DENSITY GRADIENT STUDIES OF SOME T3 AND T7 BACTERIOPHAGES AND THEIR ANTIBODY COMPLEXES

by

MAURICE VAN MITCHELL, JR.

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Approved by:

A. Eisenstark

Major Instructor

TABLE OF CONTENTS

INTRODUCTION

Reed and Eisenstark (1962) discovered that serological reactions involving head antigens of the bacteriophages could be distinguished from those involving tail or neutralizable antigens by cesium chloride density gradient centrifugation. They found that the combination of the head proteins of the phages with their homologous serum antibodies resulted in the formation of antigen-antibody complexes having a density much less than the density of the purified phages.

They observed that the T3B and T3C coliphages possessed common neutralizable antigens. The T3B phages were neutralized and precipitated by the anti-T3C serum. The T3C phages were not neutralized, but were precipitated by the anti-T3B serum. The T3C phages possibly possessed non-neutralizable tail antigens which were similar or identical to neutralizable antigens of the T3B phages. Their head antigens did not cross react, as shown in the density gradient experiments and verified by complement fixation tests. Figure 1 is a photomicrograph of T3C.

The T3B and T3C phages were only two of several coliphages being studied in the Kansas State University Virology Laboratory. It was decided that a study of the antigenic relationships among the head and tail antigens of additional phages would be both profitable and interesting. The T3Bhe, DF32m and T7(ATCC) phages were selected for study along with the T3B and T3C phages.

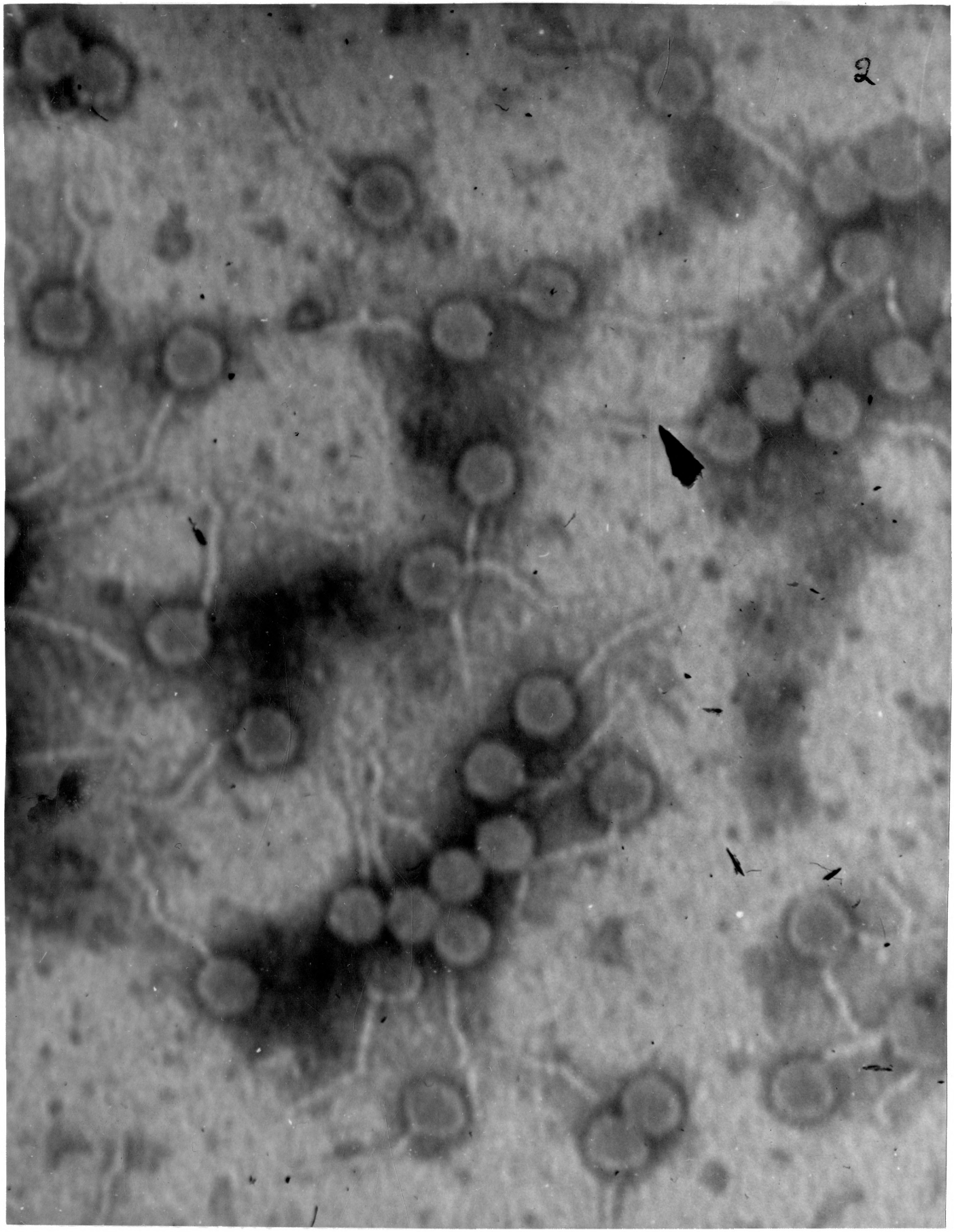

Figure 1. Electron micrograph of the T3C bacteriophage.
The long tails and hexagonal heads can be seen in many of the phages.

REVIEW OF LITERATURE

Structure and Components of Bacteriophages

The current ideas about the structures and components of bacteriophages and their functions have been arrived at by assimilating the results of electron microscopy, chemical analyses, experiments dealing with multiplication processes of phages and the antigen-antibody reactions of phages and phage parts. The model phage is T2. It has been the most widely studied, and more is therefore known about it than about any other.

Electron micrographs reveal that many of these viruses (including T2 and T3) consist basically of a head and a tail (Luria and Anderson, 1942; Anderson, 1946; Williams and Fraser, 1953; Eisenstark, Maaløe, and Birch-Anderson, 1961). The heads are polyhedral in shape. The tails range from short and stubby to long and slender, some having tail fibers attached (Champe, 1959).

