two high speed centrifugations (equal to about 800 ml of the original crude lysate) appeared to be the maximum amount for an ECTEOLA column measuring 0.9 x 13 cm. The results of experiments on standardization of the ECTEOLA cellulose columns are shown in Table III.

TABLE III

YIELDS OF λ+ FROM ECTEOLA CELLULOSE COLUMNS AFTER DIFFERENT TREATMENTS OF THE LYSATE

Treatment of Lysate before Loading on Columns (0.9 x 30 cm)	TOTAL PHAGE INPUT		PHAGE OUTPUT				
	Volume (ml)	Concentration (p.f.u./ml)	Phage Present in Peak Eluting Before Salt Gradient Total p.f.u.	% of Input	Phage Present in Peak Eluting After Salt Gradient Total p.f.u.	% of Input	Total
None	10	1.5×10^9	5.3×10^9	35%	Not examined		Not examined
None	100	1.8×10^9	6.4×10^{10}	35%	Not examined		Not examined
Dialyzed into starter buffer	10	1.4×10^9	none[1]		9.25×10^9	64%	64%[2]
None	10	1.5×10^9	3.75×10^9	25%	7.8×10^9	52%	77%
One High Speed Centrifugation	10	5.8×10^{10}	none		5.45×10^{11}	94%	94%
One High Speed Centrifugation	10	1.2×10^{11}	none		8.3×10^{11}	69%	69%

1 Fractions suspected of containing phage were given a preliminary assay by spotting on a lawn of the permissive bacterial host RC600.

2 The low total yields from the ECTEOLA cellulose columns may be due to loss of infectivity of the phage during the course of purification on the column.

Separation of Normal Lambda Heads on ECTEOLA Cellulose Columns

The appearance of a sample of purified concentrated lysate of sus M87 applied to the ECTEOLA columns is shown in figure 4. It was separated by the column into two fractions (figure 5). Examination of the peak fractions from the column with the electron microscope showed that the first peak contained only normal lambda heads (2028 normal lambda heads in plates from 20 columns) and the second peak contained petit lambda heads contaminated by less than 5% normal lambda heads (3345 petit lambda heads and 149 normal lambda heads in plates from 28 columns). In the second ECTEOLA peak, a small amount of non-phage particulate matter was detected, but this in no instance exceeded the amount of normal lambda present. The appearance of the material in the two ECTEOLA peaks is shown in figure 6.

Separation of Other Phage Structures by a Salt Gradient

The salt concentration necessary to elute the different phage structures from the ECTEOLA cellulose column was monitored by the refractive index. The relationship between the refractive index and the sodium chloride concentration in the starter buffer is shown in figure 7.

Figure 4

Sample of Sus M87 after Concentration by High Speed Centrifugation

Normal (n) and Petit (p) lambda heads are present and are contaminated by bacterial debris (b). Magnification 100,000 x.

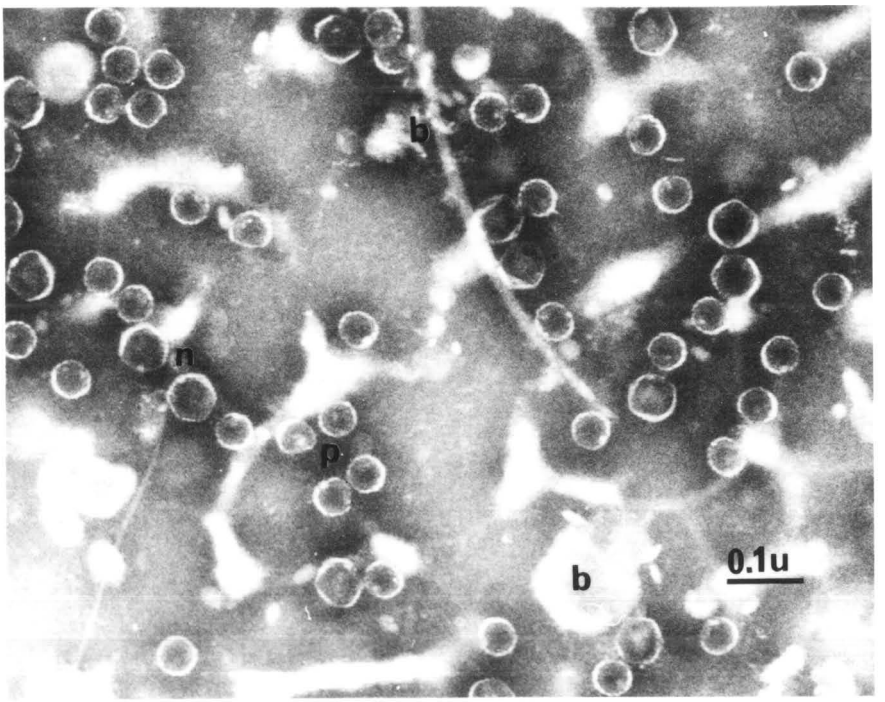


FIGURE 4

Figure 5

Separation of Lambda Heads by ECTEOLA Cellulose Chromatography

The fractions eluting from the ECTEOLA cellulose column were monitored by absorbance at 280 nm. The salt concentration was determined from the refractive index of the fractions.

_____ Absorbance at 280 nm

--------- Sodium chloride concentration

FIGURE 5

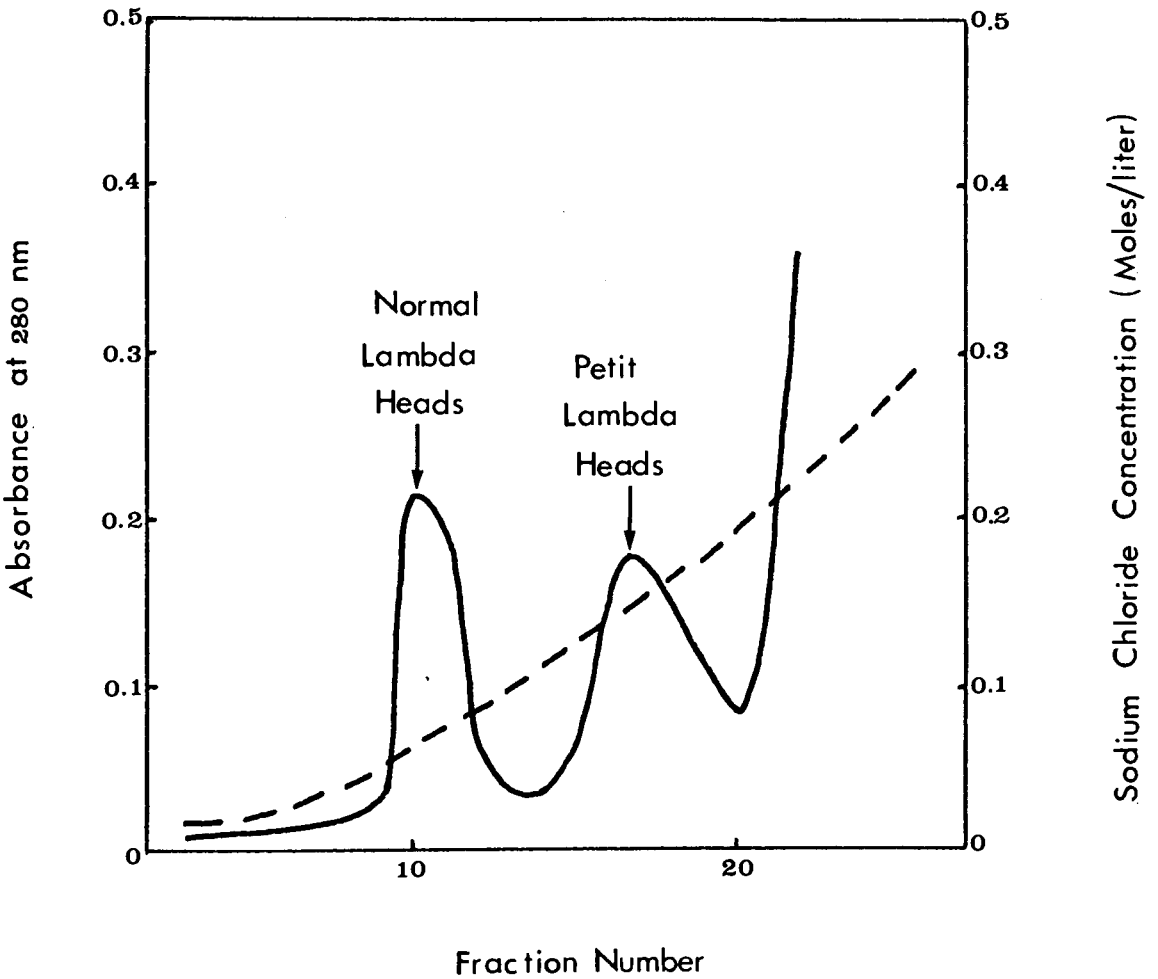

0.5
0.4
0.3
0.2
0.1
0
Absorbance at 280 nm
Normal
Lambda
Heads
Petit
Lambda
Heads
10
20
Fraction Number
Sodium Chloride Concentration (Moles/liter)

Figure 6

Entities Present in Peaks of ECTEOLA Cellulose Column

(a) First peak from the ECTEOLA cellulose column. Only normal lambda heads are present. Magnification 100,000 x.

(b) Second peak from the ECTEOLA cellulose column. The fraction is mainly petit lambda but approximately 5% of the total number of heads are normal lambda. Magnification 100,000 x.

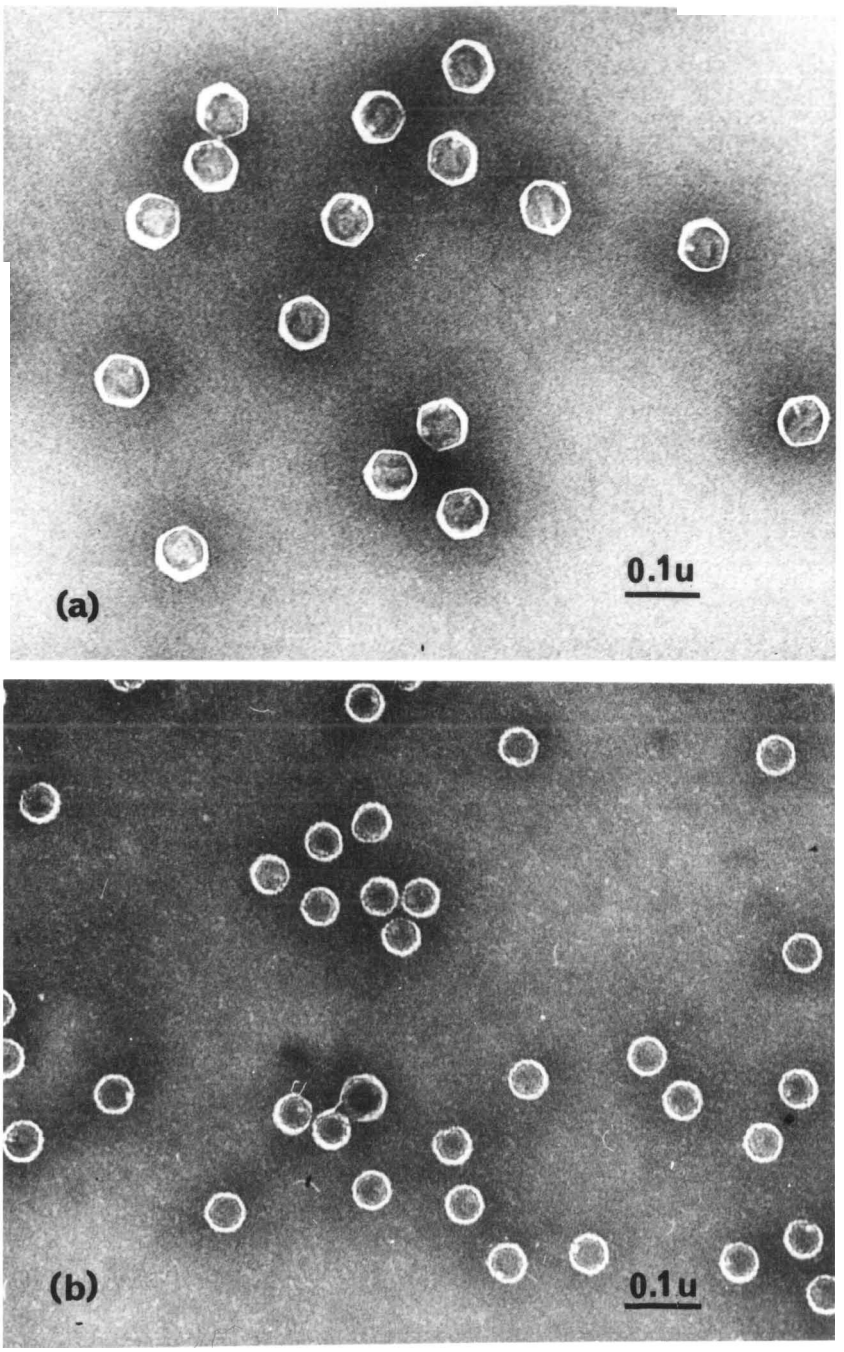


FIGURE 6

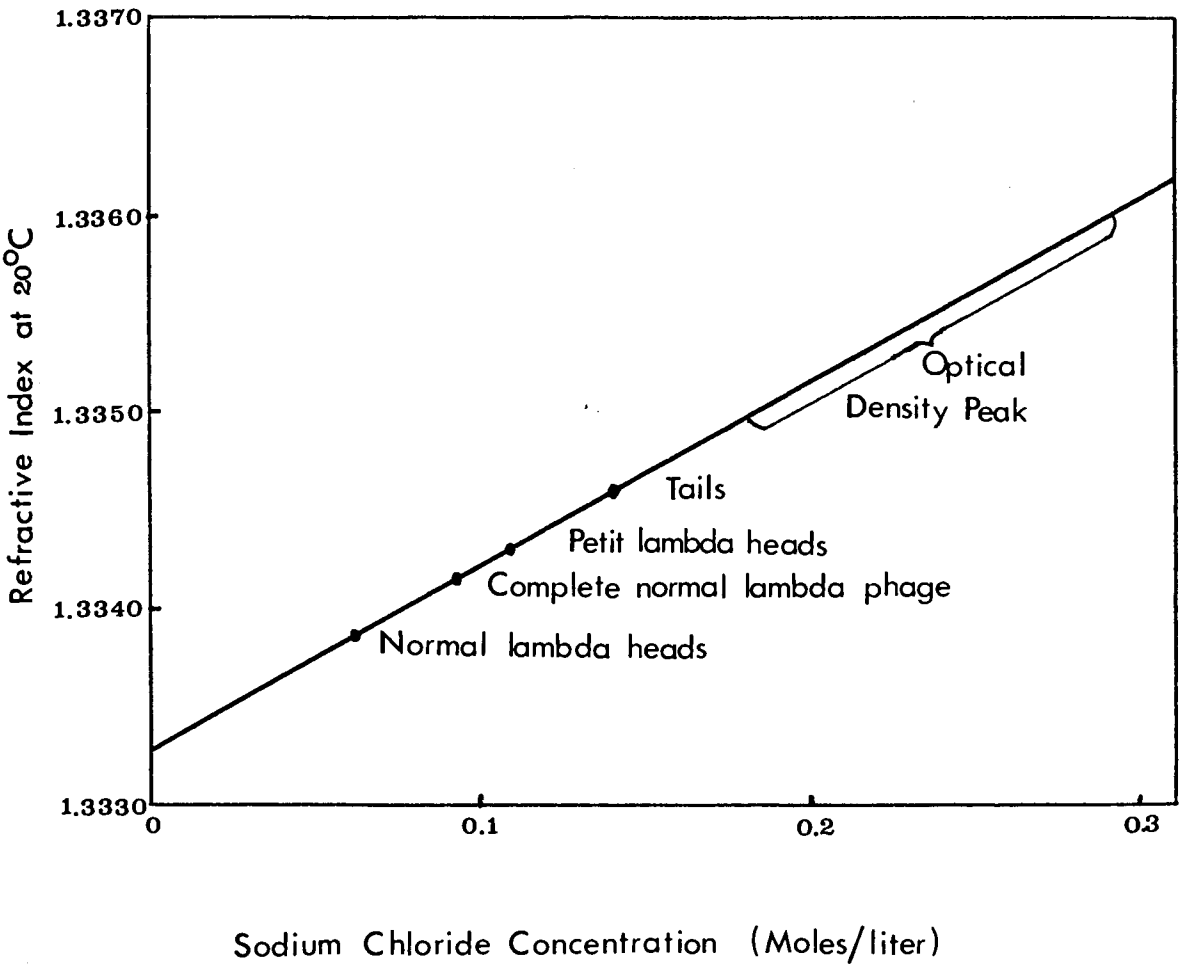
Figure 7

Separation of Lambda Phage Entities on ECTEOLA Cellulose by a Sodium Chloride Gradient

This figure is a composite of various phage entities prepared from different lambda mutants and run on different columns.

The optical density peak revealed no identifiable phage structures in the electron microscope and the ratio of the optical density at 280 and 260 nm was that expected for nucleic acids.

FIGURE 7


1.3370
1.3360
1.3350
1.3340
1.3330
Refractive Index at 20°C
0
0.1
0.2
0.3
Optical
Density Peak
Tails
Petit lambda heads
Complete normal lambda phage
Normal lambda heads
Sodium Chloride Concentration (Moles/liter)

The different phage components eluted in the following order with increasing salt concentrations: normal heads (sus M87), normal whole phage (λ+), petit lambda heads (sus M87), and tails (T 61). The results are summarized in figure 7. They indicate that the net negative charge on the phage structures increases in the order stated. The detailed data from which figure 7 was constructed are enumerated in Table IV.

The elution patterns of lambda components on an ECTEOLA column is similar to that for T2r found by Creaser and Taussig (1957) where a peak corresponding to phage protein occurred at approximately 0.1 M NaCl and a peak corresponding to nucleic acids, at 0.25-0.3 M NaCl. The optical density peak obtained on chromatography of the lambda heads, eluted at a NaCl concentration of about 0.2M and had an O.D. 280/260 ratio less than 0.5, that of nucleic acids (Layne, 1957).

DEAE Cellulose Column

When DEAE cellulose was used to separate a concentrated sample of sus M87 heads, higher salt concentrations were required to elute the phage structures, but the separation was not as marked as on ECTEOLA. DEAE was therefore not used

TABLE IV

SEPARATION OF LAMBDA PHAGE ENTITIES ON ECTEOLA CELLULOSE BY A SODIUM CHLORIDE GRADIENT

Entity	Characteristic Measured	Column Number	Peak Tube	Range	Refractive Index of Peak Tube	Range
		0.9 x 30 cm				
Infective Lambda Phage (λ+)	Infectivity (p.f.u./ml)	ECT V	20	15 - 40	1.3344	1.3339 - 1.3362
		ECT VII	16	13 - 37	1.3343	1.3339 - 1.3364
Normal Lambda Heads (λ sus M87)	Optical Density at 280 nm	ECT VII		12 - 13		1.3337 - 1.3339
		ECT VIII		12 - 12		1.3337 - 1.3340
		ECT X	11	10 - 13	1.3338	1.3337 - 1.3340
		ECT XI	11,12	10 - 15	1.3337	1.3336 - 1.3340
		ECT XII	11	10 - 15	1.3336	1.3335 - 1.3338
		ECT XIII	11	10 - 13	1.3337	1.3336 - 1.3339
		ECT XIV	11	8 - 13	1.3337	1.3334 - 1.3339
		ECT XV	17	12 - 18	1.3338	1.3340 - 1.3343
		ECT XVII	11	11 - 14	1.3338	1.3336 - 1.3339
		ECT XX	14	11 - 15	1.3336	1.3335 - 1.3338
		1.5 x 30 cm				
		ECT XXIII	29	26 - 31	1.3338	1.3334 - 1.3339
		ECT XXIV	38	36 - 40	1.3338	1.3337 - 1.3340

(continued)

TABLE IV (continued)

Entity	Characteristic Measured	Column Number	Peak Tube	Range	Refractive Index of Peak Tube	Range
Petit Lambda Heads (λ sus M87)	Optical Density at 280 nm	0.9 x 30 cm				
		ECT VIII	16	14 - 17	1.3343	1.3341 - 1.3346
		ECT X	17	14 - 18	1.3343	1.3340 - 1.3344
		ECT XI	17	14 - *	1.3342	1.3340 - *
		ECT XII	16	15 - 18	1.3339	1.3338 - 1.3340
		ECT XIV	15	13 - *	1.3341	1.3339 - *
		ECT XV	19	18 - 25	1.3344	1.3343 - 1.3352
		ECT XVII	15	14 - 16	1.3339	1.3339 - 1.3340
		ECT XX	16	15 - 17	1.3338	1.3337 - 1.3339
		1.5 x 30 cm				
		ECT XXIII	38	35 - 43	1.3344	1.3342 - 1.3348
		ECT XXIV	45	40 - 50	1.3344	1.3340 - 1.3348
Tails T61	Presence of tails in fractions examined by the electron microscope	0.9 x 30 cm				
		ECT IX		21 - 25		1.3342 - 1.3348

* not measured

routinely. The profile of a DEAE cellulose column is shown in figure 8.