Chemical analyses show that bacteriophages are composed mostly of protein and nucleic acid (Northrup, 1939; Polson and Wycoff, 1948; Hershey and Chase, 1952; Wyatt and Cohen, 1952). The nucleic acid is usually deoxyribonucleic acid, but some phages containing ribonucleic acid have been reported (Loeb and Zinder, 1961; Davis, Strauss, and Sinsheimer, 1961). All of the work described in this thesis deals with DNA-containing phages.

The heads of T2 phages appear to be composed of about two thousand protein subunits, each having a molecular weight of about 80,000 (Van Vunakis, Baker and Brown, 1958; Brenner and Barnett, 1959; Champe, 1959). The tail sheaths are made up of some two hundred subunits, each having a molecular weight of approximately

50,000. Each of the tail fibers is thought to consist of a single polypeptide with a molecular weight probably greater than 100,000 (Champe, 1959).

In addition to the structural proteins and DNA, bacteriophages have a serologically distinct "internal" protein containing about 3% of the total phage protein sulfur (Hershey, 1955) and representing 4-6% of the total phage protein (Levine, Barlow and Van Vunakis, 1958). Other minor internal constituents have also been reported (Hershey, 1957; Ames, Dubin and Rosenthal, 1958). From lysates of T2 phages a lysozyme which was presumed to be a part of the phage structure was isolated (Barrington and Kozloff, 1956; Champe, 1959).

Because in phages only the DNA contains phosphorous and only the protein contains sulfur, it has been possible to use radioisotopes of these two elements to discover the roles of DNA and protein in the virus multiplication cycle. All studies showed that bacteriophages transfer most of their DNA, but very little of their protein constituents to their progeny (Putnam and Kozloff, 1950; French, Graham, Lesley and Van Rooyen, 1952; Mackal and Kozloff, 1952; Hershey and Chase, 1952; Kozloff, 1953; Watson and Maaløe, 1952; Kozinski, 1961). The Hershey and Chase (1952) experiments showed that most of the S^{35} remained outside the bacterial cells during and after infection, and could be sheared from the surfaces of the bacterial cells in a Waring blendor without affecting normal multiplication of the viruses. The process of phage multiplication was surmised to consist of adsorption of the phages to the host cells by the tips of their tails

(Anderson, 1952, 1953), with subsequent contraction of the tail sheaths, projection of the tail cores through the tips of the tails and apparently into the cells, and injection of DNA and other internal contents into the cells through the tail cores (Champe, 1959).

In summary, the present concept is that bacteriophages are composed of an outer protein coat whose inner contents consist mostly of nucleic acid (usually DNA). The tail sheath, tail core, tail fibers and head covering are the evidently different proteins that make up the outer protein coats of the phages (Champe, 1959).

Host Range of Bacteriophages

The species and strains of bacteria in which a given phage will grow are called hosts, and constitute the host range of that particular phage. Each phage will grow in a very limited number of different strains of bacteria, and these bacteria are usually members of a single genus and species (Kalmanson and Bronfenbrenner, 1942; Lazarus and Gunnison, 1947).

Almost all species of bacteria are subject to infection by phages. Viruses have been found which grow in at least one member of each of the following groups of bacteria: Bacillus, Brucella, Chromobacterium, Clostridium, Corynebacterium, all genera of the Enterobacteriaceae, Gaffkya, Lactobacillus, Micrococcus, Mycobacterium, Neisseria, Nocardia, Pseudomonas, Rhizobium, Xanthamonas, and others. None have been found that will infect the pneumococci, spirochaetes and molds. Zymophages which grow in yeasts have been reported (Lindegren and Bang, 1961).

The most widely studied of all the bacteriophages are the T-coliphages which infect different mutant strains of the Escherichia coli species of Enterobacteriaceae (D'Herelle, 1961). Most of these phages are capable of growing on several strains of E. coli. Table 1, taken from "General Virology" by Luria (1953), gives a partial list of the bacteria which constitute the host range of the T-phages.

Host range is an expression of the mutual affinity of complementary chemical groups occurring on the tips of the phage tails and on the cell walls of the host bacteria (Garen and Puck, 1951; Puck, 1955). It has been found that chemical agents are capable of blocking the chemical groups which are essential to successful adsorption of the bacteriophages, both on the surfaces of the bacterial cells and on the tips of the phage tails (Gough and Burnet, 1934; Levine and Frisch, 1934; Puck and Tolmach, 1954). The bacterial cell wall may be visualized as a two-dimensional sheet of regularly occurring chemical groups arranged in recurring patterns over its whole surface (Weidel, 1953). These chemical groups cannot be considered as independent molecules, though they act as if they were. They are, individually and in groups, subject to mutation. All of the bacterial mutants shown in Table 1 were derived from the wild type by a single mutational step. The number of phage exclusions in each mutation shown ranges from one to six. Actually, within the species, E. coli, many more strains are resistant to a particular phage than are susceptible to it.

Bacteriophages are subject to similar mutations, and vary in their host ranges within their own groups. For example, not all

Table 1. Mutants of *E. coli* B resistant to various T-coliphages[a]

Strains of *E. coli*	T-coliphages						
	T1	T2	T3	T4	T5	T6	T7
Wild type	+[b]	+	+	+	+	+	+
B/1	-	+	+	+	+	+	+
B/6	+	+	+	+	+	-	+
B/1, 5	-	+	+	+	-	+	+
B/3, 4	+	+	-	-	+	+	+
B/3, 4, 7	+	+	-	-	+	+	-
B/1, 3, 4, 7	-	+	-	-	+	+	-
B/1, 3, 4, 5, 7	-	+	-	-	-	+	-
B/2, 3, 4, 6, 7	+	-	-	-	+	-	-
B/1, 2, 3, 4, 6, 7	-	-	-	-	+	-	-

[a]Data adapted from Luria, S. E. 1953. In General Virology, John Wiley & Sons, New York.

[b]Symbols: + = bacteria susceptible to infection; - = bacteria not susceptible to infection.

T3 phages have the same host range (Eisenstark et al., 1961). Because such mutations occur constantly in all groups of phages, and also in bacterial strains, the host range property is not suitable for bacteriophage taxonomy (Weidel, 1953). Nevertheless, it has practical value in assays for determining the number and kinds of host range mutants present in a phage mixture, or for determining that a particular phage suspension consists of only one host range type.