Purity of the Bacteriophage Lambda Heads

The purity of the bacteriophage lambda heads was assessed by several means to determine the level of particulate and soluble contaminants.

The elution of the normal lambda heads as a single, well-defined peak from the ECTEOLA cellulose column in itself constitutes a criterion of purity. The proximity of the second peak (mostly petit lambda) to the large optical density peak indicates that the degree of purity in this later peak is lowered by contamination with material eluting in the large optical density peak.

Contamination of the heads by particualte matter is at a low level. Electron microscope counts of entities visible in the first and second ECTEOLA peaks have already been given. No contamination was seen in the normal lambda peak, and less than 5% contamination in the petit lambda fraction.

Disc gel electrophoresis using standard and SDS gels containing 7 1/2% acrylamide (Chapter III, p. 52) indicated that soluble proteins were not present in detectable amounts in

Figure 8

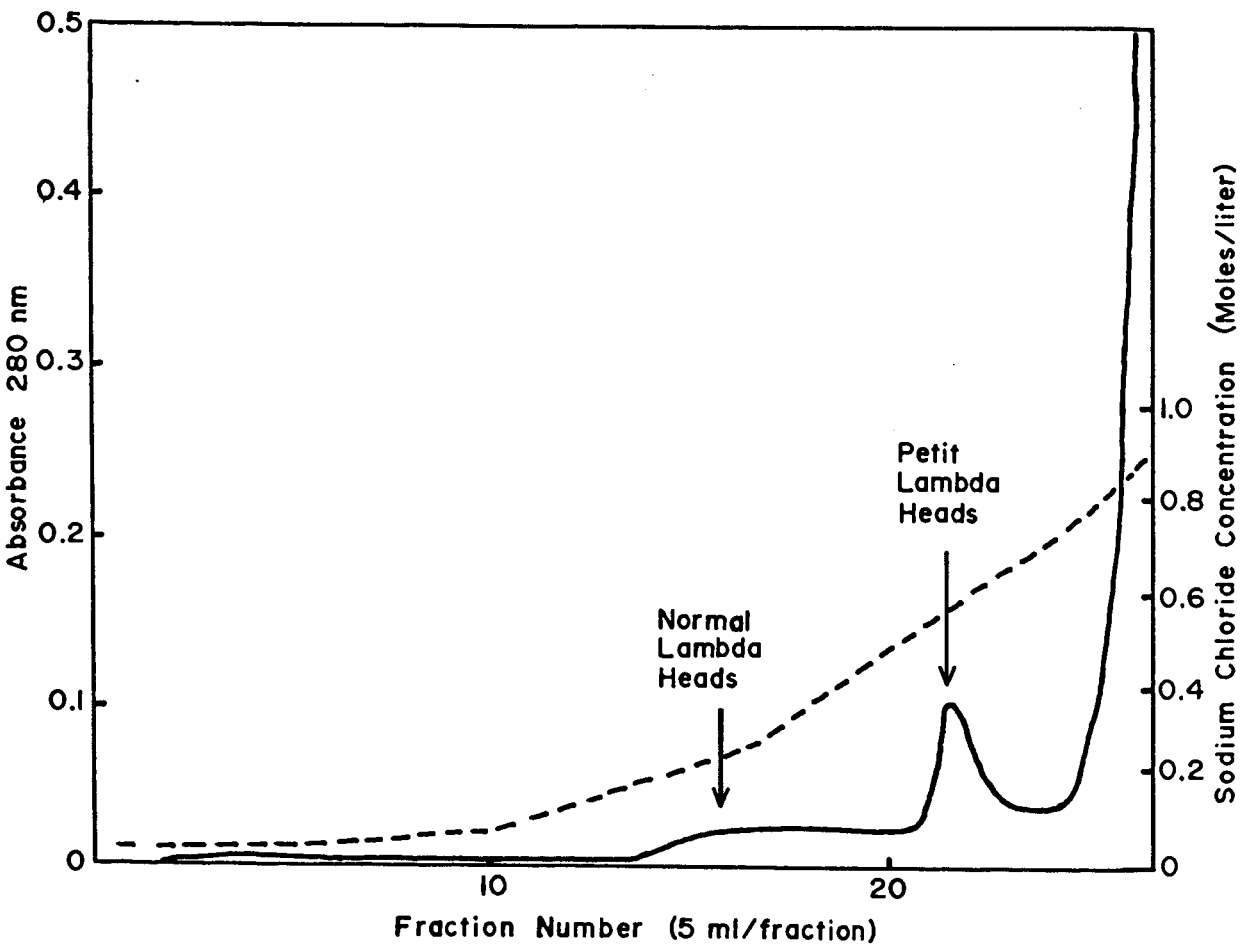
Profile of DEAE Cellulose Column

The elution profile is that of a single DEAE cellulose column. The fractions eluting from the column were monitored by absorbance at 280 nm. The salt concentration was determined from the refractive index of the fractions.

_____ Absorbance at 280 nm.

------- Sodium Chloride Concentration.

FIGURE 8


0.5
0.4
0.3
0.2
0.1
0
Absorbance 280 nm
Normal
Lambda
Heads
Petit
Lambda
Heads
1.0
0.8
0.6
0.4
0.2
0
Sodium Chloride Concentration (Moles/liter)
10
20
Fraction Number (5 ml/fraction)

material eluting in either the first or second peaks from the ECTEOLA cellulose column. The data are shown in Table V.

The presence of tail protein in the lysates is negligible on theoretical grounds. In phage ghosts, tail protein has been estimated to be present in amounts of approximately 15% of the total phage protein (Dyson and van Holde, 1967). The amount of normal phage produced is indicated by the titers of the lysates on RC600, the permissive host for sus mutants, and is about 10^4 whereas the amount of heads produced by W3350 (sus M87) is reported to be more than 3 times the normal amount of phage-specific structures relative to concentrations in normal lysates (+++) (Kemp, Howatson and Siminovitch, 1968), or about 10^{10}. How much unpolymerized tail protein is produced by the gene M mutant is not, however, known. It would, however, presumably be discarded with the supernatant of the high speed spins. Small amounts would be assumed to be negligible in comparison with the formed heads produced. Other soluble proteins would also be assumed to be removed in the high speed centrifugations on the same grounds unless they were actually trapped within or adsorbed to the heads.

TABLE V

PURITY OF BACTERIOPHAGE LAMBDA HEADS DETERMINED BY STANDARD AND SDS 7½% ACRYLAMIDE GELS

Sample	Size (ug)	Type of Gel	Appearance of Gel
Untreated Normal Lambda Heads (First ECTEOLA Peak)	200 100 140	Standard Standard Standard	No bands, but starter gel stained darkly
Untreated Petit Lambda Heads (Second ECTEOLA Peak)	185	SDS	No bands, but dark stain at top of gel which did not enter

Analytical Ultracentrifuge Studies

Three samples were run on the analytical ultracentrifuge, Spinco Model E. The data of the runs are summarized in Table VI and the Schlieren patterns of these runs are shown in figure 9. The purified normal lambda heads showed a major peak of 468-493 S and the petit lambda fraction, a major peak at 484 S. In addition, the normal lambda heads had minor peaks at 718 and 148-191 S.

Density of Bacteriophage Lambda Heads

When empty normal bacteriophage lambda heads from the first ECTEOLA peak were centrifuged to equilibrium in a cesium chloride density gradient, a single band was observed, lying at a position 38 cm from the bottom of a 52 cm tube. Measurement of the refractive index of fractions containing this band (1.3604 and 1.3608) when compared to the standard curve relating density and refractive index (Figure 10) showed that material in the band had a density of approximately 1.27 g/cm^3.

TABLE VI

DETERMINATION OF SEDIMENTATION COEFFICIENTS

OF NORMAL AND PETIT LAMBDA

Sample and Concentration	Buffer	Speed of Run R/min	Temperature °C	Peaks	Sedimentation Coefficient S 20,w
Normal lambda head from *sus* M87 488 μg/ml	Phosphate	30,000	20	Major Slow Fast	493 191 718
Normal lambda heads from *sus* M87 496 μg/ml	Tris	20,000	23	Major Slow	468 149
Mixture of normal and petit lambda heads from *sus* M87 137 μg/ml	Phosphate	30,000	20	Single	484

The buffers used in the sedimentation centrifugation were 0.01 M Tris, pH 7, or phosphate sedimentation velocity buffer (containing 1.380 g NaH_2PO_4 and 2.68 g $Na_2HPO_4.7H_2O$ per liter of distilled water).

Figure 9

Schlieren Patterns from Ultracentrifuge Runs of Normal and Petit Lambda Heads

(a) Normal Lambda Heads in Phosphate Buffer from first ECTEOLA Peak. The centrifuge was run at 30,000 rpm with the temperature 23°C. The pictures were taken at 2 min intervals at a bar angle of 50° or 10°.

(b) Normal Lambda Heads in Tris Buffer from first ECTEOLA Peak. The centrifuge was run at 20,000 rpm with the temperature 20°C. The pictures were taken at 2 min intervals at a bar angle of 50° or 20°.

(c) Petit Lambda Heads in Phosphate Buffer from the second ECTEOLA Peak. The centrifuge was run at 30,000 rpm, with the temperature 23°C. The pictures were taken at 2 min intervals at a bar angle of 50° or 10°.

FIGURE 9

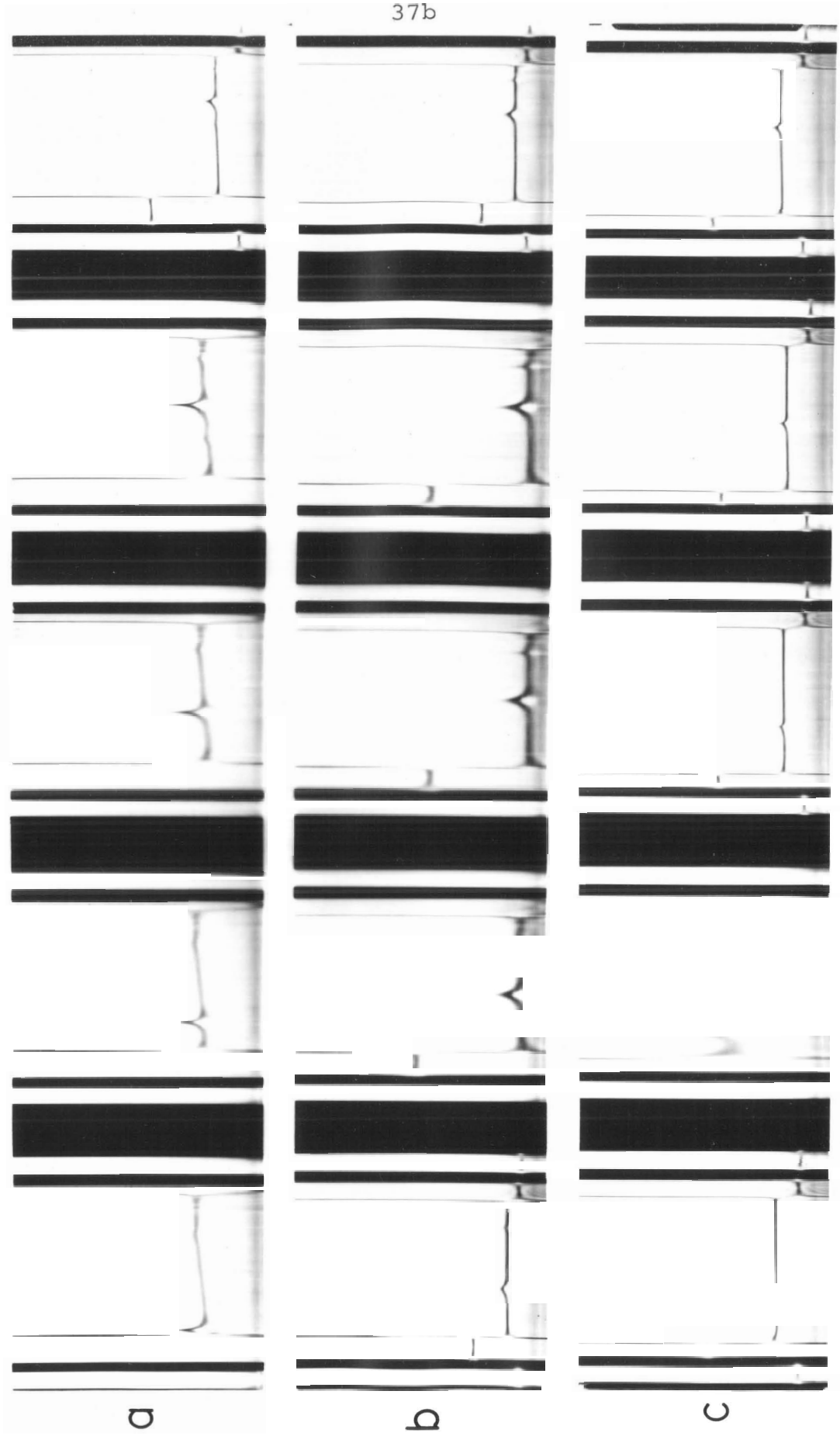
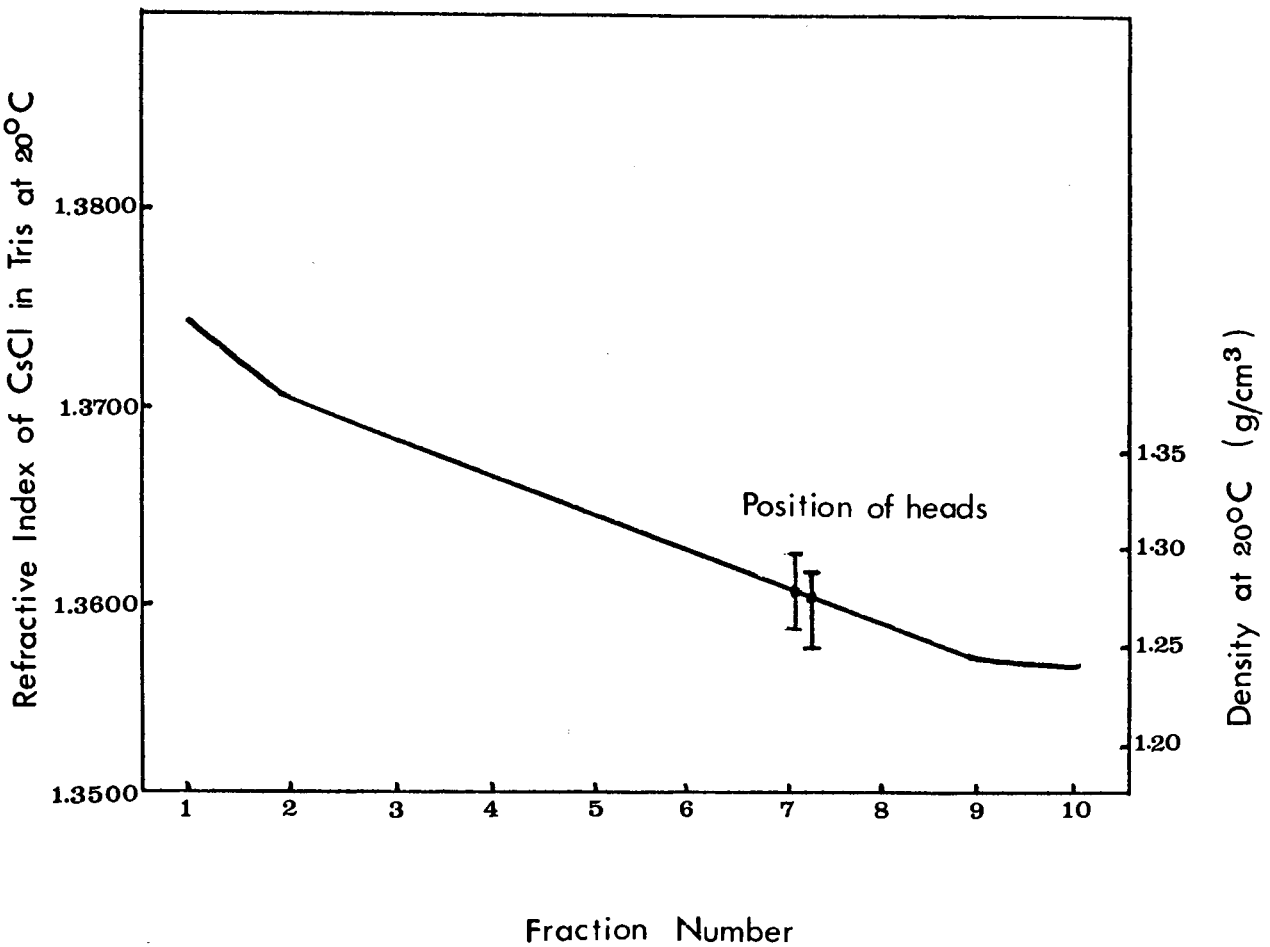


Figure 10

Density Gradient Centrifugation of Normal Lambda Heads

The points superimposed on the standard curve correspond to the refractive index of the fraction containing the visible band in each run. The error bars indicate the refractive indices of the adjacent fractions (also containing a small amount of lambda heads) which were pooled when the sample was washed and concentrated following the density determination.

FIGURE 10


Refractive Index of CsCl in Tris at 20°C
1.3800
1.3700
1.3600
1.3500
Position of heads
1.35
1.30
1.25
1.20
Density at 20°C (g/cm³)
1
2
3
4
5
6
7
8
9
10
Fraction Number

Discussion

Normal lambda heads, containing no DNA, have been prepared free from contamination by petit lambda and soluble proteins. Differences in surface qualities enable the normal and petit lambda heads to be separated on the ECTEOLA cellulose columns. The charge differences causing the differential affinity towards the ECTEOLA cellulose may be ascribed to a minor protein present throughout the head lattice, to a small entity situated in a certain part of the head, i.e., in the region where tail attachment occurs, or to the molecular conformation of the capsid protein subunits (Chapter III, p.97). A low molecular weight factor must be present in the medium in order for *in vitro* head and tail assembly to occur (Bode and Harrison, 1969). This factor may be attached to a specific region of the head, causing changes in the surface charges of normal heads.

The immunological evidence shows that petit lambda heads can be precipitated by an antiserum to whole lambda (Karamata *et al.*, 1962) and normal lambda heads, by an antiserum to petit lambda (Kemp, unpublished). Although most of the lambda antibody is directed towards the tail, a small amount is directed toward the head. This is what is believed to react

with petit lambda indicating an antigenic relatedness between the normal and petit lambda heads. The antigenic similarity between the two heads may be due to the presence of the same major protein subunit within them.

The preparation of purified normal lambda heads has enabled determinations to be made for the values of sedimentation coefficient (indicating the size or "molecular weight" of the particle) and density. The few attempts that have been made to determine these values for lambda have mostly involved complete lambda ghosts rather than isolated structural components (Kaiser, 1966; Villarejo et al., 1967; and Dyson and van Holde, 1967).

During sedimentation, the sample of normal lambda heads prepared in phosphate buffer separated into a major and two minor Schlieren peaks while that prepared in tris buffer showed only the two slower components. The presence of both full and empty heads in electron micrographs of the phosphate buffer preparation could account for the three peaks in the first run if full heads separated into empty heads and DNA sedimenting as separate peaks. Electron micrographs of the tris buffer preparations revealed only empty heads. The fast

peak with a sedimentation coefficient of approximately 718 S may have been caused by the presence of some full heads in the sample in phosphate buffer. Weigle (1966) reported a $S_{20,W}$ of 650 for lambda heads. The major peak in the first and second runs is assumed to be empty heads which sediment more slowly than full ones. No definite conclusion can be made as to the nature of the small, slow-running peak in these runs, with a sedimentation coefficient of 149-181, although it approximates that of whole lambda ghosts ($S_{20,W}$ = 141), Dyson and van Holde, 1967) Buchwald et al. (personal communication) have indicated that the $S_{20,W}$ of empty normal lambda heads is 113 S. Their results would indicate that the major peak with a $S_{20,W}$ of about 480 S may consist of head aggregates, but does not explain why such aggregates are so uniform in size.

The similarity between the values for the petit lambda peak and the major peak for normal lambda would give rise to the rather unlikely suggestion that both head types can form essentially the same size and type of aggregate.

The density of the normal lambda heads from the ECTEOLA cellulose column is the density of protein, indicating that these heads are free from DNA. Weigle (1968) reported, on

the basis of density gradient centrifugation in cesium chloride, a value for the density of lambda tails containing protein only, of 1.28 g/cm^3, and for full lambda heads, containing DNA, of 1.53 g/cm^3. Kaiser (1966) stated that the density of DNA-free lambda ghosts is 1.3 g/cm^3.

Once the purified normal and petit lambda heads were available, breakdown studies could proceed to determine the number and nature of the protein subunits present in them. Earlier work (Villarejo et al. 1967; and Dyson and van Holde, 1967) on breaking down the phage to its protein subunits was done on whole ghosts, where unequivocal results as to the nature of the major head protein subunit could not be made due to the presence of contaminating tail protein.

Summary

Purified bacteriophage lambda heads have been produced from tail-less mutants by differential centrifugation and anion exchange chromatography. Normal and petit lambda heads differ in the surface charge they carry; petit lambda is bound more tightly to the ECTEOLA anion exchange cellulose than normal lambda, indicating that, under the conditions of

elution, it carries a lower net positive charge. The empty normal lambda heads have a sedimentation coefficient of approximately 480 S, and a density of 1.27 g/cm^3.

CHAPTER III

THE PROTEIN SUBUNIT OF BACTERIOPHAGE LAMBDA HEADS

Introduction

Several chemicals, known to attack different types of bonds, have been used in attempts to dissociate the purified normal and petit lambda heads into their smallest uniform protein components.

Four kinds of interaction which determine the quaternary structure of proteins are known: (1) sulfhydryl bridges between two cysteine residues of the same or different chains, (2) hydrophobic attraction between apolar groups resulting in an infolding of these, (3) hydrogen bonding between polar groups, and (4) salt bonding between acidic and basic residues exposed on the surface of the subunit molecule. Any or all of these forces may hold the phage subunits together.

Reagents, known to break specifically the various types of

bonds, have been used to dissociate viruses. Reducing agents, such as 2-mercaptoethanol, dithiothreitol, and lithium borohydride, which break sulfhydryl bridges have been used to degrade viruses (Holowczak and Joklik, 1967; Kellenberger, 1968; and Hare and Chan, 1968).

Hydrophobic bonds may be dissociated by the use of organic solvents such as dioxane, but little success has been reported in virus breakdown using these reagents (Dyson and van Holde, 1967).

Reagents such as concentrated urea (8M) or guanidine hydrochloride (6M) are thought to dissociate aggregated proteins by replacing one of the polar groups involved in a hydrogen bond, thereby unfolding that region of a peptide and changing its conformation so that subunits can no longer attach to one another. Urea has been used by a number of workers in the breakdown of viruses (Kellenberger, 1968; Hare and Chan, 1968; Dann-Markert et al., 1966; Duesberg and Rueckert, 1965; and Lin et al., 1967). Guanidine hydrochloride has much the same mode of action in breaking down viral structures as has urea (Kellenberger, 1968; and Nathans, 1965).

The detergent, sodium dodecyl sulfate (SDS), is believed

to break down proteins to their subunit structure by reacting with the charged groups of dibasic amino acids (Lauwers, 1967). Its effectiveness is thus governed by the accessibility of such groups. Several workers have succeeded in breaking down viruses with SDS although most of these have occurred at a pH greater than 10 or in the presence of other reagents such as urea or mercaptoethanol or in conjunction with heating (Brenner et al., 1959; Carusi and Sinsheimer, 1963; Holowczak and Joklik, 1967; and Fine et al., 1968). SDS has been used in attempts to dissociate whole lambda ghosts, but the reported results are contradictory. Villarejo et al. (1967) reported that at neutral pH, SDS had no effect on the lambda ghosts, while at alkaline pH it caused them to swell without disrupting the inter-subunit bonds. Dyson and van Holde (1967) found that most of the lambda ghost protein dissociated in the presence of SDS at pH 7.5 in a matter of minutes.

The reagents used to break salt bonds act at extremes of pH, in either the very acidic or very basic ranges outside the secondary pK_A's of the bifunctional amino acids. The secondary dissociation constants of those amino acids acting in salt bond formation, i.e., between pH 3.68-4.25 and pH

10.07-12.48 (Mahler and Cordes, 1966). Below the secondary pK_A's of the acids and above those for the bases, the secondary functional groups are uncharged and no longer take part in salt bond formation.

The protein of several viruses has been solubilized in 67% acetic acid (Fraenkel-Conrat, 1957; Enger and Kaesberg, 1965; Roberts and Steitz, 1967; and Kellenberger, 1968). Bacteriophage lambda has also been broken down in the presence of acidic buffers. Villarejo et al. (1967) reported that lambda phage ghosts dissolved slowly in citrate buffer at pH 2.5, and more rapidly in this buffer in the presence of urea, to produce three component proteins of whole phage. Dyson and van Holde (1967) described dissociation of intact lambda ghosts by a pH 2.5 glycine-hydrochloric acid buffer.

Breakdown of viruses has been achieved at high alkaline pH in systems containing sodium hydroxide pH 11-13 either alone (Rossomando and Zinder, 1968) or in the presence of 8M urea and a reducing agent (Kellenberger, 1968). At the lower alkaline pH of 10.5, several viruses and other proteins have been degraded either at low ionic strength (Anderer et al., 1968) or in the presence of succinic anhydride which reacts specifically with the ε-amino group of lysine (Friesham et al.,

1967).

Before the dissociation of bacteriophage lambda was attempted, the amino acid composition of the lambda heads was ascertained, to determine which residues capable of contributing to tertiary and quaternary structure were present in the protein. The results are shown in Table XVIII (p. 85), but the following salient features may be noted at this time:

(1) Nearly one-half of the residues are hydrophobic, short chain aliphatic or aromatic amino acids.

(2) Approximately one-third of the amino acids are dibasic or dicarboxylic, and of these, the dicarboxylic acids and their amides outnumber the dibasic ones.

(3) There is an absence of cysteine in the lambda head in agreement with the work of Villarejo et al. (1967) and Dyson and van Holde (1967).

In addition to indicating the reagents that might be useful in phage breakdown, the amino acid analysis in conjunction with tryptic peptide data may be used to obtain a value for the molecular weight of the protein subunit (Hull et al., 1969; Miki and Knight, 1968).

Materials and Methods

Analytical Methods

Following a specific chemical treatment, the phage heads were examined by three methods: electron microscopy, gel filtration, and disc gel electrophoresis to determine the nature and extent of breakdown.

Electron Microscopy

The heads were examined first in the electron microscope to see if they had undergone any gross structural change.

Procedure: Following chemical treatment of the heads, the material was desalted on Sephadex G-100. It was then negatively stained with 2% phosphotungstic acid, pH 6.5, (Kemp et al., 1968) and examined in a Zeiss EM 9A or RCA EMU 3H electron microscope. Photographs or random areas were taken at instrument magnifications of about 20,000.

Gel Filtration

Gel filtration on Sephadex of various porosities gives an estimate of the size and relative amount of the products of chemical breakdown of the lambda heads. In this study Sephadex G-100 was used and the columns standardized with blue dextran 2000 as the totally excluded marker substance (Andrews,

1964). Untreated heads and most forms retaining some semblance of head structure are totally excluded from this Sephadex whereas breakdown products are generally retarded and may thus be characterized.

Procedure: Sephadex columns (0.9 x 30 cm or 2.5 x 45 cm) were eluted by downward flow and care was taken that the operating pressure head did not exceed the maximum specified by the manufacturer. The material from the column was monitored by absorbance at 280 nm as it passed through a flow cell (path length 3 mm) and 15 drop (approximately 1 ml) fractions were collected in a fraction collector (LKB Uvicord and Ultrarac). In each column, void volume was determined and then the samples applied. A quantitative estimate of each fraction could be made by evaluating the area under the peak.

Disc Gel Electrophoresis

Disc gel electrophoresis was used to determine the number of protein types present following a specific treatment of the phage heads. In normal disc gel electrophoresis (Ornstein, 1964), proteins are separated on the basis of their mobilities through a porous gel. The mobility is related to the charge

on the molecule and also its size (molecular diameter) in relation to the pore size of the gel, and hence its frictional resistance in the system. The standard gel system consists of a large pore sample gel in which the material is applied, a spacer gel in which the components of the sample are concentrated to narrow bands and layered on top of each other, and finally the smaller pore running gel in which the protein components are separated. The concentration of monomer (acrylamide) and cross-linker (bis-methylene acrylamide) may be varied in the gels so that the size of the molecules able to penetrate the pores of the gel may be controlled.

In the presence of sodium dodecyl sulfate (SDS), the charge differences on the proteins are minimized and separations occur on the basis of molecular size (Shapiro et al., 1967; Dunker and Rueckert, 1969; and Weber and Osborn, 1969). The theory involved in this system is that reaction of the protein with 1% SDS at a relatively elevated temperature will cause unfolding of the protein exposing the charged groups of dibasic amino acids (such as the guanidino group of arginine) with which the SDS reacts to form a protective cloud. If all the charges are protected in this way the proteins of a homologous series will migrate in a manner analogous to gel filtration except

that the driving force will be the electrical attraction to the anode (+) of the negatively charged SDS enshrouded protein. The resolution of the proteins present in a mixture is thus solely on the basis of their size and shape.

Procedure: Standard Gels: Standard analytical gels, 7½% acrylamide, were run at pH 8.6 with tris-glycine, pH 8.1, as the tray buffer and bromophenol blue as the tracking dye (Steward et al., 1965). The chemicals used were obtained from Canalco. The gels were run in glass tubes of 0.6 cm internal diameter, with the running gel 2.8 ml, and the stacking gel 0.2 ml. The sample gel varied from 0.2-0.6 ml and contained 50-200 μg of protein. The gels were run at 5 milliamperes per tube for about 1 hour or until the tracking dye had migrated 5 cm. The gels were stained with 0.7% (w/v) aniline blue black in 7% (v/v) acetic acid, and destained electrophoretically in 5% (v/v) acetic acid.

1% SDS Gels: Gels 5-10% acrylamide, in which 1% SDS was incorporated, were used in estimations of the molecular weight of the proteins (Shapiro et al., 1967). Staining was with aniline blue black as in the standard gel system.

For both types of gel, the position of the dye front and

of the protein bands, and the length of the gel before and after staining were measured with a millimeter rule. The ratio of migration of the protein bands with reference to the dye front (Rf) was calculated (Weber and Osborn, 1969). The position and relative intensity of the bands in later gels was measured with a Joyce-Loebl microdensitometer equipped with an integrator.

The proteins used in the standard curves and their source are listed in figure 11 (p. 58).

Method of Breakdown of Phage Heads

Early Experiments on Breakdown of Normal Lambda Heads

Aliquots of 0.1-0.3 ml of purified lambda heads (125-260 μg protein) were treated with the appropriate reagent: 12 M 2-mercaptoethanol, 8 M urea, 6 M guanidine hydrochloride, 1% (w/v) SDS, 67% (v/v) glacial acetic acid, or 1.25 N NaOH, pH 13. The treated samples were examined by disc gel electrophoresis, using standard 7½% acrylamide gels.

Standard Analytical Treatment

The material used in the breakdown studies came from either the first ECTEOLA peak (normal lambda heads) or the

second ECTEOLA peak (petit lambda heads contaminated by less than 5% normal lambda heads). The material from the second ECTEOLA peak was used in the original treatments because it was present in larger amounts than the purified normal lambda heads from the first ECTEOLA peak. The material from the second ECTEOLA peak which had been desalted on Sephadex G-50 and showed no bands on 7½% acrylamide SDS gels (Chapter II) will hereafter be referred to as "the petit lambda preparation" (bearing in mind that contamination from soluble protein external to the head has not been detected in the gels and that normal lambda heads are present in an amount of 5% of the total number of particles).

Desalted lyophilized heads, 1 or 2 mg, were dissolved in 1 ml reagent and reacted 30-60 min (Table VII). They were then run through a Sephadex G-100 column (0.9 x 30 cm) and eluted in the appropriate buffer. Larger samples (9-13.2 mg) were dissolved in 2-3 ml reagent, reacted for 120 min and run through a 2.5 x 45 cm Sephadex G-100 column. The peaks eluting at void volume were examined with the electron microscope. The peak fractions were pooled, lyophilized and run on 5-10% acrylamide gels containing 1% SDS or on standard 7½% acrylamide gels.

TABLE VII

TREATMENTS USED TO BREAK DOWN LAMBDA HEADS CHEMICALLY

Treatment	Reaction Time Min	Temperature °C	Eluent Used on Sephadex G-100 column	Type of gel	Acrylamide %
Distilled water	30	45	0.5M urea	Standard	$7^1/_2$
8M urea	30	45	0.5M urea	Standard	$7^1/_2$
1% SDS, pH 7	30	45	0.5M urea	Standard	$7^1/_2$
1% SDS, pH 7	30	80	0.5M urea	Standard	$7^1/_2$
0.1M Citric acid-Na Citrate, pH 2.6	30	45	0.1M Formic acid-Na formate	1% SDS	5
0.1M Glycine-HCl, pH 2.6	30	45	0.1M Formic acid-Na formate	1% SDS	5
0.1M Citric acid-Na citrate, 4M urea, pH 3.2	30	45	0.1M Formic acid-Na formate	1% SDS	5
0.1M Glycine-HCl, 4M urea, pH 2.6	30	45	0.1M Formic acid-Na formate	1% SDS	5
0.1M $NaHCO_3$-Na_2CO_3, 1% SDS, pH 10.6	30, 60 or 120	45	0.15M NH_4OH, pH 11	1% SDS	5,10
0.1M $NaHCO_3$ - Na_2CO_3, 0.1% succinic anhydride, pH 10.6	30	45	0.15M NH_4OH, pH 11	1% SDS	5
1.25M NaOH	30	45	0.5M urea	Standard	$7^1/_2$

Amino Acid Analysis

Lyophilized heads, 0.5-4.0 mg, were dissolved in 0.8 ml of 6N HCl, and hydrolyzed in sealed tubes under nitrogen 18-24 hr at 110°C. The HCl was then removed under nitrogen in a boiling water bath. The samples were taken up in citrate buffer pH 2.2, and analyzed on the Beckman-Spinco amino acid analyzer 120B.

Tryptic Peptide Analysis

Lyophilized heads, 3-4 mg, were dissolved in 2% $NaHCO_3$, pH 8.0, containing 60-80 μg trypsin (Worthington) and reacted under sterile conditions for 48 hours at 37°C (Brenner et al., 1959). Following hydrolysis, the samples were lyophilized and the powder taken up in 25 μl of distilled water and spotted on a sheet of Whatman 3MM filter paper (20 x 34 cm). Electrophoresis was carried out at 20 V/cm for 2½ hours using the buffer, pyridine: acetic acid: water, 25:1:225, pH 6.4 (Ingram, 1958). The paper was then dried overnight, and subjected to ascending chromatography in n-butanol: acetic acid: water, 3:1:1 for 2½-3 hours. After drying, the chromatograms were sprayed with Ninspray, the spots developed at 80°C, and recorded before fading occurred.

Results

Standardization of Acrylamide Gels for Use in Determining Molecular Weights

Standard curves were constructed for 5 and 10% acrylamide gels containing 1% sodium dodecyl sulfate (SDS) by plotting the relative migration of the proteins (Rf) of known molecular weight against their molecular weight. The data from which the standard curves in figure 11 were drawn is enumerated in Tables VIII and IX.

The amount of stainable material detected in the gels varied for the standard proteins. Ovalbumin (125 μg) produced a total integrated value of 258 when the gel was scanned; pepsin (250 μg), a value of only 3; and cytochrome c(125 μg), a value of 192. For any one protein, however, a linear relationship exists between the amount applied to the gel and the integrated value for stainable material on the gel (Wiens and Nair, personal communication).

Chemical Breakdown of the Normal and Petit Lambda Heads from the ECTEOLA Cellulose Columns

The degree of purity of the two peaks from the ECTEOLA

Figure 11

Standard Curves for 5 and 10% Acrylamide SDS Gels

The molecular weights of the standard proteins are those listed by Dunker and Rueckert (1969): Bovine Serum Albumin (67,000), Ovalbumin (45,000), Pepsin (35,500), α-Chymotrypsin (24,500), Trypsin (23,000), β-Lactoglobulin (17,500), Lysozyme (14,400), and Cytochrome c (12,400). The bovine serum albumin, ovalbumin, β-lactoglobulin, and lysozyme were obtained from Nutritional Biochemicals Corporation; the pepsin and cytochrome c, from General Biochemicals; and the α-chymotrysin, and trypsin from Worthington Biochemical Corporation.

The error bars indicate the maximum variation for all samples of any particular protein run.