Serology of Bacteriophages

D'Herelle (1961) first reported the antigenicity of the bacteriophages.

The percentage law of Andrewes and Elford (1933a) stated that "A given strength of antiserum neutralizes, over a wide range, a definite percent of phage, irrespective of the quantity of phages present." However, Fodor and Adams (1955) declared that at 10^8 or more particles/ml the lytic assays showed that both neutralization and agglutination reactions occurred, resulting in an apparent increase in the neutralization rate.

Adams (1959) described a neutralization constant, $k = 2.3\ D/T \log P_o/P$, which could be used (and is used in this thesis) to measure the strength of an antiserum against a number of different viruses. In the formula k is the neutralization constant, D is the reciprocal of the antiserum dilution (e.g. when the antiserum is diluted 1/100, D = 100), T is time in minutes from mixing the phages and antiserum to diluting the aliquot to stop the neutralization reaction, P_o is the number of plaque forming units per unit volume at time zero (before

addition of antiserum), and P is the number of plaque forming units per unit volume at time T.

Kalmanson, Hershey and Bronfenbrenner (1942) reported that the rate of serum neutralization was independent of phage concentration, progressed logarithmically up to 99% of completion, resembled a bimolecular reaction, had a Q_{10} of two from 0° C. to 37° C., and was not affected by pH in the range from 5.0 to 10.0. They found also that heat inactivation gave the same curve as serum neutralization, indicating that there were no semi-neutralizing antibodies in the antisera.

Several workers have stated that the bacteriophage and the antibody form a complex during neutralization which does not dissociate at a measurable rate (Hershey, Kalmanson and Bronfenbrenner, 1943; see also Andrewes and Elford, 1933b; Jerne and Skovsted, 1953). Most agreed that the neutralization reaction appeared to be either entirely irreversible, or did not reach equilibrium under ordinary experimental conditions. Serum neutralization was shown not to destroy the phages, however, since full infectivity could be restored by treating the neutralized phages with papain (Kalmanson and Bronfenbrenner, 1943) or sonic vibrations (Anderson and Doermann, 1952).

Hershey (1943 and 1946) reported that phages which were distinguishable on the basis of host range sometime stimulated the production of indistinguishable antibodies. He stated also that neutralized phages may adsorb to host cells, indicating that more than just adsorption is involved in effective invasion of bacteria by the phages.

Delbruck (1945) also declared that neutralized phages may adsorb to host cells. He demonstrated that antibacterial antibodies did not inactivate phages but when reacted with the bacteria prior to infection by phages they did protect the bacteria from lysis. He reported that antiphage antibodies did not prevent lysis by combining with bacterial cells.

From experiments with T2 coliphages Tanami and Miyajima (1956) concluded that one antibody was sufficient to neutralize a virus particle. Their experiments reflected different neutralization rates of the same phage-anti-phage system when different hosts were used for assaying survivors, indicating that T2 had a complex, perhaps heterogeneous, tail structure. Studies of Streisinger (1956b) were in accord with these findings.

Neutralization may be described as the irreversible bimolecular reaction or combination of bacteriophage and neutralizing antibody whereby the antibody becomes attached to a sensitive, functional site on the bacteriophage tail in such a manner as to prevent infection of the host bacteria, though not always preventing adsorption.

Working with T5 coliphages Lanni and Lanni (1957) found that the "serum-sensitive portion of the T5 particle contains an ensemble of somewhat independently functioning not necessarily heterogeneous antigens, whose collective structure can vary mutationally in at least two distinct ways." Cross-breeding experiments by Fodor and Adams (1955) with T5 and PB produced bacteriophages with more complex and heterogeneous antigenic

structure than either parent, indicating that neutralizable antigens were genetically controlled.

Streisinger (1956a) found that host range and serological specificity were not genetically separable in T2. Therefore, changes in host range are usually genetically determined (Hershey, 1943), but are not always of such a nature as to be detectable serologically. Host range mutations evidently do not always result in such gross changes in tail protein structures as to produce different antigens. It has been suggested that host range specificity could be as much a matter of spatial configuration as of chemical composition (Weidel, 1953), but changes in spatial configurations are known in some instances to result in serologically detectable differences in antigens (Carpenter, 1956).

To interpret the various serological findings with exactness it is necessary to know the number of distinctly different antigens possessed by phages. DeMars, Luria, Fisher and Levinthal (1953), by growing phages in the presence of proflavine, produced incomplete phage parts called "doughnuts" which they considered to be immature phage heads. Their studies with whole phages and phage parts led them to conclude that the bacteriophage T2 possessed at least two distinct surface antigens: a head antigen and a tail antigen. The tail antigen was a neutralizable antigen, sensitive to neutralizing antibody.

Lanni and Lanni (1953a, b) also stated that T2 bacteriophages possessed at least two distinct antigens, largely confined to the outer area of the skin, which stimulated the production of two

types of antibodies. The head antigen could fix complement and stimulate the production of antibodies capable of precipitating phages. The tail antigen could fix complement and stimulate the production of antibodies capable of precipitating and neutralizing the phages. The head antigen and tail antigen were dissimilar and did not cross react.

Champe (1959) by chemical chromatographic analysis, found that phage coats were made up of three different proteins, these proteins being dissimilar in amino acid composition, structure and molecular weight. His experiments were conducted with T2 and T4 phages. He reported that the corresponding proteins of these two phages, when compared by electrophoretic and chromatographic separations of their tryptic peptides, were quite similar, although minor amino acid peptide differences were detected. It was his opinion that considerable latitude was possible in head protein assembly, but that sheath protein (which has a more active function) had a relatively more restricted structure. He believed that tail fibers and sheath interact in a specific way during the process of adsorption to the bacterial cell, and that this interaction is controlled by genetic alteration of a protein in either the fibers or the sheath.