FIGURE 11

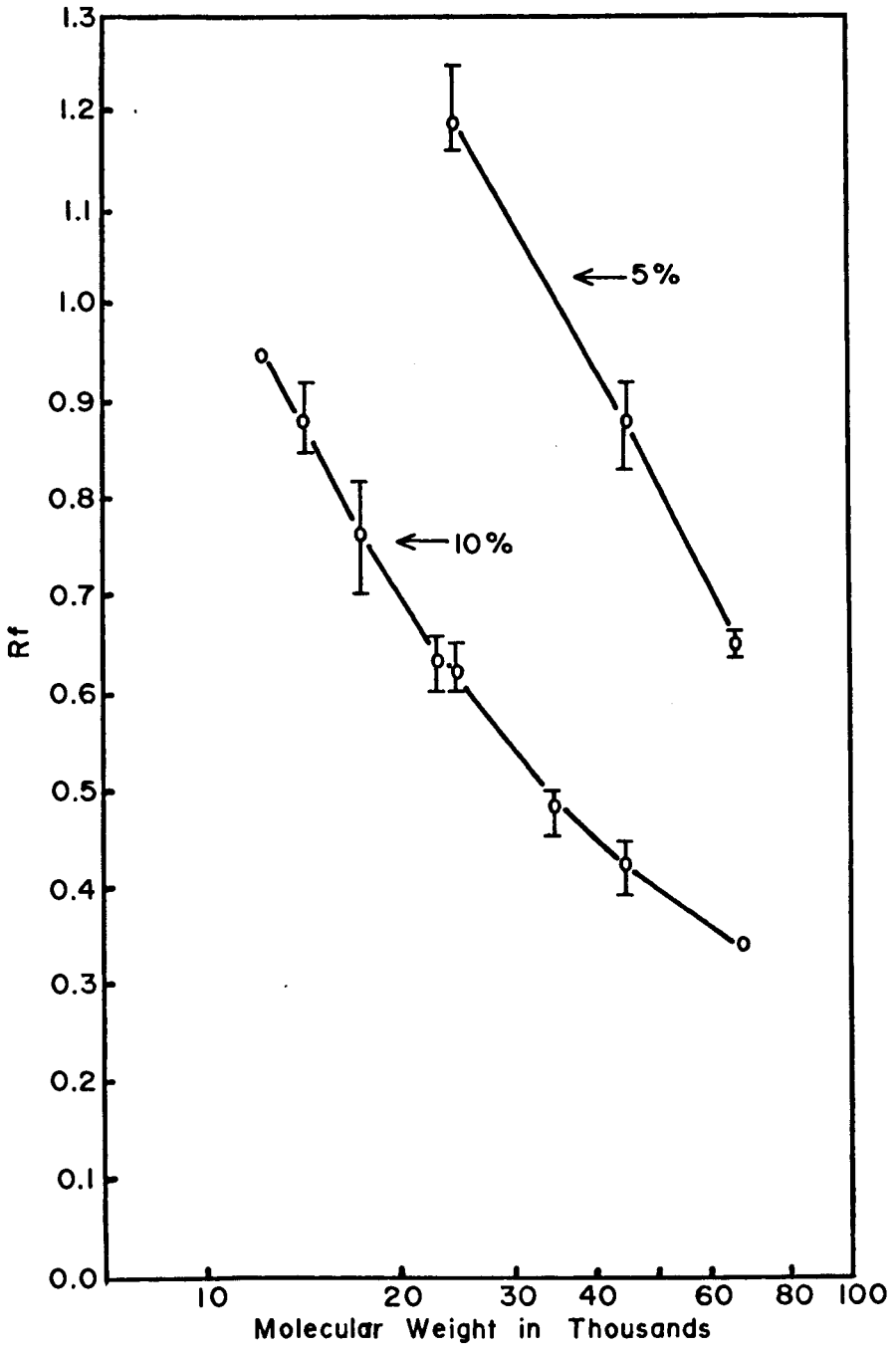

1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Rf
5%
10%
10
20
30
40
60
80
100
Molecular Weight in Thousands

TABLE VIII

RF'S OF PROTEINS OF 5% ACRYLAMIDE SDS GELS FROM WHICH STANDARD CURVES WERE CALCULATED

Molecular Weight[1] (Thousands)	Standard Protein	Sample Size (ug)	Migration Relative to Dye Front[2] Rf	
67	Bovine Serum Albumin	50	.66	
		100	.64	
		Mean	.65	
45	Ovalbumin	50	.60	.88[3]
		100	.58	.88
		100	.60	.90
		125	.57	.88
		50		.90
		125	.59	.90
		125	.69	.92
		500	.60	.84
		Mean	.60	.88
24.5	α-Chymotrypsin	50	1.25	
		100	1.18	
		100	1.16	
		Mean	1.19	
14.4	Lysozyme	50	1.06	1.2

1. The molecular weight values are those listed by Dunker and Rueckert (1969).

2. The error in measurement of the position of both the dye front and the band did not exceed 1 mm. Errors in the values of the Rf's therefore ranged from $\pm$ .03 in a value of .65 to $\pm$ .05 in a value of 1.19.

3. Other bands, presumably representing higher polymers, were present in some gels. Only the two fastest bands representing the monomer and the dimer of the standard are listed.

TABLE IX

RF'S OF PROTEINS IN 10% ACRYLAMIDE SDS GELS FROM WHICH STANDARD CURVES WERE CALCULATED

Molecular Weight 1 (Thousands)	Standard Protein	Sample Size (μg)	Migration Relative to Dye Front 2 Rf
67	Bovine Serum Albumin	125	.20 .34
45	Ovalbumin	125	.43 (.58)[3]
		100	.39 (.48-53)
		125	.34 .42
		125	.34 .41 (.48)
		125	.31 .45
		125	.36 .43 (.53)
		Mean	.33 .42
			Outer range (.48-.58)
35.5	Pepsin	125	No bands
		250	.45-.50
24.5	α-Chymotrypsin	125	.65 .83 [4]
		100	.60 .74 .85
		Mean	.62 .84
		125	.92-.99
		125	.94
		Mean	.94
23	Trypsin	125	.60 .73
		125	.66 .73
		125	.63 .96
		125	.63 .99
		Mean	.63
17.5	β-Lactoglobulin	125	.44 .76
		125	.40 .70-.82
		125	.45 .78
		Mean	.43 .76

TABLE IX (continued)

Molecular Weight 1 (Thousands)	Standard Protein	Sample Size (μg)	Migration Relative to Dye Front Rf
14.4	Lysozyme	125	.61 .88
		100	.60 .87
		125	.62 .92
		125	.58-.63 .85
		125	.61 .89
		Mean	.61 .88
12.4	Cytochrome C	125	.46 .66 .95

1. The molecular weight values are those listed by Dunker and Rueckert (1969).

2. The error in measurement of both the dye front and the position of the band did not exceed 1 mm. Errors in the values of the Rf's therefore ranged from $\pm$.03 in a value of .34 to $\pm$.04 in a value of .95.

3. Other bands, representing higher polymers were present in some gels. Only the two fastest bands, presumably representing the monomer and the dimer of the standard are listed. In the case of ovalbumin there was considerable range or spreading of the band. The positions of monomer and dimer have been calculated.

4. Some breakdown of the α-chymotrypsin standard appeared to have occurred. The faster moving bands may correspond to the B chain (MW 13,927) and C chain (MW 10,157) (Weber and Osborn, 1969).

cellulose column has been discussed in Chapter II (p. 31). In review, the first ECTEOLA peak consists of normal lambda free from contamination by either particulate matter or soluble proteins at the levels examined. The second ECTEOLA peak consists of petit lambda heads contaminated by normal lambda heads in an amount less than 5% of the total particles, other particulate matter to an even smaller degree, and no soluble proteins at the levels examined.

The results of chemical degradation of both types of head have been classified according to the degree of breakdown the various chemical procedures produced.

Treatments Producing No Breakdown of the Heads

As a control, the properties of the petit lambda preparation were examined. All the material eluted from the Sephadex G-100 column at void volume (figure 12); the electron microscope showed the head forms to be unbroken (figure 13); and a standard 7½% acrylamide gel revealed no bands.

After normal lambda heads were treated with 2-mercaptoethanol, no bands were seen on a standard 7½% acrylamide gel, also indicating that this reagent did not break down these heads to products sufficiently small to enter the gel.

FIGURE 12

EULTION PATTERN ON SEPHADEX G-100 OF THE PETIT LAMBDA PREPARATION AFTER VARIOUS TREATMENTS PRODUCING NO BREAKDOWN, PARTIAL BREAKDOWN OR EXCESSIVE BREAKDOWN

A 1 mg sample of the petit lambda preparation was dissolved in 1 ml of the appropriate reagent, reacted, applied to a Sephadex G-100 column (0.9 x 30 cm), and eluted with the appropriate eluent.

Legend	Reagent	Reaction time/ temperature	Eluent
———	distilled water	negligible/ room temperature	0.5 M urea
------	8M urea 1% SDS	30 min/45°C 30 min 45° or 80°C	0.5 M urea 0.5 M urea
—·—·—·—	0.1 M glycine-Hcl, pH 2.6	30 min/45°C	0.1 M formic acid - ammonium formate, pH 2.6
.....	0.1 M citric acid-sodium citrate, pH 2.6	30 min/45°C	0.1 M formic acid - ammonium formate, pH 2.6
××××××	1.25 N NaOH	30 min/45°C	0.5 M urea

FIGURE 12

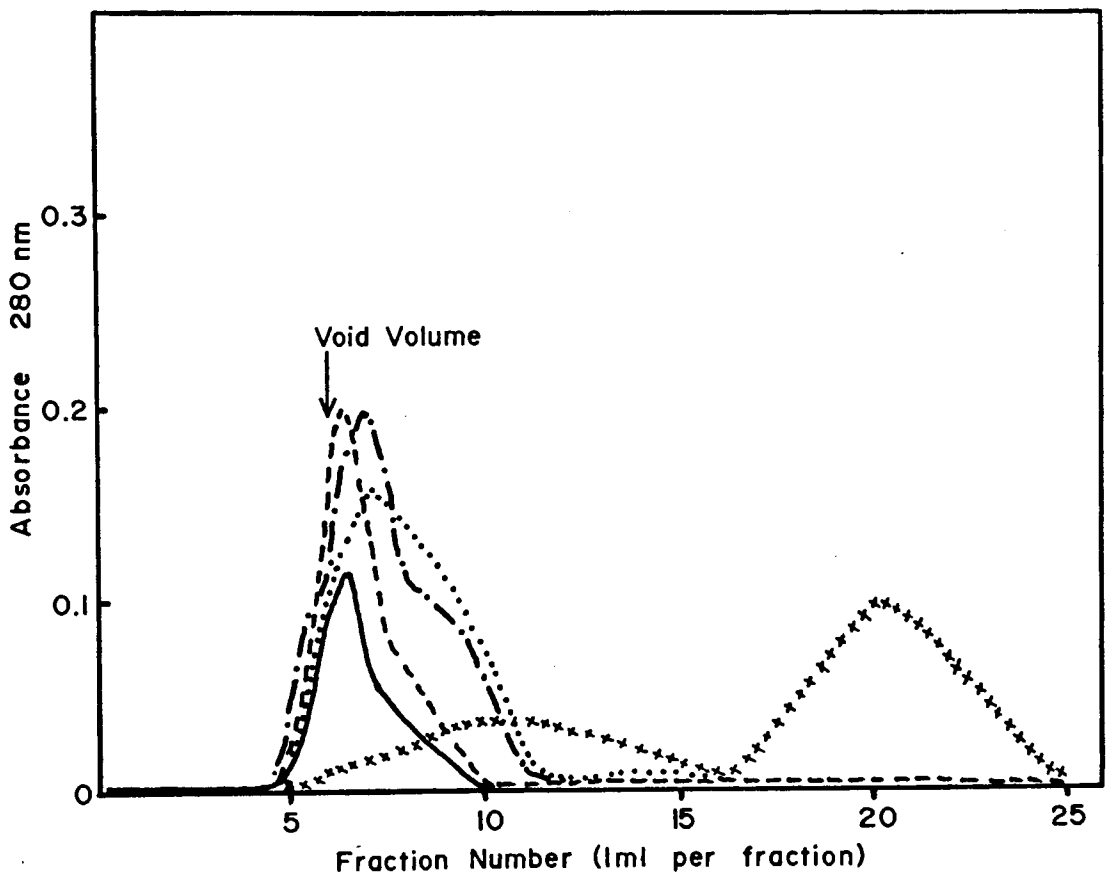

Absorbance 280 nm
0.3
0.2
0.1
0
Void Volume
5
10
15
20
25
Fraction Number (1ml per fraction)

Figure 13

Electron Micrographs of Partially Broken Down Heads from the Petit Lambda Preparation

The heads in the photographs appeared in the void volume peak from the Sephadex G-100 volumns. The heads from the petit lambda fraction (second ECTEOLA peak) were treated with the following reagents before loading on the column:

(a) Distilled Water

(b) 8M Urea

(c) Sodium Dodecyl Sulfate

(d) Citrate Buffer, pH 2.6

(e) Glycine-HCl Buffer, pH 2.6.

All magnifications are 100,000 x.

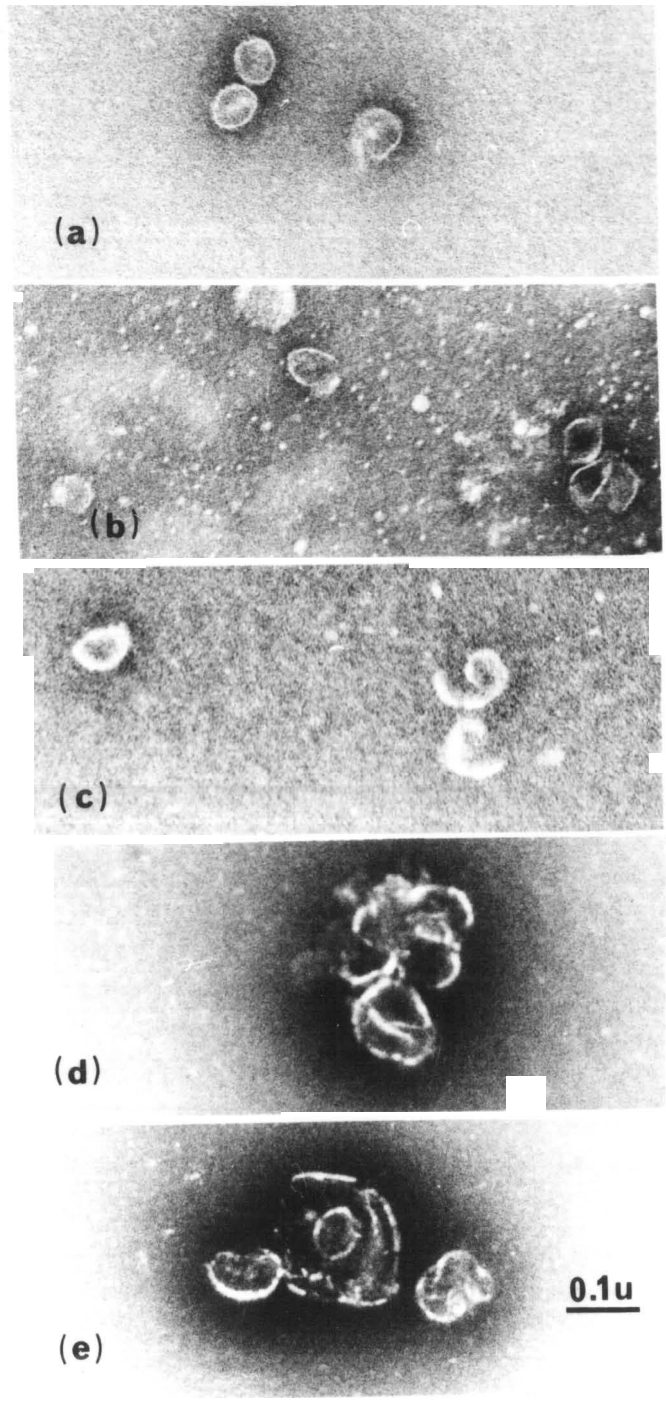


FIGURE 13

Treatments Producing Partial Breakdown of the Heads

Treatments of samples of the petit lambda preparation with urea, guanidine, SDS at neutral pH, and acidic buffers all produced partial breakdown of the heads. Most of the material in the reaction mixtures eluted from Sephadex G-100 at void volume although there was evidence of a small shoulder following the main peak in certain cases (figure 12). The material eluting at void volume resembled the petit lambda heads although the morphology had been altered to varying degrees (figure 13).

Very little material was retarded by the Sephadex G-100 column of the petit lambda samples treated with urea or SDS. However, in petit lambda preparations treated with acidic buffers, although most of the material eluted from the Sephadex as a broad peak at void volume, a marked shoulder or spreading of the peak occurred. Most of the material from the early part of this peak did not enter 5% acrylamide SDS gels. The small amount of material migrating into the gels corresponded to proteins of molecular weights of 45,000 and higher. In the shoulder of the peak, a major band was present corresponding to a molecular weight of about 45,000 (Table X).

TABLE X

ACRYLAMIDE GEL PATTERNS OF PETIT LAMBDA HEAD PROTEIN FOLLOWING SEVERAL TREATMENTS WHICH PRODUCED PARTIAL BREAKDOWN

Type of Gel	Treatment	Sephadex[1] Fraction	Sample Size	Rf of Bands	Integrated Value
Standard 7 1/2 %	Urea, 8M 30min/45°C	7 - 9	Not calculated	no bands	
Standard 7 1/2 %	1% SDS, pH 7, 30 min/45°C	7 - 9	"	.48[2]	
	30 min/80°C	7 - 9	"	.48	
SDS 5%	Glycine-HCl Buffer, pH 2.6 30 min/45°C	5 - 7	144 µg	.89/4	
		8 - 10	173 µg	.66/39	.88/39
SDS 5%	Citrate Buffer, pH 2.6	7 - 11	350 µg	.68/100	.89/20

1. From 0.9 x 30 cm Sephadex G-100 column as described in figure 12.

2. Densitometer tracings were not made on these gels.

In contrast, treatment of the normal lambda heads with urea appeared to release a low molecular weight protein (Table XI). (The bands produced may be compared with those in figure 19, p.107). Treatment with SDS alone may also have had the same result. Guanidine and 67% acetic acid did not appear to release the low molecular weight protein, but to result in the production of small amounts of a protein with a mobility corresponding to a molecular weight of 45,000.

Treatments Causing Breakdown to a Major Subunit

Two types of treatment were found to cause breakdown of both the normal and petit lambda heads (the first and second ECTEOLA peaks respectively) to a uniform major subunit. They were acidic buffers in the presence of 4M urea and alkaline buffers containing either SDS or succinic anhydride. The results of these experiments will be discussed in detail in a later section of this chapter.

In all cases, however, there were no heads recognizable when the material from the peak eluting at or near void volume from the Sephadex G-100 columns was examined in the electron microscope.

TABLE XI

ACRYLAMIDE GEL PATTERNS OF NORMAL LAMBDA HEAD PROTEIN FOLLOWING SEVERAL TREATMENTS WHICH PRODUCED PARTIAL BREAKDOWN

Type of Gel	Treatment	Rf of Bands [1]
Standard 7½%	8M Urea 30 min/45°C	.33 .41 .53* .65* .76 1.02
SDS 10%	8M Urea, 30 min/45°C +SDS Gel sample buffer	.07 .39 .63* .88*
SDS 10%	8M Urea, 30 min/45°C + SDS Gel sample buffer heated to 60°C	.07 .39 .63* .88*
Standard 7½%	Guanidine-HCl 6M	.30 .40 .52 .96 1.00
Standard 7½%	SDS, pH 7 30 min/45°C	.03 .09 .16 .29 .38 .48 .64 .80 1.0 1.09 1.4
Standard 7½%	67% Acetic acid	.30 .40 .50 (.60) (.92) 1.00

1. Densitometer tracings were not made on these gels.
   denotes the presence of a band stronger than the others in the gel.
   ( ) denotes the presence of a very faint band at this position.

Also, there were no bands present in SDS 5% acrylamide gels of the material from the void volume peak of samples treated with acid-urea (Table XVI, p. 82). The material was probably too large to enter the gel, and therefore larger than the thyroglobulin molecule (molecular weight 165,000) which is among the largest proteins able to enter these gels (Shapiro et al., 1967). After treatment with the alkaline buffers, a major band with an Rf of .88, was present in the first peak, which corresponded to a protein of molecular weight about 45,000 (Table XVI).

Treatment Producing Excessive Breakdown

The normal and petit lambda heads were treated with 1.25N sodium hydroxide at a pH greater than 13, a procedure which degraded bacteriophage T4 to its major subunit (Kellenberger, 1968). This treatment resulted in breakdown of all the heads to material which was retarded by Sephadex G-100 (figure 12). One or two fast-moving bands running at the dye front were present in standard 7½% acrylamide gels run on material treated in the same manner, but not separated on Sephadex G-100. These bands, although seen in the urea treated samples, were enhanced by the treatment with alkali. The bands were absent

in corresponding control gels lacking protein, run to check that they were not caused by chemicals present in the system. Results of these experiments are shown in Table XII.

Sedimentation analysis of aliquots of the same samples in the analytical ultracentrifuge (Spinco Model E) yielded no Schlieren peaks although the optical density indicated that there was sufficient material in them (160-600 μg/ml) to see a uniform peak if it was present.

Indications were, therefore, that the head protein was hydrolyzed to several shorter peptides and amino acids, no longer detectable as protein by aniline blue black staining, and that the entities formed were too heterogeneous in size to sediment as a single peak in the ultracentrifuge.

Breakdown of Normal and Petit Lambda Heads to their Major Protein Subunits

Experiments on Purified Normal Lambda Heads (Material from the First ECTEOLA Peak

The profile of normal lambda heads degraded with alkaline SDS and eluted from Sephadex G-100 with ammonium hydroxide, pH 11.0, is shown in figure 14. The first peak eluted just after

TABLE XII

ACRYLAMIDE GEL PATTERNS OF NORMAL LAMBDA HEAD PROTEIN AFTER BREAKDOWN WITH STRONG ALKALI

Treatment	Rf of Bands on Standard 7 1/2% Gel
1.25 N NaOH 30 min 45°C	.98 * .96 **
	1.09** 1.09* 1.09*

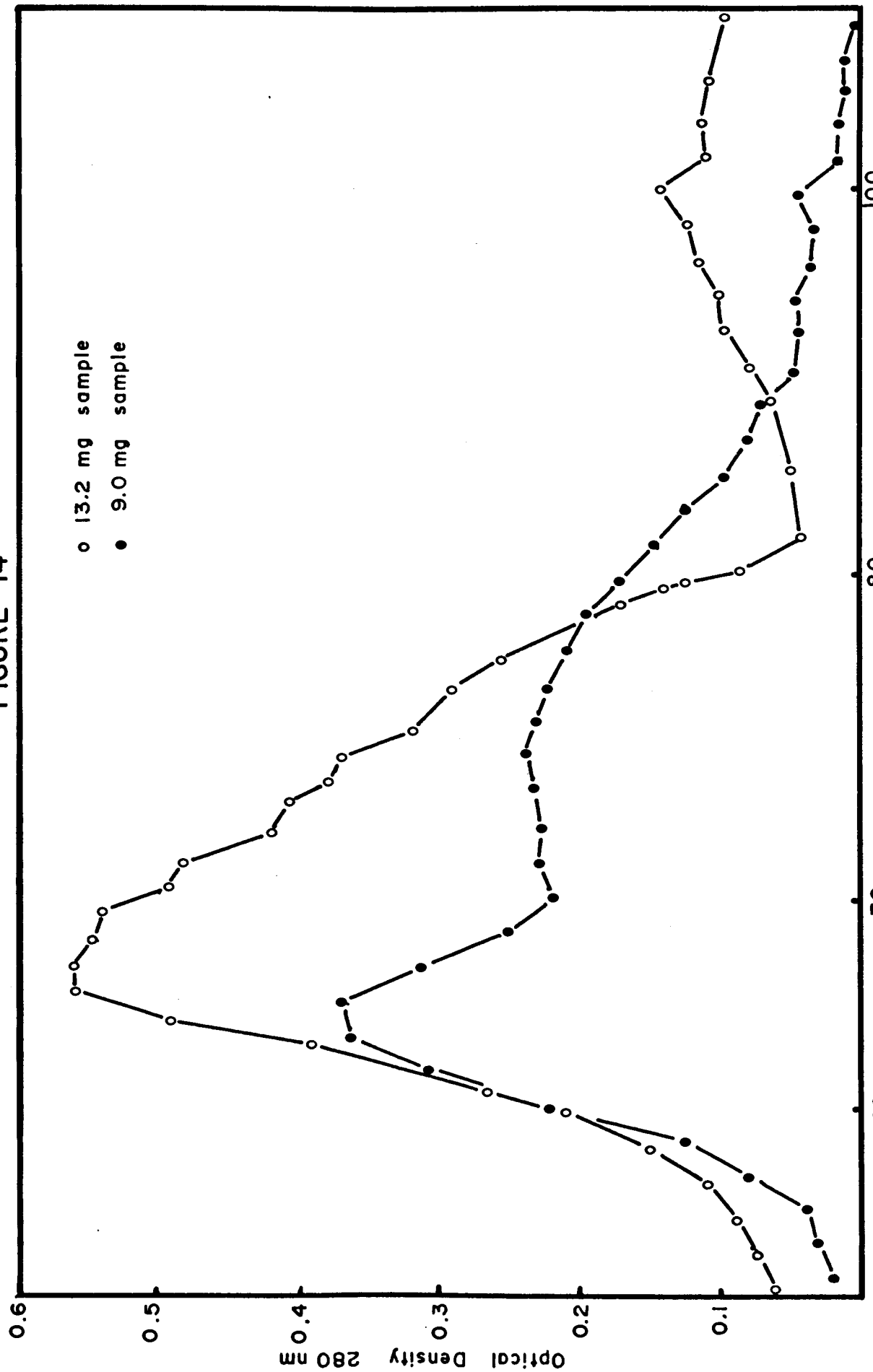
The * indicates the presence of a strong band; a ** indicates an even stronger band. These results are presented in a qualitative manner because of a lack of measuring equipment when the experiments were performed.

Figure 14

Elution Pattern on Sephadex G-100 of Large Samples of Normal Lambda Heads Degraded by Alkaline SDS

Two samples (9.0 and 13.2 mg) were dissolved in 2 and 3 ml of 1% SDS in 0.1M sodium carbonate buffer, pH 10.6. They were eluted from the Sephadex G-100 column (2.5 x 45 cm) with 0.1M NH_4OH, pH 11. The fractions were normalized so that the peaks of the two samples corresponded. (Fractions were based on drop number and the drop size varied because of the presence of SDS in some of the samples.)

FIGURE 14


o 13.2 mg sample
• 9.0 mg sample
Optical Density 280 nm
0.6
0.5
0.4
0.3
0.2
0.1
55
60
70
80
100
Normalized Fraction Number

void volume, followed by a second peak or shoulder (the degree of separation depending on the amount of sample applied to the column), and a third peak at just under two void volumes.

Material not fractionated on Sephadex as well as material from these peaks was examined by 10% acrylamide SDS gels. Densitometer tracings of the gels from one column are compared with a tracing of material not fractionated on Sephadex in figure 15. The data are tabulated in Table XIII. Both the first peak and the second peak or shoulder consist mainly of a protein which migrates with an Rf of .41. The third peak consists almost entirely of a different protein migrating with an Rf of .91. Comparison with the standard curve for the 10% acrylamide gels in figure 11 indicates that the protein migrating with an Rf of .41 has a molecular weight of approximately 45,000, while that with an Rf of .91 has a molecular weight of approximately 14,000.

The analysis of breakdown products of normal lambda heads degraded by several alkali and acid treatments on 5% acrylamide SDS gels is shown in Table XIV. Again, the presence of a major protein of molecular weight about 45,000, and its polymers, is evident, as well as the presence of a

Figure 15

Densitometer Tracings of 10% Acrylamide SDS Gels of Material Applied to and Separating as Peaks from the Sephadex G-100 Columns of Degraded Normal Lambda Heads

Densitometer Tracings are shown of various peaks from the Sephadex G-100 column depicted by the open circles (o) in figure 14.

(a) Material not fractionated on Sephadex - treated for 10 min at 65^{o}C with 1% SDS.

(b) The first peak- Fraction 64 (normalized position 64).

(c) The shoulder - Fraction 74 (normalized position 75).

(d) The third peak - Fraction 92 (normalized position 98).

FIGURE 15

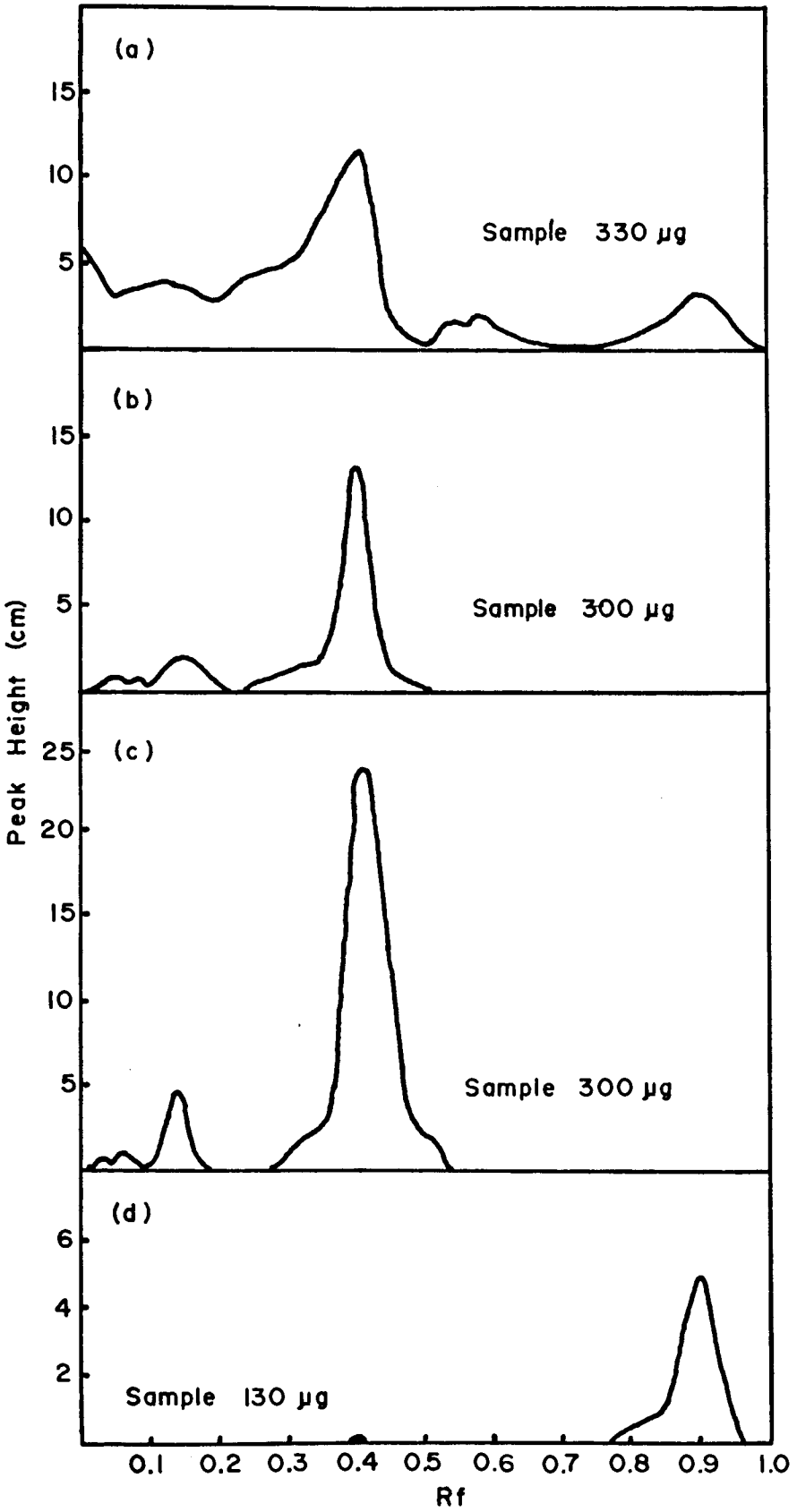

(a)
Sample 330 µg
(b)
Sample 300 µg
(c)
Sample 300 µg
(d)
Sample 130 µg
Peak Height (cm)
Rf
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

TABLE XIII

INTEGRATED VALUES OF AREAS UNDER PEAKS FROM DENSITOMETER TRACINGS OF 10% ACRYLAMIDE SDS GELS FROM PEAKS OF SEPHADEX SEPARATION OF DEGRADED NORMAL LAMBDA HEADS

Peak Number	Column Number[1]	Rf of Band <.2	.34	.40	.58	.90	Total Integrated Value
Unfractionated Sample		819 (33%)		1249 (51%)	87 (4%)	302 (14%)	2450
1	2	32 (16%)	26 (13%)	136 (70%)	- -	- -	195
2 or Shoulder	1	- -	8 (15%)	44 (85%)	- -	- -	52
	1	- -	5 (15%)	28 (85%)	- -	- -	33
	2	50 (<15%)	13 (<4%)	>260 (>80%)	- -	- -	323
3	1	- -	- -	- -	- -	20 (100%)	20
	2	-	-	.1 (2%)	- -	44 (98%)	45

Column 1 • in figure 14.
Column 2 o in figure 14.

TABLE XIV

RF'S ON 5% ACRYLAMIDE SDS GELS OF MATERIAL FROM THE BREAKDOWN OF NORMAL LAMBDA HEADS (FROM THE FIRST ECTEOLA PEAK)

Treatment	Sephadex [1] Fraction	Sample Size (µg)	Integrated Value/ Rf of band
SDS in $Na\text{-}CO_3$ buffer, pH 10.6	6 - 8	74	4/ .67 26/ .87
	9 - 11	76	14/ .86 3/ 1.21
Further treatment of fractions 6-8 preceding with SDS in $Na\text{-}CO_3$ buffer, pH 10.6	7 - 8	100	1/ .60 30/ .90
Glycine-HCl-Urea, pH 3	7 - 8	30	nil
	9 - 10	100	3/ .12 5/ .41 9/ .62 10/ .88 8/ 1.35
	11 - 14	50	<1/ .12 2/ .38 5/ .59 7/ .89 9/ 1.26
Further treatment of fractions 9-10 preceding with Glycine-HCl-Urea	6 - 7	100	2/ .63
	8 - 10	87	8/ .61 6/ .83
Further treatment of fractions 11-14 preceding with Glycine-HCl-Urea	7 - 9	43	nil
	11 - 14	60	10/ .68 9/ .81 13/ 1.18

1. The Sephadex fractions were 1 ml from a 0.9 x 30 cm column.

protein of lower molecular weight. The molecular weight of the second protein could not be estimated accurately by the 5% gels as it was out of their range (figure 11).

Repeated treatment of normal lambda heads with either the alkaline-SDS or the acid-urea reagents did not increase the number of protein types present although it did increase the amount of the faster migrating material able to enter the gels, a result indicative of further breakdown to the subunit (Table XIV).

From a comparison of the Sephadex G-100 elution profile and the relative amounts of the proteins present in the peaks as determined by densitometric tracings of the 10% acrylamide SDS gels, an estimate of the relative amounts of the two proteins present in broken down normal lambda heads may be calculated (Table XV). The high molecular weight protein comprised 63-72%, and the low molecular weight protein 6-14% of the heads eluting from the Sephadex column, which were degraded to material able to enter the 10% acrylamide gels. In comparison, the unfractionated material (figure 15) showed 56% as the 45,000 molecular weight protein, 25% as slower migrating molecules, and 15% as the low molecular weight protein.

TABLE XV

ESTIMATED RELATIVE AMOUNTS OF THE TWO PROTEINS IN BROKEN DOWN NORMAL LAMBDA HEADS

	COLUMN I			COLUMN II		
	Relative Amount in gel [1] (%)	Estimated Amount of Protein in Peak [2] (mg)	Absolute Amount of Protein (mg)	Relative Amount in gel [1] (%)	Estimated Amount of Protein in Peak [2] (mg)	Absolute Amount of Protein (mg)
Major Protein:						
Peak 1	70	2.5	1.75	65	2.4	1.56
Peak 2	85	1.9	1.62	80	5.6	4.40
Total			3.37 (72%)			5.96 (63%)
Low Molecular Weight Protein:						
Peak 3	100	0.3	0.30 (6%)	100	1.3	1.3 (14%)
Total Protein:		4.7 (100%)	3.67		9.3 (100%)	7.26

1. From Table XIII.

2. The peak or shoulder was resolved by eye and the $O.D._{280}$ of the relevant fractions totalled to get an estimate of the amount of protein in the peak.

Both proteins were run on 7½% acrylamide standard gels. Densitometer tracings are shown in Chapter IV (p.107). Both proteins are similar in this system, each having bands with Rf's of .53 and .66.

Experiments on Petit Lambda Heads (Material from the Second ECTEOLA Peak)

The elution profile of petit lambda heads on Sephadex G-100 after alkaline SDS degradation is shown in figure 16. As in the breakdown of the normal lambda heads (figure 14), there was a peak eluting just after void volume with a shoulder, but, in contrast to normal lambda, there was no later peak.

The broken down petit lambda heads were studied by disc gel electrophoresis on 5 and 10% acrylamide gels containing 1% SDS. An aliquot of the entire peak after either alkaline-SDS or acid-urea breakdown was applied to the gel. Densitometer tracings of 5 and 10% gels show that petit lambda consists mainly of a single protein (figure 17). Results of other gels are given in Table XVI. Comparison of the lambda protein bands in the 5 and 10% acrylamide SDS gels with the standard curves (figure 11, p. 58) showed that the major protein had a

Figure 16

Elution Pattern on Sephadex G-100 of Large Samples of Petit Lambda Heads Degraded by Alkaline SDS

Petit lambda heads (from the second ECTEOLA peak), 12.0 mg. were dissolved in 2 ml of 1% SDS in 0.1M sodium carbonate buffer, pH 10.6. They reacted for 2 hr at 45°C. They were eluted from the Sephadex G-100 column (2.5 x 45 cm) with 0.1M NH_4OH, pH 11, and fractions of approximately 1 ml were collected.

FIGURE 16

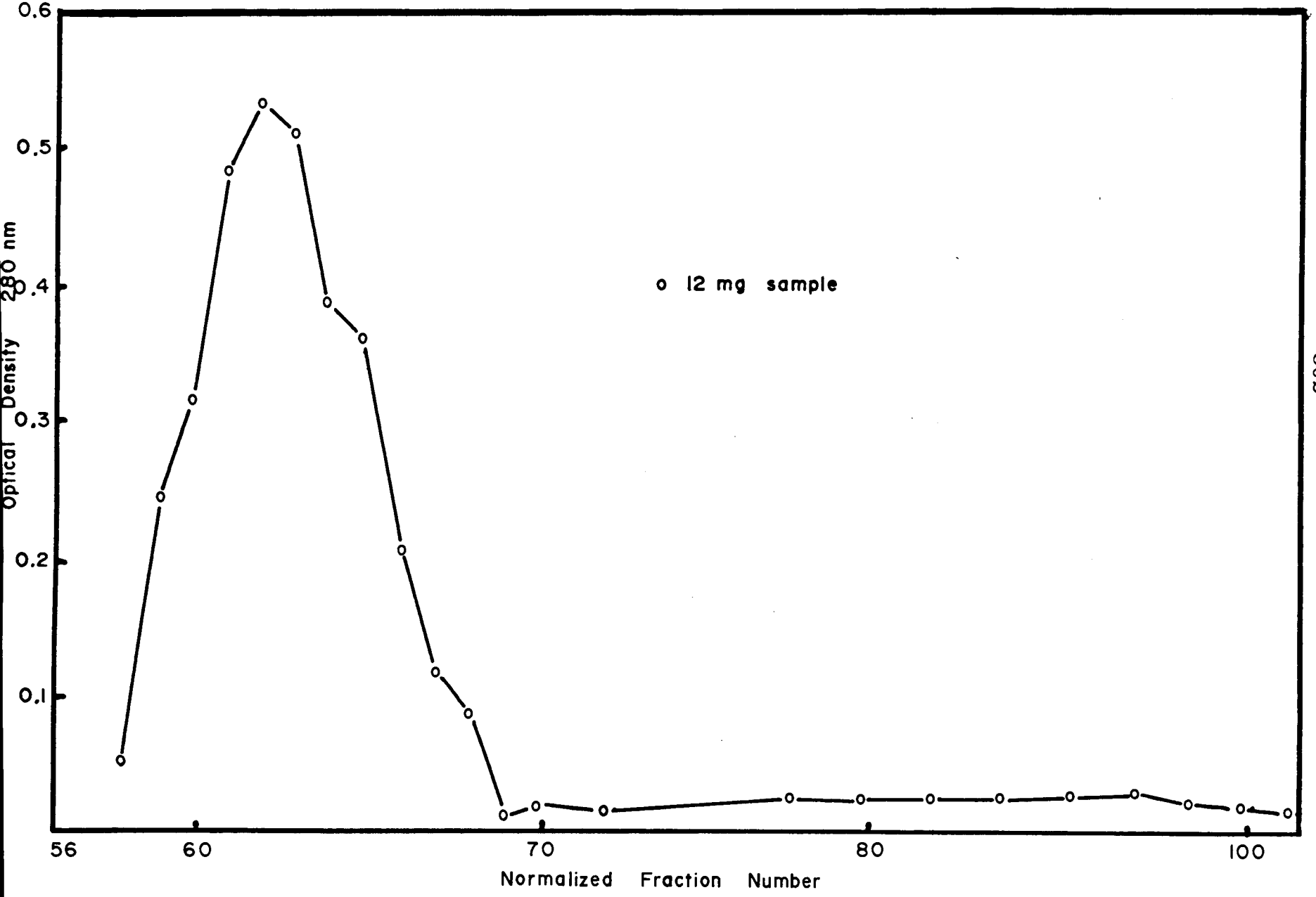

0.6
0.5
0.4
0.3
0.2
0.1
Optical Density 280 nm
o 12 mg sample
56
60
70
80
100
Normalized Fraction Number
808

Figure 17

Densitometer Tracings of Acrylamide Gels of Material Not Fractionated and Fractions from the peak after Sepadex G-100 Separation of Proteins of Degraded Petit Lambda Heads

(a) SDS Breakdown: The petit lambda heads were treated for 10 min at 65°C with 1% SDS, and run on a 10% acrylamide gel.