The idea that bacteriophages have at least two separate surface antigens was given further support by the work of Reed and Eisenstark (1962). Eisenstark et al. (1961) and Lindell (1961) reported host range and serological properties of four T3 coliphages: T3B, T3Bhe, T3C and T3M. They found that the short-tailed phage, T3B, produced a long-tailed host range mutant, T3C,

with a frequency of 1×10^{-6}. When injected into rabbits T3C stimulated the production of antibodies which neutralized both T3B and T3C, but antibodies stimulated against T3B did not neutralize T3C. Serum blocking power tests revealed, however, that T3C during mutation retained a surface antigen capable of adsorbing anti-T3B neutralizing antibodies. Anti-T3C serum and anti-T3B serum produced precipitation reactions with both phages. Because of the change in host range and the differences in serological characteristics of the tail proteins of the two phages, Reed and Eisenstark (1961) considered the possibility that T3C consisted basically of a T3B phage with a slightly altered tail protein structure.

Expecting to find either that the increase in tail length of T3C (supposedly by increase in total amount of phage protein) or a loss of genetic material (deletion) during the process of mutation from T3B to T3C might have changed the ratio of protein to DNA in the T3C phage, Reed (1962) attempted to find a difference in their densities by isopycnic cesium chloride density gradient centrifugation. Contrary to expectations, he found that if either phage were less dense it was T3B.

The most interesting part of his work was the finding that anti-T3B serum greatly decreased the density of T3B phages, and anti-T3C serum greatly decreased the density of the T3C phages. Under his experimental conditions neither antiserum apparently decreased the density of the other phage. He attributed the observed decreases in density to the reaction of head antigens

and antibodies, since they occurred only in the homologous reactions. He verified these results by complement fixation tests. This work constituted two vital contributions: it added more weight to the idea that bacteriophages have at least two distinct surface antigens (one on the head and the other on the tail), and it introduced isopycnic cesium chloride density gradient centrifugation as a technique for detecting bacteriophage head antigen-antibody reactions.

Density Gradient Centrifugation

Density gradient centrifugation refers to techniques wherein particles are centrifuged through a liquid column which has a density gradient. The gradient column is a centrifuge tube. The purpose of the density gradient is to prevent convection and to keep the virus and other particles localized in zones. The possible modifications of density gradient centrifugation techniques have been classified in three categories. The term "isopycnic gradient centrifugation" (Anderson, 1955) is used for methods in which centrifugation is continued until each particle has reached a point where the suspending medium has a density equal to that of the particle. The supporting concentration gradient may be formed prior to or during centrifugation (Meselson, Stahl and Vinograd, 1957). Isopycnic gradient centrifugation separates particles on the basis of their densities and may also be used for the determination of densities.

The other two categories of methods have been called "rate zonal centrifugation" and "equilibrium zonal centrifugation."

In these methods the viruses are floated in a layer on top of a preformed gradient column before centrifugation. Each type of particle sediments as a zone through the column at a rate dependent on its size, shape and density. When the centrifuge is stopped while the virus is still sedimenting rapidly, the technique is called "rate zonal centrifugation" (Brakke, 1956). Particles are separated on the basis of their sedimentation rates, which depend on their sizes, shapes and densities. This modification has been termed "gradient differential centrifugation by Anderson (1955). The term "equilibrium zonal centrifugation" has been used for methods in which centrifugation is continued until most of the particles approach isopycnic positions. The separation is based mainly on the densities of the particles, even though not all are at true equilibrium at isopycnic positions. Long before this, small differences in densities are magnified into large differences in sedimentation rates (Brakke, 1960).

Brakke may be considered the father of density gradient centrifugation virus study. Using the equilibrium zonal centrifugation method and a sucrose density gradient column he has purified, concentrated and studied barley stripe mosaic, potato yellow-dwarf, wheat streak mosaic and other viruses (Brakke 1953, 1956, 1958a, 1958b, 1959; Brakke and Staples, 1958).

A variety of other plant viruses have also been studied by using density gradient centrifugation techniques. With sucrose gradient columns Bancroft and Kaesberg (1960) worked

with alfalfa mosaic virus, and Sinclair, Gail and Kaesberg (1957) performed experiments with wild cucumber mosaic virus.

Density gradient centrifugation has also been useful in animal virology. Breese, Trautman and Bachrach (1960) analyzed the infectivity of foot-and-mouth disease virus in migrating zones. Their density gradient columns consisted of heavy water (D_2O) and ammonium acetate. Trautman, Savan and Breese (1959) also used density gradient centrifugation in experiments with this virus. Other animal viruses investigated by means of density gradient centrifugation techniques include Rous sarcoma virus (Crawford, 1960; Kohler, Byron, Boyd and Maloney, 1954; Crawford and Crawford, 1961); poliomyelitis virus (Polson, Ehrenberger and Cramer, 1958); influenza virus (Friedwald and Pickels, 1944); African horsesickness virus (Polson and Madsen, 1954); and Shope papilloma virus (Watson and Littlefield, 1960).

Investigators have also employed density gradient techniques in experiments with bacteriophages (Kozinski and Szybalski, 1959; Sinsheimer, 1959; Meselson and Weigle, 1961; Reed and Eisenstark, 1962).

Other substances subjected to density gradient centrifugation analysis include DNA (Meselson and Stahl, 1958; Sueoka, 1959; Rolfe and Meselson, 1959); cell particulates (Anderson, 1955; Holter and Moller, 1958); and proteins (Hogeboom and Kuff, 1954; Anderson, 1958).

Density gradient columns in most cases have consisted of sucrose and water (Brakke, 1953, 1956, 1958a, 1958b, 1959; Sinclair et al., 1957; Bancroft and Kaesberg, 1960;

Kohler et al., 1954; Friedwald and Pickels, 1944; Hogeboom and Kuff, 1954). The next most frequently used substance has been cesium chloride and water (Weigle et al., 1959; Davern and Meselson, 1960; Watson and Littlefield, 1960; Meselson and Weigle, 1961; Reed and Eisenstark, 1962; Kozinski and Szybalski, 1959).

Other substances used to form gradient columns include potato starch (Anderson, 1958), hemocyanin (Polson et al., 1958; Polson and Madsen, 1954), rubidium chloride (Crawford and Crawford, 1961), and rubidium chloride and sodium bromide (Meselson et al., 1957).

MATERIALS AND METHODS

Materials

Media.