Rf of Peak	Integrated Value (%)
.02	14.5
.13	22.5
.43	56
.58	7

(b) Alkaline-SDS Breakdown: The petit lambda heads were treated for 1 hr at 45°C with 1% SDS in sodium carbonate buffer, pH 10.6, and run on a 5% acrylamide gel.

Rf of Peak	Integrated Value (%)
.13	7
.34	2
.45	6
.61	20
.86	55
.93	7

(c) Acid-Urea Breakdown: The petit lambda heads were treated for 30 min at 45°C with Glycine-HCl buffer, pH 2.6, containing 4M Urea, and run on a 5% acrylamide gel.

Rf of Peak	Integrated Value (%)
.57	11
.85	83
.91	6

FIGURE 17

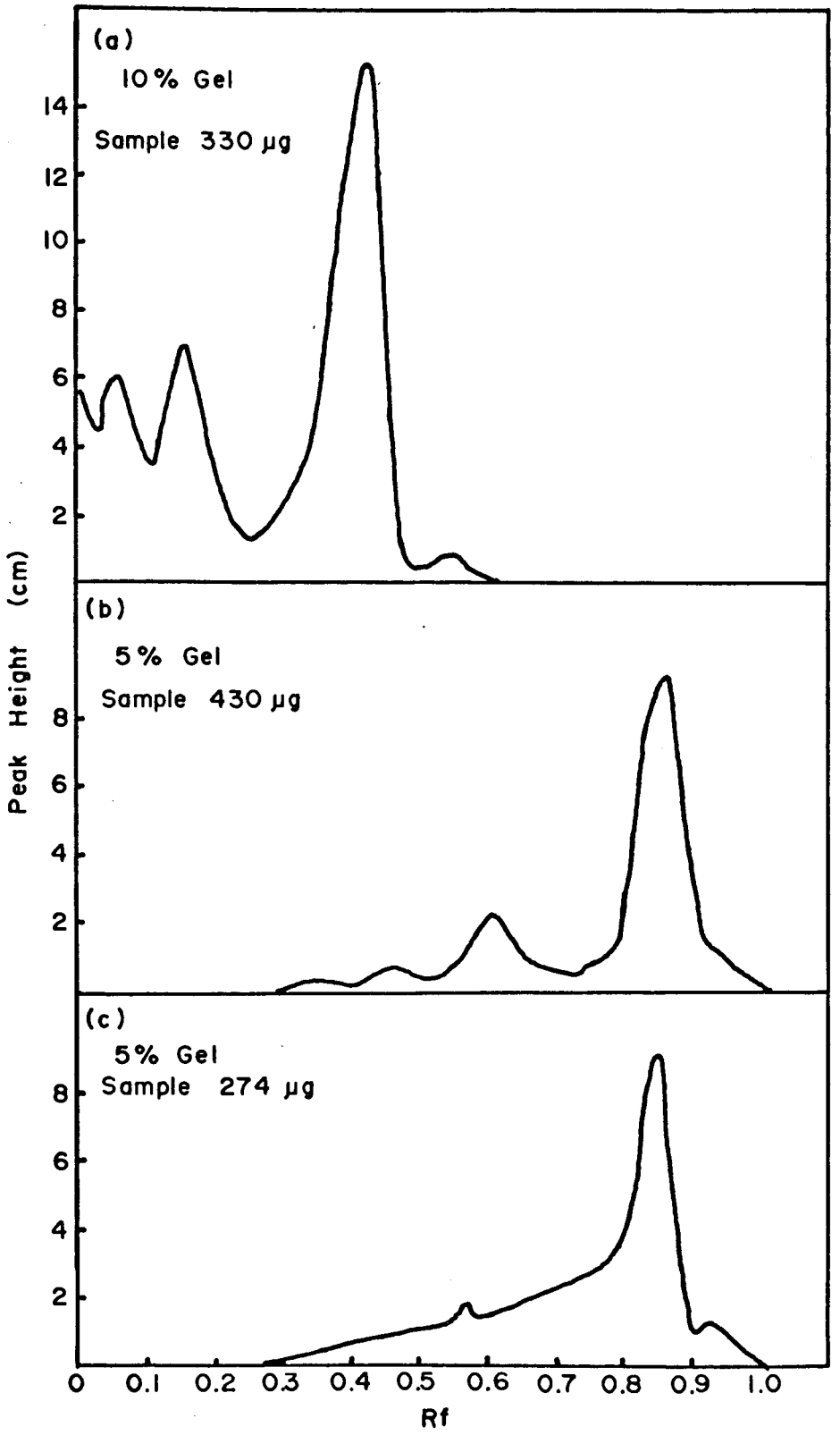

(a)
10% Gel
Sample 330 µg
(b)
5% Gel
Sample 430 µg
(c)
5% Gel
Sample 274 µg
Peak Height (cm)
Rf
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
2
4
6
8
10
12
14

TABLE XVI

RF'S ON 5% ACRYLAMIDE SDS GELS OF MATERIAL FROM THE BREAKDOWN OF PETIT LAMBDA HEADS (FROM THE SECOND ECTEOLA PEAK)

Treatment	Sephadex Fraction [1]	Sample Size (μg)	Integrated Value/ Rf of band
Citrate-Urea Buffer, pH 3.2	6 - 7	120	nil
	8 - 11	200	33/ .51
			75/ .75
			24/ .86
Glycine-HCl-Urea, pH 2.6	4 - 5	137	nil
	6 - 9	274	23/ .57
			170/ .85
			12/ .91
SDS in $Na\text{-}CO_3$ Buffer, pH 10.6	7 - 9	430	14/ .13
			9/ .34
			13/ .45
			42/ .61
			112/ .86
			14/ .93
	10 - 11	127	5/ .88
	12 - 16	144	1/ .88
Succinic Anhydride in $NaCO_3$ Buffer, pH 10.6			
Run I	8 - 9	340	13/ .88
Run II	7 - 8	200	1/ .53
			4/ .88

1. The Sephadex fractions were 1 ml from a 0.9 x 30 cm column.

molecular weight of 45,000, although it was preceded by a small peak corresponding to a molecular weight of about 40,000 in the case of the 5% gels. In the 10% gels, the faster peak corresponded to a molecular weight between 30,000 and 35,000. The slower migrating peaks probably represent aggregation products of the major protein subunit (see discussion, p. 92).

To follow the course of the breakdown of the petit lambda heads with alkaline SDS, samples were taken after various reaction times and examined on SDS 10% acrylamide gels. The results, tabulated in Table XVII, indicate that breakdown of the petit lambda heads is complete as soon as they dissolve in the reagent. The major protein peaks comprised about 80-90% of the total protein present in petit lambda heads. Other, slower migrating bands could represent aggregation products of the major protein.

Amino Acid Analysis and Tryptic Peptide Mapping of Normal and Petit Lambda Heads

The amino acid composition of normal and petit lambda heads and their components is given in Table XVIII. The values for petit lambda and the major (45,000 molecular weight) protein are very similar, the variation of analyses within

TABLE XVII

SEQUENTIAL BREAKDOWN OF PETIT LAMBDA (MATERIAL FROM THE SECOND ECTEOLA PEAK)

Molecular Weight (Thousands)	on 10% Acryl-amide SDS Gel	Integrated Value 0 hr	%	2 hr	%	4 hr	%	8 hr	%	12 hr	%
> 100	.14-.17	15	20	10	15	13	24	9	6	4	9
45	.41-.45	37	50)	33	50)	26	48)	30	52)	23	50)
			)80		)92		)76		)85		)91
30	.51-.57 (.55)	22	30)	23	42)	15	28)	19	33)	19	41)
Total Integrated Value		74		66		54		58		46	

The sample size in each case was 0.1 ml, corresponding to 400 mg of protein in the original reaction mixture.

TABLE XVIII

AMINO ACID COMPOSITION OF NORMAL AND PETIT LAMBDA HEADS AND NORMAL LAMBDA HEAD COMPONENTS

Amino acid	Normal Lambda[1]		Petit Lambda[1]		Major Protein[1]		Minor Protein[2]	
	Molar Ratio	Residues	Molar Ratio	Residues	Molar Ratio	Residues	Molar Ratio	Residues
Lysine	.052	5	.052	7	.058	10	.058	7
Histidine	.010	1	.008	1	.006	1	.016	2
Arginine	.052	5	.064	8	.054	9	.023	3
Aspartic acid	.101	10	.112	14	.127	21	.094	16
Threonine	.098	10	.065	8	.076	13	.151	20
Serine	.063	6	.066	8	.068	11	.082	10
Glutamic acid	.108	11	.104	13	.120	20	.077	10
Proline	.052	5	.055	7	.062	10	.061	8
Glycine	.082	8	.079	10	.078	13	.086	11
Alanine	.118	12	.091	11	.102	17	.168	21
Cysteine	-	-	-	-	-	-	-	-
Valine	.063	6	.069	9	.075	12	.044	6
Methionine	.015	1	.014	2	.011	2	.011	1
Isoleucine	.032	3	.030	4	.032	5	.026	3
Leucine	.071	7	.080	10	.078	13	.068	8
Tyrosine	.038	4	.043	5	.031	5	.004	1
Phenylalanine	.040	4	.044	6	.046	8	.043	6
Tryptophan[3]	(.99%)	1	(1.3%)	1	N.D.		N.D.	
Average Minimum Molecular Weight	12,675	100	15,952	124	21,251	170	15,180	133

1. The individual runs, of which the molar ratio values for normal lambda, petit lambda, and the major protein, are means are given in Table XIX.

2. Value from a single run.

3. Tryptophan was determined on duplicate runs by the method of Spies and Chambers (1948).

N.D. Not Determined.

petit lambda fractions being as great as the variation between the petit lambda and major protein fraction. The individual runs are shown in Table XIX. Comparison of the amino acid composition of the normal and petit lambda heads reveals an excess of threonine and alanine in the normal lambda preparations. This excess can be accounted for by the excess of threonine and alanine in the amino acid composition of the minor protein associated with the normal lambda head. The average minimum molecular weight of the lambda head proteins, based on the amino acid compositions has been determined and included in Table XVIII.

A composite map of the tryptic peptides found in normal and petit lambda heads is shown in figure 18. Comparison of four sets of tryptic peptide maps has revealed a maximum of 35 peptides to be common to the normal and petit lambda preparations. The minimum number of spots counted on a map of petit lambda was 29. In addition, a maximum of 6 other spots was seen on the normal lambda maps. The number of distinct spots was dependent on the amount of material applied to the paper; smearing (incomplete resolution) accompanied the identification of weakly stained, well separated spots on heavily loaded chromatograms. The value of 35 peptides, assigned as common to normal and petit heads is thus a

TABLE XIX

INDIVIDUAL DETERMINATIONS OF AMINO ACID COMPOSITION OF NORMAL LAMBDA HEADS, PETIT LAMBDA, AND THE MAJOR PROTEIN

Amino Acid	Normal Lambda			Petit Lambda		Major Protein		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 1	Run 2	Run 3
Lysine	.052	.050	.055	.053	.051	.058	.041	.057
Histidine	.011	.010	.008	.037	.008	.006	.007	.006
Arginine	.058	.048	.051	.060	.068	.053	.059	.054
Aspartic acid	.102	.093	.108	.114	.109	.129	.113	.125
Threonine	.102	.101	.091	.066	.064	.074	.089	.073
Serine	.060	.065	.065	.066	.066	.066	.067	.070
Glutamic acid	.102	.107	.113	.086	.122	.113	.110	.126
Proline	.062	.043	.051	.047	.063	.028	.061	.063
Glycine	.082	.083	.081	.080	.078	.076	.080	.079
Alanine	.115	.126	.112	.094	.088	.106	.126	.099
Cysteine	-	-	-	-	-	-	-	-
Valine	.045	.068	.066	.069	.069	.075	.078	.076
Methionine [1]	.004	.001	.0003	.008	.011	.003	-	.001
Methionine [2]	(.037)	(.016)	(.015)	(.030)	(.022)			
Isoleucine	.029	.032	.033	.031	.030	.031	.037	.034
Leucine	.059	.076	.077	.080	.081	.075	.079	.081
Tyrosine	.044	.038	.033	.044	.042	.031	.008	.010
Phenylalanine	.038	.040	.041	.044	.044	.042	.046	.046

1. Methionine peak only.

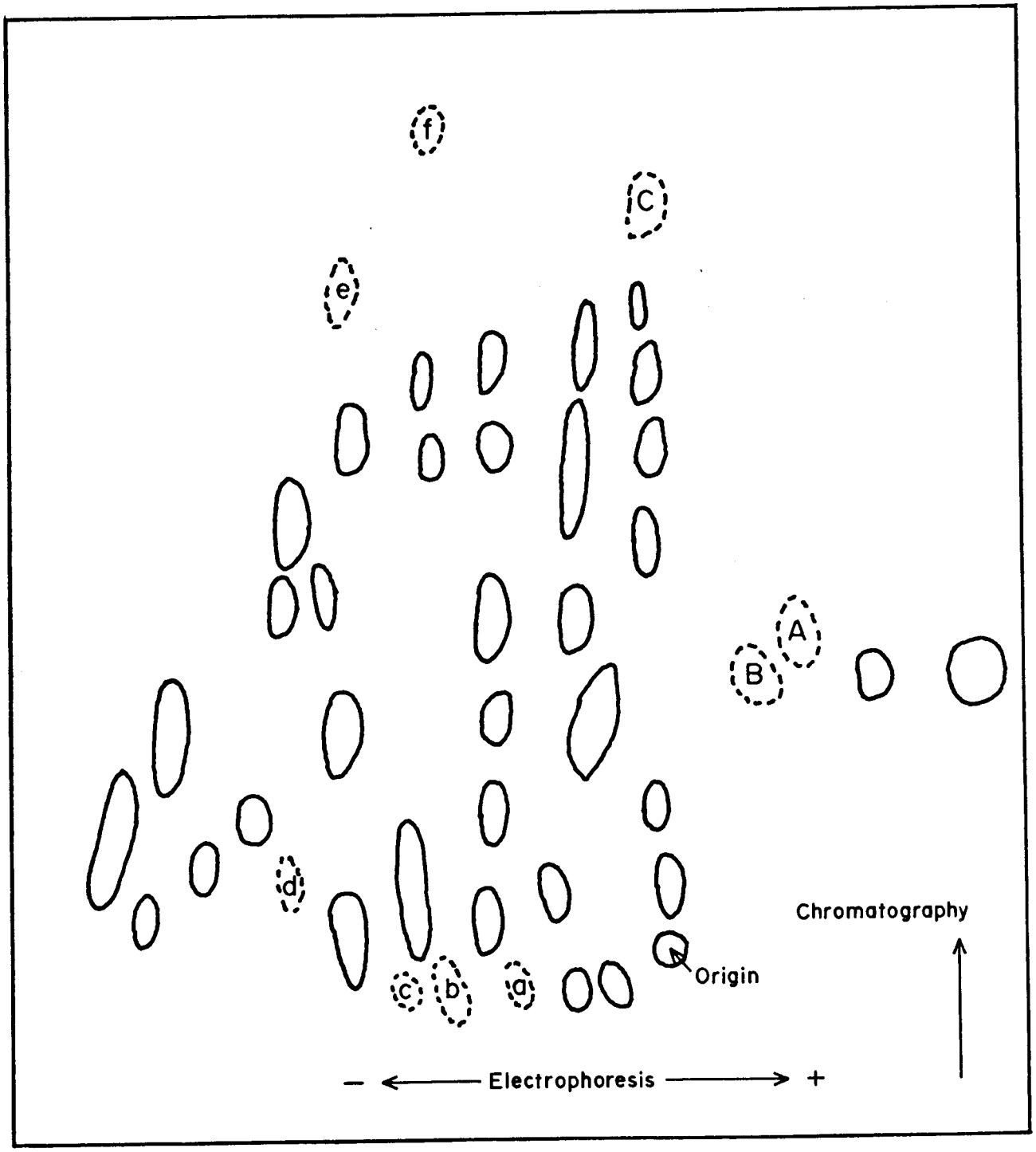
2. Includes peaks for methionine sulfones and sulfoxones.

Figure 18

Tryptic Peptide Map of Hydrolyzed Normal and Petit Lambda Heads

The peptides with solid outlines are common to both normal and petit lambda heads (35). The dotted peptides came either from the normal lambda head hydrolysate (lower case, 6 peptides) or from the petit lambda head hydrolysate (upper case, 3 peptides).

FIGURE 18



composite number based on all peptide maps made.

The molecular weight of the major protein of both the normal and petit lambda heads is estimated to be 45,000 on the basis of migration is SDS acrylamide gels. Since the petit lambda head has been shown to be essentially a single protein (figure 17), an estimate of the molecular weight based on the amino acid composition and the number of tryptic peptides is possible. The average minimum molecular weight obtained from the amino acid composition of petit lambda is 15,952. The estimated number of lysine residues is 7 and of arginine residues 8, a total of 15 potential sites of cleavage by trypsin. A maximum of 16 peptide spots is expected rather than the 29-35 identified on the peptide maps. Therefore, the molecular weight of the major protein of petit lambda is estimated to be 3 x 15,952 or about 48,000.

Since the maximum number of peptide spots is not usually detected by ninhydrin spraying (Hull et al., 1969), a factor of 3 corresponding to a theoretical total of 48, rather than a factor of 2, corresponding to 32, was used. In any event, the maximum number of observed spots, 35, exceeded 32, ruling out the factor of 2.

The amino acid composition of the isolated major protein indicates a minimum molecular weight of 21,251. By anology with the petit lambda calculations, a molecular weight estimate

for the major protein monomer of normal heads is 42,500. The two values are in fair agreement with each other and with the estimates from SDS gels; therefore, the molecular weight of about 45,000 for the major protein of both petit lambda and normal lambda heads is reasonable.