Nutrient broth : 8 gm dehydrated nutrient broth, Difco
5 gm NaCl
1000 ml distilled water

Nutrient agar : 8 gm dehydrated nutrient broth, Difco
5 gm NaCl
15 gm agar, Difco
1000 ml distilled water

Tryptose overlay: 8 gm tryptose with thiamine, Difco
5 gm NaCl
8 gm agar, Difco
1000 ml distilled water

Saline. 8.5 gm NaCl
1000 ml distilled water

Cesium chloride. This pure compound was obtained from the Fairmount Chemical Company, 117 Blanchard Street, Newark 5, New Jersey. Solutions of varying concentrations (densities) were prepared.

Bacterial Hosts. E. coli B, B/1, and K12 HfrC were obtained from the University of Copenhagen, Denmark, by Dr. A. Eisenstark.

Bacteriophages. T3B, T3C, T3Bhe and T7-cop were obtained by Dr. A. Eisenstark from the University of Copenhagen, Denmark. T3(DF32m) was isolated from T3(DF32) obtained from Dorothy Fraser, Massachusetts Institute of Technology, Cambridge, Massachusetts. T7(ATCC) came from the American Type Culture Collection,

Washington, D.C.

Antisera. Antisera against T3C and T7-cop were available from the Kansas State University Virology Laboratory. Antisera against the other phages were prepared by injecting approximately 1 x 10^{11} phages in Freund's incomplete adjuvant per inoculation for three inoculations into rabbits. The inoculations were given intramuscularly every three days. Before beginning the injections all rabbits were bled and their sera were tested for the presence of neutralizing antibodies against all the phages to be used in the experiments.

Eight days after the last injection the rabbits were bled by cardiac puncture. The whole blood was placed in sterile 125 ml Erlenmeyer flasks and allowed to clot at room temperature. After one hour the clots were released from the sides of the flasks with sterile applicator sticks. The flasks were incubated overnight at 37° C., then incubated 12 hours at 4° C. The serum expressed from the clots during this treatment was transferred with sterile pipettes to sterile bottles and stored at 4° C.

Equipment. For all high speed centrifugations a Model L Spinco preparative ultracentrifuge was used. For low speed centrifugations a Servall Automatic Superspeed centrifuge was employed.

Methods

Maintenance and growth of bacterial cultures. Bacterial cultures were maintained on nutrient agar slants in screwcap tubes. Suspensions used for growing phages were prepared either

from freshly grown agar slant cultures or by aerating young nutrient broth cultures.

Growth and preparation of virus cultures. All of the viruses were maintained in a frozen state in the Kansas State University Virology Laboratory.

Preparation of stock cultures. Each virus culture was prepared in such a manner as to insure that the ultimate stock culture was "pure", exhibiting the proper host range properties.

T3B was titered on E. coli B/1. A clear, well-isolated plaque on the plate having the highest dilution was touched with a sterile toothpick. The toothpick was dipped into a small tube of nutrient broth. This single plaque isolate broth culture was titered on B/1, HfrC, and on a mixture of B/1 and HfrC. No plaques appeared on plates inoculated with HfrC, as was expected, since the mutation rate of T3B to T3C or to T3Bhe was reported at approximately 1×10^{-6}. Usually the number of infectious units picked up on the end of a toothpick was about 5×10^{5}. The number of plaques on plates inoculated with B/1 and on the plates inoculated with the mixture of B/1 and HfrC was essentially the same. The plaques growing on B/1 were clear, while those growing on the mixed culture were cloudy. A cloudy plaque on the mixed host was touched with a toothpick and the toothpick was dipped into a small tube of nutrient broth. This single plaque isolate was the stock culture from which all T3B phages were grown. E. coli B/1 served as host for cultivating the T3B phages.

T3C was titered in the same manner as T3B. No plaques appeared on E. coli B/1. Plaques on HfrC and on the mixed host, B/1 and HfrC, were essentially the same in number. Only clear plaques appeared on HfrC. Only cloudy plaques appeared on the mixed host. A cloudy plaque on the mixed host was touched with a toothpick and the toothpick was dipped into a small tube containing sterile nutrient broth to form the stock culture from all T3C phages were grown. E. coli K12 HfrC served as host for cultivating the T3C phages.

T3Bhe grew equally well on both B/1 and HfrC. A clear plaque on a mixture of these two hosts was selected for preparing the single plaque broth stock culture. For cultivation HfrC was used. In all assays the mixed host was employed.

T3(DF32) was titered on E. coli B and a pinpoint size plaque was selected after 12 hours growth for preparing the single plaque broth stock culture. Because of the "minute" size of the plaque the culture was named "DF32m". These phages were cultivated on E. coli B.

T7(ATCC) was grown from the beginning of the experiments on E. coli B, since its host range was not known. During the investigation of its host range the phage population was found to consist of three host range mutants. All three were isolated in pure cultures.

T7-cop was cultivated on E. coli B from a frozen stock maintained in the Kansas State University Virology Laboratory.

Cultivation and concentration of viruses. For the neutralization experiments, fresh single plaque isolates of each of the phages were prepared as described to be sure that whole virus particles were the only antigenic materials present.

For the density gradient experiments virus suspensions containing a large number of viruses per ml were required. The method used in this laboratory for preparing high titer phage stocks was to pour 10-15 ml melted nutrient agar into each of a large number of petri dishes and allow the agar to solidify. In the meantime, a series of tubes, each containing 2 ml melted tryptose overlay, 3-5 x 10^9 host bacterial cells and 1200-1500 infectious virus particles was prepared and held at 45° C. in a constant temperature water bath. The contents of each inoculated tube of melted tryptose soft agar overlay was poured into one of the petri dishes containing the solidified nutrient agar, and spread evenly over the surface of the agar by gentle swirling or rocking of the dish. When the soft agar had solidified the petri dishes were incubated at 28° C. until semi-confluent lysis had occurred over all the plates. (It was found that plates inoculated with too many virus particles became completely clear and usually produced a very low yield of phages.)

To harvest the phages, the soft agar layer was scraped from each of the plates with a bent glass rod into large centrifuge tubes containing 2.5 ml saline for each plate.