The minor protein, accounting for about 15% of the protein of the normal lambda heads, has a molecular weight of about 14,000 based on SDS acrylamide gel migration. From the amino acid composition, a minimum molecular weight of 15,180, which agrees well with the value from electrophoresis, was calculated (Table XVIII).

Discussion

Many reagents have been used in the preceding experiments in the attempts to degrade normal and petit lambda heads to their smallest uniform subunits. All the treatments resulting in any degree of breakdown (with the exception of the strong alkali) have released a protein of molecular weight 45,000. This protein migrates at approximately the same position as the ovalbumin standard (molecular weight 45,000) on 5 and 10% acrylamide SDS gels. The estimate may not, however, be representative of the molecular weight, since gel filtration techniques separate on the basis of molecular diameter, and the SDS gels are a variation of gel filtration, rather than

electrophoresis. The molecular weight estimate of 45,000 by disc gel electrophoresis is, however, in good agreement with the value of 42,500 found by the combined techniques of amino acid analysis and tryptic peptide mapping. The value of 45,000 is close to that of 47,000 (Villarejo et al., 1967) but somewhat lower than that of 55,000 (Dyson and van Holde, 1967), attributed to the head protein on the basis of studies using whole lambda ghosts.

A band corresponding to a molecular weight between 30,000-35,000 in 10% gels and 38,000-40,000 in 5% gels was found after treatment of petit and normal lambda heads with acid and urea, neutral SDS at 80°C, and SDS at pH 10.6. This band appeared only when the 45,000 molecular weight fraction was present and may be a version of this protein folded differently due to the rather harsher treatments of the combined reagents or elevated temperature. This band may thus represent a different conformational form of the 45,000 molecular weight protein. Buchwald et al. (personal communication) indicate that the major protein of the head of bacteriophage lambda has a molecular weight of 37,500 by disc gel electrophoresis. Their method of breakdown, heating in a boiling water bath in the presence of SDS and mercaptoethanol, may have produced a change in molecular configuration of the

major protein.

Bands corresponding to smaller entities were also seen in the gels. After several of the treatments, two fast moving bands were present in the standard 7½% acrylamide gels. They correspond to the two fast bands of the low molecular weight protein of molecular weight about 14,000 (figure 19, p.107). The protein of low molecular weight was seen only in samples of broken down normal lambda heads.

Also present in many of the gels from partially degraded normal or petit lambda heads was a band corresponding to a molecular weight of about 70,000 (Tables XI, XIV and XVI). In the case of petit lambda heads (Table XVI), breakdown resulted mainly in the production of the 45,000 molecular weight protein, although some 70,000 molecular weight protein was present. A band, at the same position in the ovalbumin standard gel, was probably caused by an ovalbumin dimer (Shapiro et al., 1967). The 70,000 molecular weight lambda protein may likewise represent dimerization of the 45,000 molecular weight components. Changes in conformation during dimerization, producing a molecule smaller than two separate monomer units coupled, could result in an incorrect molecular

weight estimate. The molecules may assume the "38,000 molecular weight" conformation prior to dimerization.

In the case of normal lambda heads, repeated treatment decreased the 70,000 molecular weight band and resulted in relative increases of both the 45,000 and 14,000 molecular weight bands (Table XIV). The results may indicate the presence of a complex within the capsid of the 14,000 and 45,000 molecular weight proteins, in addition to the possible dimer of 45,000 units. The persistence of the 70,000 molecular weight band in certain instances may, however, indicate the presence of yet another minor protein of molecular weight about 70,000.

The most prevalent components produced by degradation of the lambda heads have now been discussed. What are the forces which act to hold them together? The measure of success in the degradation of the heads by various reagents may give an insight into the types of bond contributing to head structure. Urea and SDS, known to break hydrogen bonds, when acting alone have little effect on the morphology of the heads although they release the minor protein from normal lambda heads. It is interesting to note that Buchwald et al. (personal communication) reported using urea to remove petit lambda from

their normal lambda head preparations and coincidentally detected no minor protein in the normal lambda head. In our system, the reagents used indicate that hydrogen bonding has little effect on the integrity of either the normal or petit lambda capsids, but dissociates the 14,000 molecular protein from the normal lambda head.

More important in holding the capsid subunits together are salt bonds. Alkaline and acidic solutions at pH's beyond the secondary dissociation constants of the dicarboxylic and dibasic amino acids result in destruction of the morphology of both types of head with the release of the 45,000 molecular weight subunit. Complete breakdown occurred rapidly with combined salt and hydrogen bond breaking reagents indicating that hydrogen bonds also have a role in maintaining head structure, but they cannot be broken unless they are first made accessible by disrupting the salt bonds.

What are the possible roles of the 45,000 and 14,000 molecular weight subunits in the lambda heads? The 45,000 molecular weight protein is certainly the major structural protein of the normal lambda head and possibly the only structural protein present in the petit lambda head. Gel

data suggest that, in petit lambda, the 45,000 molecular weight subunit molecules could form dimers which could then aggregate by self assembly to produce the icosahedral petit lambda heads.

The function of the 14,000 molecular weight protein is not so easily defined. It could be a minor structural protein located on the surface of the normal lambda capsid. The minor protein, which appears to be more basic (carrying a higher positive charge, Villarejo et al., 1967), would explain the differential affinity of ECTEOLA cellulose for the two types of head. Normal lambda, containing the minor protein, is less tightly bound to the N^+ groups of the anion exchanger and petit lambda, more tightly bound.

At 42°C, or upon removal of magnesium, tubular head forms of lambda can convert from those with a petit lambda type of packing to the normal lambda head type of packing (Kemp, unpublished). The conversion could be explained if the longheads contained an abnormal low molecular weight protein which assumed a more normal shape in the absence of magnesium. In the presence of magnesium, the subunits containing the low molecular weight protein would appear identical to the 45,000 dimers and thus, on assembly, the two would assume the petit

lambda type of packing. In the absence of magnesium the difference of the two types of subunits would be manifested as a change to the normal lambda type of packing. Magnesium concentration has been found to affect the conformation of protein subunits in the β-galactosidase system (Ullman and Monod, 1969).

The amount of the 14,000 molecular weight protein, if it is a structural component on the capsid surface, would be expected to be constant; but the amount of the minor protein varies in different preparations (figure 14). If the minor protein is situated on the surface of the head, it is broken off relatively easily by urea without affecting the head morphology. The 14,000 molecular weight protein is thus probably not an integral part of the capsid structure but may be a morphopoietic factor present on the surface of the capsid but not necessary for its structural integrity once it is formed (Kellenberger, 1968).

Alternatively, the minor protein might not be present in the capsid of normal lambda heads, but be situated inside the head as a core around which the DNA is wound (Kaiser, 1966). Such cores, if attached to the DNA, would be expected to be

lost with it rather than remaining within the heads. The density of the heads described in the experiments, 1.27 g/cm^3 (figure 10), indicated there was no DNA remaining within them. Also, no evidence of cores has been detected in the samples by the electron microscope, the electron micrograph shown in figure 4 being typical of the appearance of the purified normal lambda heads. If, however, the minor protein of the normal lambda head is core protein, its presence in limiting amounts would result in the formation of normal lambda heads when it is present and petit lambda when it is absent. It could form some type of morphopoietic core such as Kellenberger et al. (1968) have postulated for the T-even phages.

The minor protein does, as pointed out earlier, affect the surface properties of the normal lambda head. The presence of a morphopoietic core within the heads could affect the conformation of the protein (45,000 molecular weight form or 38,000 molecular weight form) prior to aggregation to form a normal or petit lambda head. The folding of the protein subunits in turn could affect the surface charge and antigenic properties of the whole head. The change in molecular conformation could also explain the longhead

conversion on the basis of a change of the major protein from the "45,000" to the "38,000" molecular weight form or *vice versa*.

If the 14,000 molecular weight protein forms part of an inner core, the experimental results show that the capsid is resistant to urea degradation while the core can be broken down when urea penetrates the capsid.

The 45,000 molecular weight protein is the major structural component of both normal and petit lambda heads. The 14,000 molecular weight protein may have either a surface or an internal or core role in the formation of the normal lambda head, but the experimental results do not enable distinction between the two. In a normal lysate, the petit lambda heads could result from the low molecular weight protein being present in limiting amounts, condensation of both types of protein leading to the functional normal lambda head, and self-assembly of the major protein subunits, to the nonfunctional petit lambda.

Summary and Conclusions

(1) Both normal and petit lambda heads consist of the same major protein monomer as determined by disc gel electrophoresis of the products of chemical degradation, and also by tryptic peptide mapping.

(2) The major protein has a molecular weight of about 45,000 as determined by SDS disc gel electrophoresis. Amino acid analysis of the isolated major protein from normal lambda heads as well as amino acid analysis of petit lambda heads in conjunction with tryptic peptide mapping gave a value of approximately 43,000 for the molecular weight.

(3) A minor protein of different amino acid composition is present in normal lambda heads only. It has a molecular weight of about 14,000 as determined by disc gel electrophoresis and about 15,000 by amino acid analysis. The function and position of the minor protein within the normal lambda head is not elucidated by our experiments. It may be present in the normal lambda capsid lattice conferring surface charge and antigenic properties or it may be the protein of the core around which it is postulated that DNA is wound.

(4) Salt bonds are of prime importance in maintaining the structural integrity of both normal and petit lambda heads. Hydrogen bonds play a minor role, but salt bonds must first be broken in order to make them accessible to the reagents. Hydrogen bonds appear to act in binding the minor protein loosely to the normal lambda heads.

CHAPTER IV

THE GENE DETERMINING THE MAJOR PROTEIN SUBUNIT OF BACTERIOPHAGE LAMBDA HEADS

Introduction

There are no head-like structures in lysates of induced Escherichia coli cells lysogenic for bacteriophage lambda when mutations are present in lambda genes E, or possibly F (Kemp et al., 1968; Mount et al., 1968). The major proteins present in the normal and petit lambda heads have been identified by disc gel electrophoresis (Chapter III). Therefore, a comparison of the gel patterns of cell-free extracts prepared from wild type, absolute defective and conditionally lethal mutant strains should indicate which of the two genes actually controls the production of the major protein.

Gels of cell-free extracts are contaminated by bacterial proteins. The extraneous bands should be diminished by

reacting the cell-free extract with an antiserum prepared against the homologous non-lysogenic host bacterial strain. Cell-free extracts of conditionally lethal mutants pretreated with purified antisera were used in an attempt to identify phage specified protein bands by disc gel electrophoresis.

Materials and Methods

Bacterial and Phage Strains

The bacterial and phage strains used are listed in Table XX.

Preparation of Cell-Free Extract

Cell-free extracts of various bacterial strains and bacteria lysogenic for lambda mutants were prepared following irradiation of the cultures as described in Chapter II. The cells were further incubated for an optimal time as determined by lysis curves and then concentrated by centrifuging in the cold for 20 min at a g max of 10,400. The pellets were washed twice with sterile distilled water to remove the culture medium and finally resuspended in 10 ml sterile distilled water. The cells were disrupted in a French press at a pressure of 10,000 lb/in^2. The bacterial

TABLE XX

BACTERIAL AND PHAGE STRAINS

Strain	Genotype	Source	Comment
Bacteria:			
594	$pm_r^-(Su^{II-})$ sm	R. Thomas	E. coli 594, a streptomycin resistant non-permissive host for sus mutants of λ (Weigle, 1966).
Y10-1	pm^+	C.R. Fuerst	A cured derivative of Y10 and a permissive host for λ (Thomas et al., 1967; Mount et al., 1968).
Bacteriophage:			
E ts 21		C.R. Fuerst	A temperature sensitive mutant in gene E deficient in head production derived from Y 10 (Mount et al., 1968).
Sus E4 Sus F96B		C.R. Fuerst	Sus mutants of lambda deficient in head production (Campbell, 1961).
Sus E214 Sus F204		R. Thomas	Sus mutants of lambda deficient in head production (Thomas et al., 1967).

Only the strains not already listed in Table I have been listed here.

debris was then removed by centrifuging 15 min at a g max of 4,000.

Lysis Curves

Lysis curves were done on the mutant strains mentioned in Table XX, in order to determine the optimum interval between induction of bacteriophage synthesis by ultraviolet light and the preparation of the cell-free extract. Lysis occurred at 60-70 min in temperature sensitive strains at 42°C and at 80 min in defective strains, 80-90 min in sus strains in W3350, and 100 min in sus strains in 594 at 37°C. The cell-free extract was prepared just before lysis, when optimum production of phage entities had occurred and concentration of whole cells rather than proteins was possible.

Preparation of Antiserum to Host Bacterial Strains

Cell-free extracts of E. coli W3350, 594, and Y 10-1 were prepared for use as antigens. Injections of 1-2 mg total protein (as determined by the method of Lowry et al., 1951) were given intramuscularly to rabbits at weekly intervals for 8 weeks and a booster injection 6 weeks after termination of the regular schedule. The serum was then harvested at weekly

intervals and pooled if it gave a positive ring test with the homologous antigen. A purified gamma globulin fraction was prepared by separation of the serum proteins on a DEAE cellulose column (Sober et al., 1956). This fraction gave a positive ring test when reacted with an equivalent amount of antigen protein (i.e., 1 mg of antibody protein precipitated approximately 1 mg of antigen protein.)

Treatment of the Cell-Free extracts Prior to Gel Analysis

Two series of gels were run on cell-free extracts of the mutants.

Antigen only. Gels were run using antigens only (200 μg protein in the sample).

Antigen excess and alkaline SDS treatment. Antigen (approximately 2 mg) was treated with 0.1 ml purified gamma globulin (0.9-1.1 mg/ml) in a total volume of 2 ml for 60 hr at 4°C. The reaction mixture was centrifuged 30 min at a g max of 4,000 rpm and the supernatant was then lyophilized. The protein was taken up in 1 ml of 1% SDS in 0.1 M $NaCO_3$ buffer, pH 10.6, and reacted for 30 min at 45°C. The excess reagent was removed by dialysis overnight into 0.01 M sodium phosphate buffer, pH 7. Samples, 0.2 ml, containing

approximately 400 μg protein, were run on the gels.

Disc Gel Electrophoresis

Standard 7½% acrylamide gels and SDS 5% acrylamide gels were run as outlined in Chapter III.

Results

The major bands produced by breakdown products of normal lambda heads on standard 7½% acrylamide gels are shown in figure 19. They give an indication of the possible products of phage formation that might be detected in cell-free extracts of induced lysogenic bacterial strains.

Effect of Irradiation on Non-Lysogenic Bacterial Strains

Non-lysogenic strains given an "inducing" dose of irradiation and left to incubate a further 50 min prior to preparation of the cell-free extract gave gel patterns essentially identical to unirradiated controls.

Treatment of Cell-free Extracts with Antiserum

Phage specific bands in the gels of the cell-free extracts from suppressor sensitive mutants of lambda, normal

Figure 19

Densitometer Tracings of Standard 7½% Acrylamide Gels of the Two Proteins Found In Normal Lambda Heads

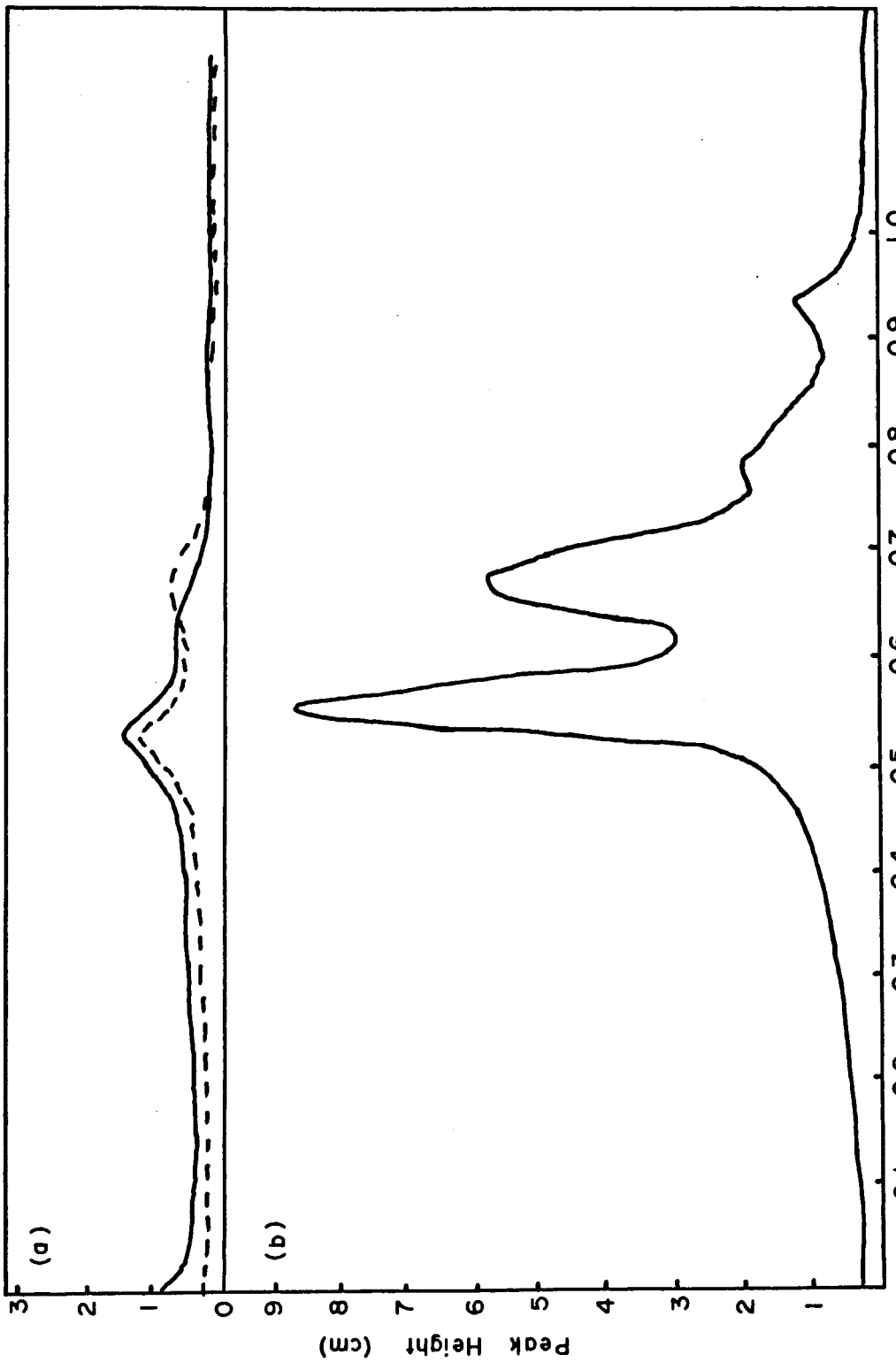
(a) The 45,000 Molecular Weight Protein (Samples both 37 μg)

Rf	Integrated Value of Peak	
	Gel 1	Gel 2
.53	20	39
.66	11	22

(b) The Low Molecular Weight Protein (Sample 100 μg)

Rf	Integrated Value of Peak
.55	96
.68	79
1.78	28
.93	7

FIGURE 19



and deficient in head production were obscured because of contamination by bacterial proteins. Some diminution of bacteria-specified bands in the nonlysogenic host strains was achieved by reacting the cell-free extract with an antiserum to the host strain prior to separating the proteins on the gels (Table XXI). However, under the conditions used, the antiserum did not react to any great degree with the bacterial antigen in the cell-free extracts of mutant phage lysogens.