After all plates were scraped the tubes were swirled gently and the sides of the tubes were rinsed with 1 ml saline for each plate. The soft agar-saline mixture was allowed to stand at room temperature 1-2 hr to permit the phages to diffuse out of the agar before centrifuging at 5,000 rev/min on a Servall centrifuge for 30 min to sediment the agar. After this low speed centrifugation the supernatant containing the phages was withdrawn from the centrifuge tubes by means of a suction pump. When very small bits of agar were found in the supernatant it was centrifuged again for 15-20 min. A good high titer virus preparation obtained by this method usually contained from 1-5 x 10^{11} infectious particles per ml, and when held up to natural light had a slight slate-grey appearance, as compared with a tube of sterile fresh straw-colored nutrient broth.

To concentrate the phages the supernatant was centrifuged at 39,000 rev/min in the 40,000 head of the Model L Spinco preparative ultracentrifuge. Pellets formed during the centrifugation were resuspended in as small an amount of sterile water as possible. This concentrated virus suspension was centrifuged at low speed to remove small particles of agar and bacterial debris which remained. The concentrated phage suspensions appeared blue-white, like a tube of water to which a drop of milk had been added. Sometimes there was a slight "off-yellow" cast.

Purification of the concentrated virus suspensions. It was found that the concentrated virus suspensions were not suitable for use in density gradient experiments involving the use of antisera. These suspensions contained not only the perfect virus particles having a density of about 1.50 but also other less dense particles. To obtain suspensions containing only the perfect virus particles a density gradient purification technique was employed. The gradient column was prepared in plastic centrifuge tubes 1/2 x 2 in. and consisted of four layers of cesium chloride of different densities. From bottom to top of the column the densities were:

Density	Ml
1.60	1.0
1.50	0.5
1.45	2.5
1.40	1.0

The densities of the cesium chloride solutions were computed from index of refraction data.

On top of the column was layered 0.5 - 1.0 ml of the concentrated virus suspension to be purified. The column, or tube, was centrifuged for 1 hr in the SW39 head of the Spinco at 25,000 - 30,000 rev/min. Usually three or four distinct migrating zones were found in each of the tubes. The zone of interest containing the bacteriophage particles was located at density 1.5, having migrated farther than the less dense, imperfect particles and extraneous substances in the concentrated

virus suspensions. The precise nature of the components of these less dense particles and zones was not determined with certainty, but experiments revealed that they were not "density mutants" of the bacteriophages.

Density gradient centrifugation. The equilibrium density gradient centrifugation technique utilized in purifying the virus suspensions was described above. The same method was employed to detect decreases in density of the viruses caused by reaction of phage head antigens and antibodies, in most of the experiments.

The isopycnic density gradient centrifugation technique was previously described by Reed (1962). In these experiments 5 ml cesium chloride having a density of 1.50-1.51 was pipetted into the centrifuge tube. The sample to be tested was layered on top of the cesium chloride and the tube was centrifuged about 24 hr at 38,000 rev/min in the SW39 head of the Model L Spinco. The density gradient was formed during the centrifugation.

Neutralization experimental procedures. Virus suspensions containing approximately 5×10^5 infectious particles/ml were prepared by touching a well isolated plaque of the virus being studied with a toothpick. The toothpick was dipped into a small tube of nutrient broth. Preparing the suspension in this manner insured that only infectious particles were present as antigenic material. Each phage culture was titered to determine the number of infectious units/ml present.

EXPERIMENTAL RESULTS

Host Range Experiments

Eisenstark et al. (1961) reported the host ranges of the T3B, T3Bhe and T3C phages. Their findings were verified during the course of these experiments.

It was discovered that the T7(ATCC) phage population consisted of three host range mutants. Table 2 shows the results of experiments designed to detect, enumerate and isolate these three mutants.

Only serological findings enabled complete differentiation among the six phages tested. However, host range properties were useful in many instances. T3Bhe and T7-cop grew equally well on E. coli B/1 and K12 HfrC. T3B could be cultivated on B/1, but not on HfrC. T3C was capable of multiplying in HfrC, but not in B/1.

DF32m was easily distinguished from all of the other phages entirely on the basis of plaque morphology and size, as shown in Figure 3. It was originally isolated from a minute plaque of the T3(DF32) culture growing on E. coli B. The progeny of this single plaque isolate produced an array of plaques ranging from about 0.1 mm to over 2.0 mm in diameter. Heterogeneous plaque morphology and size was a consistent property of many consecutive single plaque isolates. Dorothy Fraser (1957) suggested that this phage mates with the bacterial genome during multiplication, and therefore a single plaque might contain phages with many different genotypes as a result of such matings. As will be pointed out later, when injected

Table 2. Host range mutants in the T7(ATCC) phage population

Phages	Bacteria[a]		
	B/1	HfrC	B/1 + HfrC
T7B	163×10^5	0×10^5	224×10^5[b]
	26×10^6	0×10^6	25×10^6[b]
T7H	0×10^5	52×10^5	59×10^5[b]
	0×10^6	5×10^6	8×10^6[b]
T7BH	49×10^5	3×10^5	43×10^5[b]
			5×10^5
	7×10^6	0×10^6	5×10^6[b]
			1×10^6

[a]Strains of E. coli

[b]Cloudy plaques.

into a rabbit these phages failed to stimulate antibodies against their head proteins. Perhaps such a mating with the bacterial genome possibly results in a very heterogeneous population of head antigens, as well as plaque types.

Neutralization Experiments

The most convenient serological relationship among the phages to assess quantitatively is neutralization. The serological relationships among the T-coliphages are shown in Table 3 (Luria, 1953). For the T3 and T7 phages tested in these experiments, Table 4 lists the neutralization and cross-neutralization constants (k values) computed by the previously described formula, $k = 2.3\ D/T \log P_o/P$ (Adams, 1959).

To compare numerically the homologous and heterologous neutralization constants, the "Antiserum Neutralization Index" was devised. It is computed by the formula, ANI = X/Y x 10, where X is any neutralization constant of an antiserum and Y is the homologous neutralization constant of the same antiserum. ANI's are converted to the nearest whole number. The Antiserum Neutralization Index for the anti-T3 serum neutralization of the T7 phage, using data in Table 3, is $\underline{ANI} = 30/120 \times 10 = 2.5 = \underline{3}$. Since the homologous ANI is always 10, an ANI of 3 indicates that the T3 antiserum neutralized the T7 phages one-third as fast as it neutralized the T3 phages. Table 5 shows the Antiserum Neutralization Indexes computed from data in Table 3. In Table 6 are the ANI's computed from data in Table 4 which were obtained in the experiments with the mutants of the T3 and T7 phages being reported.

Table 3. Cross-neutralization constants of representative antisera against bacteriophages T1-T7[ab]

Antisera	Bacteriophages						
	T1	T2	T3	T4	T5	T6	T7
Anti-T1	80	0	0	0	0	0	0
Anti-T2	0	400	0	150	0	80	0
Anti-T3	0	0	120	0	0	0	30
Anti-T4	0	75	0	250	0	20	0
Anti-T5	0	0	0	0	80	0	0
Anti-T6	0	200	0	40	0	500	0
Anti-T7	0	0	75	0	0	0	120

[a]Adapted from original table. Luria, S. E. In General Virology. John Wiley & Sons, Inc., New York.

[b]The "T" designation was given to the coliphages by Demerec and Fano (1945).

Table 4. Cross-neutralization constants by antisera against T7 and T3 coliphage host range mutants

Antisera	Bacteriophages				
	T3B	T3Bhe	DF32m	T7(ATCC)	T3C
Anti-T3B	500	440	475	515	0
Anti-T3Bhe	570	530	495	525	0
Anti-DF32m	400	500	410	480	0
Anti-T7(ATCC)	415	480	535	505	0
Anti-T3C	395	515	495	435	385

Table 5. Antiserum Neutralization Indexes of representative antisera against bacteriophages T1-T7[a]

Antisera	Bacteriophages						
	T1	T2	T3	T4	T5	T6	T7
Anti-T1	10	0	0	0	0	0	0
Anti-T2	0	10	0	4	0	2	0
Anti-T3	0	0	10	0	0	0	3
Anti-T4	0	3	0	10	0	8	0
Anti-T5	0	0	0	0	10	0	0
Anti-T6	0	4	0	1	0	10	0
Anti-T7	0	0	6	0	0	0	10

[a]Computed from data in Table 3.

Table 6. Antiserum Neutralization Indexes of antisera against T7 and T3 coliphage host range mutants[a]

Antisera	Bacteriophages				
	T3B	T3Bhe	DF32m	T7(ATCC)	T3C
Anti-T3B	10	9	10	10	0
Anti-T3Bhe	10	10	9	10	0
Anti-DF32m	10	10	10	10	0
Anti-T7(ATCC)	8	10	10	10	0
Anti-T3C	10	10	10	10	10

[a]Computed from data in Table 4.

At the beginning of the neutralization experiments the antisera and phage suspensions were heated to 37° C in a constant temperature water bath. At zero time, 0.1 ml of the 1:10 dilution of the antiserum in nutrient broth was blown into 0.9 ml of the phage suspension. The tube containing the mixture was swirled manually five times while holding the tube in the water bath. One minute after adding the serum to the phages, 0.1 ml was withdrawn from each mixture and diluted in 9.9 ml sterile nutrient broth. The suspension was mixed well and assay plates were prepared to determine the number of surviving bacteriophages. The plates were incubated 3-5 hr at 37° C before counting the plaques.

For each phage-antiserum system a control was prepared by substituting a 1:10 dilution of normal serum for the antiserum and continuing with the same procedure as described for the antiserum.

To compare the relative ability of each of the antisera to neutralize the different phages, k values were computed using the previously described formula, $k = 2.3\ D/T \log P_o/P$ (Adams, 1959).

Precipitation reactions. No special precipitation tests were devised for these experiments. As previously reported by Reed and Eisenstark (1961) the phages combined with the antibodies in the antisera during the incubation prior to ultracentrifugation to form visible precipitates. After centrifugation the precipitates were found in distinct, narrow, white,

granular zones in the gradient columns, as shown in Figure 2. The positions in the columns depended on whether only tail antigens and antibodies had reacted, or both head and tail antigens and antibodies had reacted. The purpose of the density gradient centrifugations was to make precisely this distinction between the two types of antigen-antibody complexes.

Density Gradient Experiments

Table 7 is a tabulation of the experimental data obtained in the density gradient centrifugations. On the basis of these experimental results the reactions were easily placed into two categories, those reactions involving only phage tail antigens and antibodies and those resulting in the combination of both head and tail antigens and antibodies. Table 8 lists the former category of reactions and Table 9 shows the latter group.

The distinction between the two types of antigen-antibody complexes was their markedly different densities. Figure 2 is a photograph showing typical results of the density gradient centrifugation of both types of complexes and the unreacted viruses. Reed and Eisenstark (1962) discovered, in this laboratory, that the reaction of phage head antigens and antibodies produced a much less dense complex than the reaction of tail antigens and antibodies.

In many cases bacteriophages stocks can be differentiated from each other on the basis of their host range properties. Among the phages tested in these experiments the host ranges of several overlapped. It was therefore necessary to distinguish among these phages by serological findings. Table 10 shows this differentiation.