Proteins Detected in Defective Mutants of Lambda

A series of cell-free extracts was prepared of induced bacterial strains lysogenic for mutants of lambda defective and normal in head production. Densitometer tracings of standard 7½% acrylamide gels of the antigen from the defective head mutant are shown in comparison with that from the background nonlysogenic strain and a head producing mutant (Figure 20). Titers of lysates of these mutants are shown at the bottom of Table XXII to give an estimate of the number of wild type phage present. In figure 20, the bands migrating with Rf's of .16 and .82 are enhanced in T 1 (defective in gene M but normal with respect to head formation) and absent in T 61 (defective in gene E). Bands corresponding to both the major

TABLE XXI

NONLYSOGENIC BACKGROUND BACTERIAL STRAINS AND EFFECTS OF TREATMENT WITH ANTISERUM ON GEL BANDS

Strain	Treatment	Rf's of Bands seen in the gels					
E. coli W3350	Antigen only		.43	.48	.59	.73	.78	.95
	Antigen excess, alkaline SDS			.46	.61	.71		.92
E. coli 594	Antigen only	.27	.30	.52		.63	.80	
	Antigen excess, alkaline SDS	.23		.48		.63		

The treatments are described in the text under materials and methods.

Figure 20

Densitometer Tracings of Standard 7½% Acrylamide Gels of Mutants Defective in Head and Tail Production

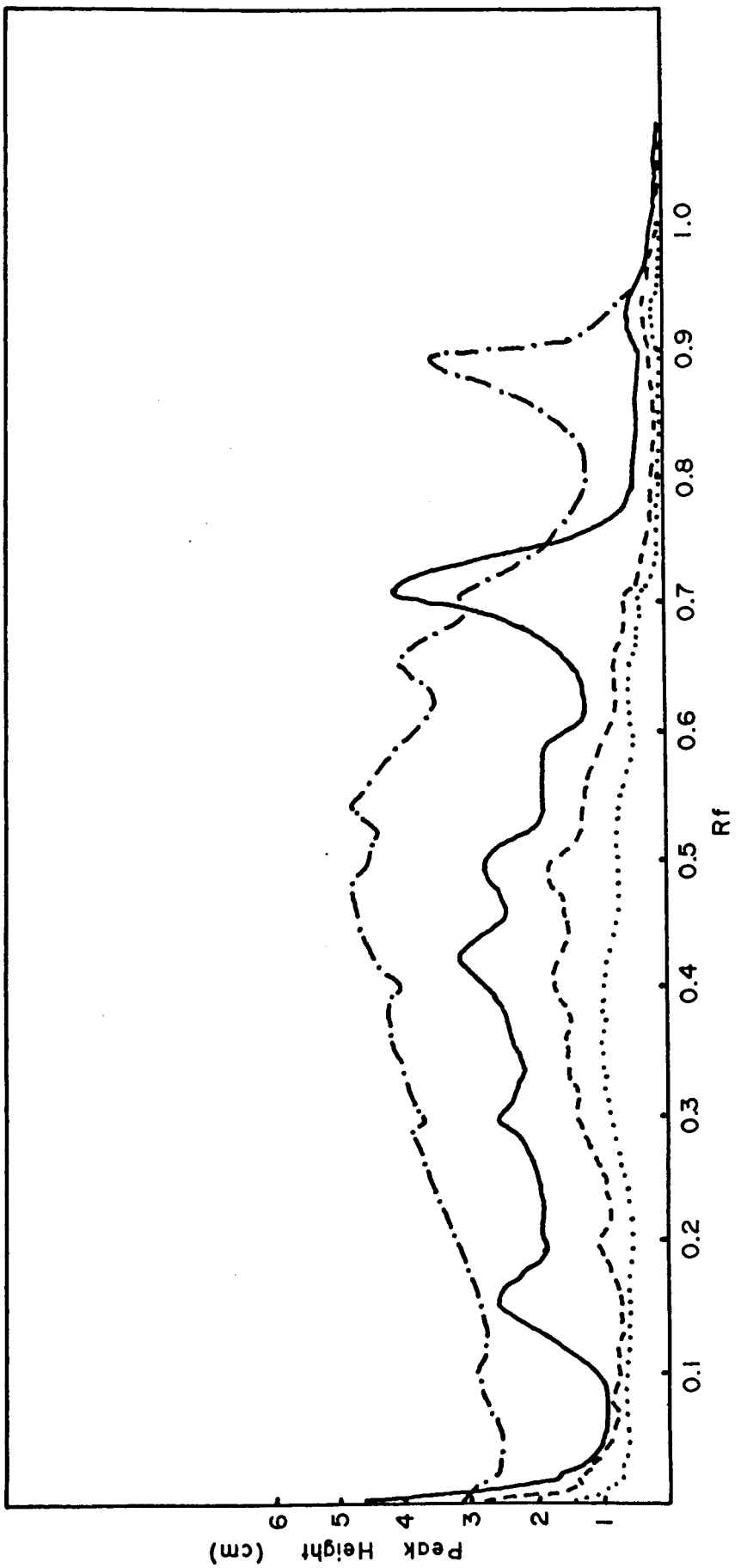
Legend	Strain	Lambda Phenotype	Sample Size[1] (μg protein)
.....	Y 10-1	Nonlysogenic	670
_____	T 1	Heads only	710
------	T 61	Tails only	700
-.-.-.-	T 7[2]	Petit lambda and tails	400

1. It is seen from the tracing that the same amount of protein did not enter all the gels.

2. The T 7 gel was run in a different experiment and thus cannot be compared quantitatively with the other tracings.

The titers of wild type phage present in the lysates of T 1 and T 61 are indicated at the bottom of Table XXI.

FIGURE 20



and minor proteins were found in a defective mutant in gene A, but which produces mainly petit lambda particles (T 7, Kemp et al., 1968).

The experiment was repeated with another set of cell-free extracts and the results were similar. In this case, all of the dye fronts were not equivalent so that the densitometer readings could not be superimposed. The Rf's were calculated and the areas under peak regions integrated and subtracted from those of the background strain. The results are shown in Table XXII. Densitometer tracings of the 7½% acrylamide standard gels run with sample gels indicated that about half the material applied remained in the sample gels (total integrated value for sample gel was equivalent to total integrated value for running gels).

From Table XXII, it is seen that, in the gene E mutant which fails to synthesize lambda heads, no protein in excess of that present in the background bacterial strain was seen running at Rf's of .53 and .66, the positions expected for the major protein (figure 19). Protein was present which migrated at .82 and at the dye front, two of the positions expected for the minor protein (figure 19) but not at the other position, .93, expected. In the gene M mutant, bands

TABLE XXII

INTEGRATED VALUE OF BANDS IN STANDARD 7 1/2% ACRYLAMIDE GELS OF CELL-FREE EXTRACTS OF DEFECTIVE LYSOGENIC STRAINS OF BACTERIOPHAGE LAMBDA

Rf of Bands	STRAIN AND PHENOTYPE				
	Y 10-1 Background	T 1 Heads		T 61 Tails	
	Total	Total	Net	Total	Net
.13-.15	137	277	140	86	Nil
.20	70	63	Nil	108	38
.27-.28	62	64	Nil	79	17
.33	37	103)[1]	116	70	42
.37-.42	159	209)		163	Nil
.48	90	37	Nil	48	Nil
.51-.57	121	180	60	115	Nil
.63-.68	85	173	138	82	Nil
.70-.76	79	101	22	71	Nil
.82-.88	76	105	29	98	22
.98-1.00	45	47	Nil	59	14
Total Integrated Value [2]	961	1359	505	988	133
Background Total		854		855	
Sample Size (µg)	240	252		245	

1. The region from .33-.42 in T 1 was present as a single peak with shoulders so the values have been bracketed in this case.

2. The total integrated value indicates that different amounts of material entered the gels. The background total (due presumably to background bacterial protein) is relatively constant; the excess protein entering the gels is thus phage-specified.

   The wild type phage present in the mutant lysate is indicated by the titer on a lawn of the permissive host strain, RC600:

   T 1 - 5.0 - 9.8 x 10^2 p.f.u./ml
   T 61 - 3.5 x 10^3 p.f.u./ml

corresponding to both the major and minor proteins were present at .53, .66, and .82.

If the cell-free extracts were pretreated with alkaline SDS (Chapter III) prior to running on the gels, qualitative examination showed similar results except that all the material migrated farther into the gels. The slower moving bands were reduced, but produced the same faster bands.

Proteins Detected in Suppressor and Temperature Sensitive Mutants of Lambda

Gels were run on cell-free extracts of lysogens carrying suppressor and temperature sensitive mutants of lambda. Densitometer tracings of protein produced by mutants in head production (genes E and F) and a mutant in gene M as a control producing heads, are shown in figure 21. The titers of the phage mutants used in these experiments is shown in Table XXIII to give an estimate of wild type phage, and hence contaminating head protein, present.

The results of the mutants in gene E are variable, but all strains show relatively little increase in the major protein bands at .53 and .66 (if the amount of protein entering

Figure 21

Densitometer Tracings of Standard 7½% Acrylamide Gels of *Sus* and *ts* Mutants Deficient in Head Production

(a) Background Bacterial Strain

——— W3350

(b) Gene E Mutants: phenotype tails only

_____ ts 21 (E)

--------- *sus* E4

..... *sus* E214

(c) Gene F Mutants: phenotype tails only

_____ *sus* F96b

--------- *sus* F204

(d) Gene M Mutant: phenotype Heads only

_____ *sus* M87

FIGURE 21

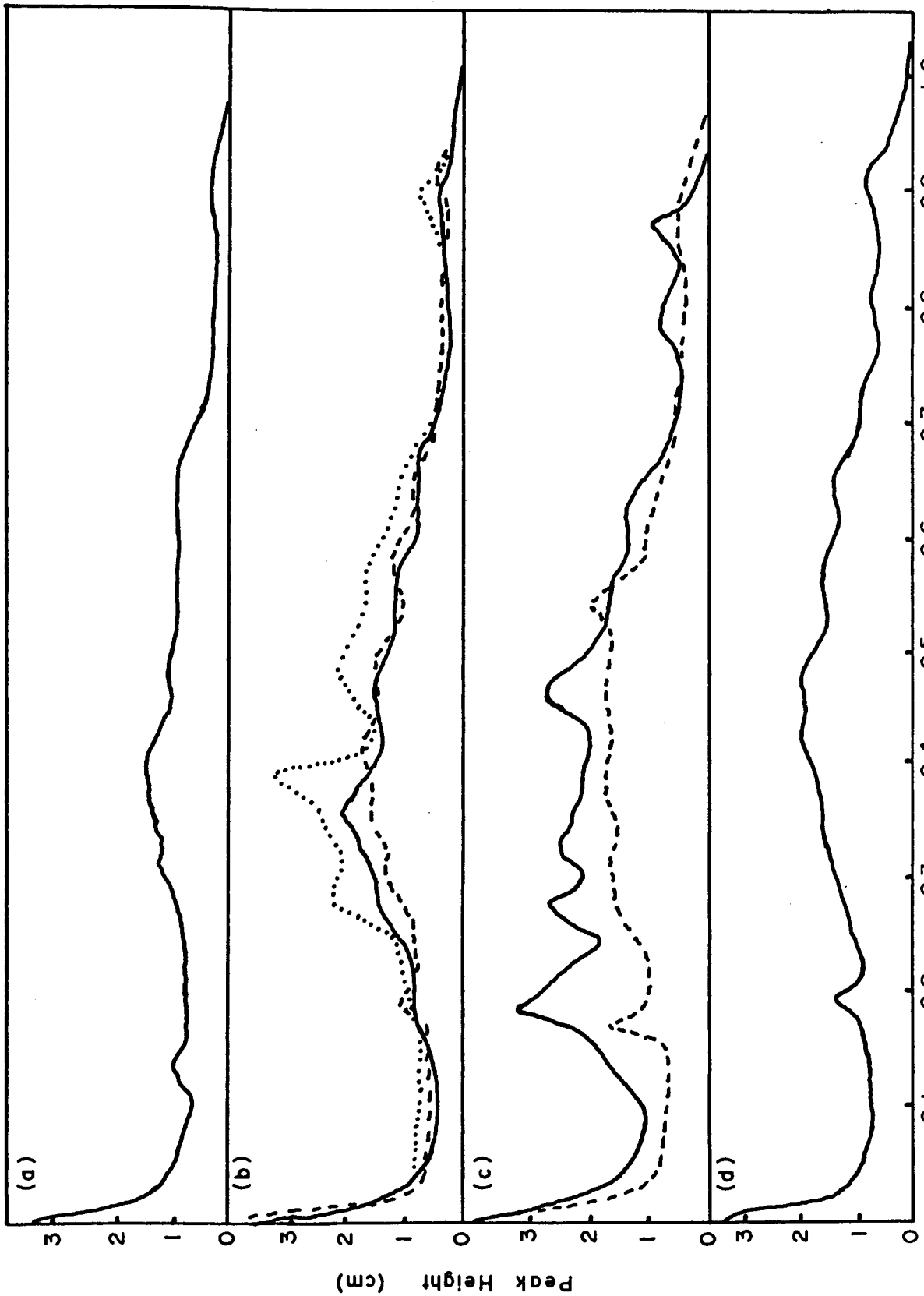


TABLE XXIII

TITERS OF LAMBDA PHAGE MUTANTS IN HEAD PRODUCTION UNDER PERMISSIVE AND RESTRICTIVE CONDITIONS

Strain	Permissive Host (*E. coli* RC600)	Restrictive Host (*E. coli* W3350)
W3350 (λ *sus* E214)	1×10^6 1×10^6	2.36×10^4 3.6×10^3
W3350 (λ *sus* E4)	1.66×10^5 1×10^4	6.1×10^3 3.2×10^3
W3350 (λ *sus* F96b)	7×10^4 1×10^5	8×10^2 2.7×10^3
	Permissive Host (*E. coli* RC600)	Restrictive Host (*E. coli* 594)
594 (λ *sus* E214	1.6×10^5	3.6×10^3
594 (λ *sus* F204)	7.6×10^4	5.8×10^3
	Permissive Temperature (30^oC)	Restrictive Temperature (42^oC)
	(Titered on *E. coli* RC600)	
W3350 (λE ts 21)	1.0×10^6	6.5×10^3

the gels is standardized so that sus E214 has the same background level). No band was produced at .80 and only a slight peak occurred at .20 in sus E4. The results are thus similar to those for the gene E defective mutant.

The tracings of the gene F mutants had a somewhat different profile. Sus F96b, which is leaky (Weigle, 1966), showed the presence of bands of Rf's .20, .53, and .66, but very little at .80 or .93, indicative that it could make the major protein which was then aggregating to higher polymers.

The gene M mutant used as a control showed bands in all the regions expected for both the major and minor proteins. The bands were not marked, presumably because the protein polymerized into larger aggregates which did not enter the gel.

Discussion

The results indicate that the major protein subunit of the head is controlled by gene E, rather than gene F. Bands corresponding to the major protein subunit were not found to an appreciable extent in the gene E mutants, but were found in the gene F mutants. The .80 band corresponding to the minor protein also does not appear to be present in the gene E mutants

and the results are inconclusive regarding its presence in the gene F mutants. The same conclusion can be reached concerning the .93 band.

Thomas et al. (1967) have ascribed a stoichiometric function to gene E, indicating that it would code for the major structural protein of the head; while they ascribed a catalytic role to the gene F product indicating that it had a regulatory role in head morphogenesis. The presence of the major protein in the gene F mutants and its absence in the gene E mutants (figure 21) would substantiate this assumption. The absence of the minor protein in both gene E and gene F mutants indicates that the control of its production is more complex. No conclusion can be reached on the basis of the data presented as to whether the function of gene F is to control the formation of the minor protein.

Gene A, on the basis of the defective mutant studied, does not seem to affect the production of either protein present in the capsid, although mainly petit lambda heads are produced (figure 20). This would indicate that something more than the major and minor proteins is required for normal lambda head synthesis. The other genes involved in head production, the

W-B-C polar group (Parkinson, 1968) and genes A and D, have not as yet been given definite functions in head morphogenesis, although the conversion of the tubular structures (Chapter III) may indicate that the gene determining the minor capsid protein is found in the W-B-C region. Salzman and Weissbach (1967) have indicated that genes A and D may have a role in making the lambda infectious.

Summary

Indications, on the basis of the disc gel electrophoresis data, are that gene E determines the major protein (of molecular weight 45,000) present in the capsid of bacteriophage lambda. No conclusion can be made from the data as to the role of gene F.

CHAPTER V

THE RELATIONSHIP BETWEEN NORMAL AND PETIT LAMBDA HEADS

Two types of head are found in a lysate of Escherichia coli lysogenic for normal bacteriophage lambda. The normal lambda heads may be filled with DNA and unite with a tail to form infectious bacteriophage. The smaller, rounder petit lambda heads, although they are three times as numerous as the normal lambda heads in the lysate of λ sus M87, do not contain DNA and never have a tail.

In this thesis certain aspects of the relationship between these two kinds of particle have been elucidated. The two types of head can be separated on ECTEOLA cellulose as a result of the differences in the surface charges they carry. Petit lambda is less positively charged and is therefore bound more tightly to the anion exchange cellulose than is normal lambda. Both normal and petit lambda heads are similar in that they are composed of the same major protein.

Normal lambda heads, free from DNA, however, contain two

proteins. The major protein, comprising about 80% of the total, has a molecular weight of 45,000 as determined by SDS acrylamide gel electrophoresis, and amino acid analysis and tryptic peptide mapping. The minor protein, about 15% of the total, has a different amino acid content and has a molecular weight of about 14,000 as determined by disc gel electrophoresis and amino acid analysis.

Only the major protein has been found in petit lambda heads. They have a tryptic peptide map similar to that of normal lambda heads, and the molecular weight of their protein subunit is about 45,000.

The minor protein may determine whether normal or petit lambda heads are formed. Its presence in limiting amounts could explain the formation of nonfunctional petit lambda heads as the "by-products" of normal head synthesis. The minor protein may be present in the normal lambda capsid lattice, causing the surface charge differences between the two types of head. Alternatively, the minor protein may be that of the core around which DNA is thought to be wound in normal lambda heads. The surface charge and structural differences of the normal and petit lambda heads could be the consequence of

either a different conformation or a different packing arrangement of the major protein in the presence or absence of a core.

At the outset of the thesis three questions were asked, of which two can now be definitely answered:

(1) Normal and petit lambda heads consist of the same major protein subunit.

(2) The normal lambda head contains a major protein and a minor protein while the petit lambda head consists only of the major protein subunit. The major protein subunit has a molecular weight of about 45,000 and the minor protein, about 14,000. The two differ in their amino acid composition.

The third question can be tentatively answered:

Disc gel electrophoresis data indicate that gene E and not gene F is the structural gene for the major protein of both types of bacteriophage lambda head.

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CURRICULUM VITAE

Name: Gael Harling Huntley (nee Stott)

Place and Year of Birth: Victoria, B.C. 1937.

Education:
Victoria College, Victoria, B.C. 1954-56
University of British Columbia, 1956-59
B. Sc. (First Class Honours in Biochemistry and Zoology) 1958
M. Sc. (Zoology) 1959
Oxford University 1960-62
B. Sc. (Oxon.) 1962

Experience:
Teaching Assistant
Department of Biological Sciences
Simon Fraser University, 1965-70

Teacher
Oxford High School for Boys
Oxford, England, 1962-63

Laboratory Demonstrator
Department of Zoology
Kings College, London, 1959-60

Research Assistant
Forest Biology Laboratory
Canada Department of Agriculture
Victoria, B.C. 1959

Laboratory Instructor
Department of Biochemistry
University of British Columbia 1959

Awards:
National Research Council Special Scholarship 1960-62
Canadian Federation of University Women Margaret McWilliams Fellowship 1959-60
National Research Council Bursary 1958-59
Woodrow Wilson Fellowship (Honorary) 1958-59
Governor General's Gold Medal 1958
Chris Spencer Special Scholarship 1954-8