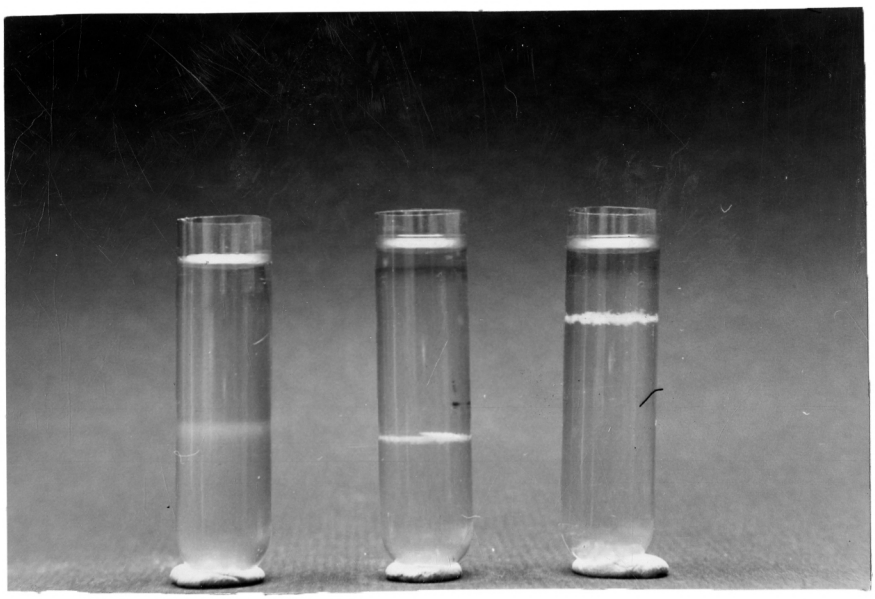

Figure 2. Photograph of three plastic centrifuge tubes showing the results of a typical equilibrium zonal centrifugation employing the artificial density gradient immediately after completion. In Tube 1 (at far left) is a zone of purified viruses. In Tube 2 (middle) is a zone containing a phage tail antigen-antibody complex. The markedly less dense zone appearing in Tube 3 (far right) contains a phage head and tail antigen-antibody complex. Qualitatively the two complexes (precipitates) are indistinguishable, but the higher position in the density gradient column after ultracentrifugation reveals that the latter complex is much less dense.

Table 7. Density gradient experiments with viruses and virus-antibody complexes

Experiment Number	Gradient Number	Sample: Phage + Antiserum	Precipitate	Distance in mm of visible zone from bottom of gradient column at termination of centrifugation
1	1	T3B + anti-T3B	+	36
	2	T3B + anti-T7(ATCC)	+	36
	3	T3B + normal serum	-	26
2	1	T7(ATCC) + anti-T7 (ATCC)	+	36
	2	T7(ATCC) + anti-T3B	+	36
	3	T7(ATCC) + normal serum	-	26
3	1	DF32m + anti-DF32m	+	21
	2	DF32m + anti-T3B	+	20
	3	DF32m + normal serum	-	19
4	1	T3C + anti-T3C	+	29
	2	T3C + normal serum	-	16
	3	T3C + H_2O	-	17
5	1	T3C + anti-DF32m	+	17
	2	T3C + anti-T3B	+	17
	3	T3C + H_2O	-	16
6	1	T7(ATCC) + anti-T7 (ATCC)	+	29
	2	T7(ATCC) + anti-T3B	+	29
	3	T7(ATCC) + H_2O	-	16

Table 7. (cont.)

Experiment Number	Gradient Number	Sample: Phage + Antiserum	Precipitate	Distance in mm of visible zone from bottom of gradient column at termination of centrifugation
7	1	DF32m + anti-DF32m	+	18
	2	DF32m + anti-T3B	+	19
	3	DF32m + normal serum	-	20
8	1	T3B + anti-T3B	+	29
	2	T3B + anti-T7(ATCC)	+	29
	3	T3B + H_2O	-	16
9	1	T3B + anti-T7-cop	+	11
	2	T3Bhe + anti-DF32m	+	13
	3	T3Bhe + H_2O	-	10
10	1	T7(ATCC) + anti-T3C	+	17
	2	T7(ATCC) + normal serum	-	16
	3	T7(ATCC) + H_2O	-	16
11	1	T3B + anti-T3C	+	17
	2	T3B + anti-DF32m	+	17
	3	T3B + H_2O	-	16
12	1	T3Bhe + anti-T3Bhe	+	34
	2	T3Bhe + anti-T3B	+	15
	3	T3Bhe + normal serum	-	14

Table 7. (cont.)

Experiment Number	Gradient Number	Sample: Phage + Antiserum	Precipitate	Distance in mm of visible zone from bottom of gradient column at termination of centrifugation
13	1	T3Bhe + anti-T3C	+	16
	2	T3Bhe + normal serum	-	16
	3	T3Bhe + H_2O	-	16
14	1	T3C + anti-T7(ATCC)	-	16
	2	T3C + normal serum	-	16
	3	T3C + H_2O	-	16
15	1	DF32m + anti-T3C	-	17
	2	DF32m + normal serum	-	16
	3	DF32m + H_2O	-	16
16	1	DF32m + anti-T3B	+	17
	2	DF32m + normal serum	-	16
	3	DF32m + H_2O	-	16
17	1	DF32m + anti-T7(ATCC)	+	27
	2	DF32m + anti-T3C	+	26
	3	DF32m + H_2O	-	26
18	1	DF32m + anti-T3Bhe	+	32
	2	T3Bhe + anti-DF32m	+	16
	3	T3C + H_2O	-	16

Table 7. (concl.)

Experiment Number	Gradient Number	Sample: Phage + Antiserum	Precipitate	Distance in mm of visible zone from bottom of gradient column at termination of centrifugation
19	1	T3Bhe + anti-T3Bhe	+	37
	2	T3B + anti-T3Bhe	+	37
	3	T3Bhe + anti-T3B	+	18
20	1	T7(ATCC) + anti-T3Bhe	+	38
	2	T7(ATCC) + anti-DF32m	+	20
	3	T3Bhe + anti-T7(ATCC)	+	19
21	1	T3B + anti-T3Bhe	+	33
	2	DF32m + anti-T3Bhe	+	33
	3	DF32m + anti-T7(ATCC)	+	20
22	1	T3B + anti-T7(ATCC)	+	38
	2	T3B + anti-DF32m	+	21
	3	T3C + anti-T3Bhe	-	20
23	1	T3C + anti-T3C	+	37
	2	T3C + H_2O	-	18
	3	DF32m + anti-DF32m	+	18
24	1	T3Bhe + anti-T3Bhe	+	37
	2	DF32m + anti-DF32m	+	19
	3	T3Bhe + H_2O	-	20

Table 8. List of serological reactions involving only tail antigens and antibodies (positive reactions only)

Antisera	Phages	Experiment Number	Gradient Column Number	Antiserum Neutralization Index
Anti-T3B	T3Bhe	12	2	1
		19	3	
	T3C	5	2	0
	DF32m	3	2	1
		7	2	
		16	2	
Anti-T3Bhe	T3C	22	3	0
Anti-DF32m	T3B	11	2	1
		22	2	
	T3Bhe	9	2	1
		18	2	
	T3C	5	1	0
	T7(ATCC)	20	2	1
	DF32m	3	1	1
		7	1	
		23	3	
		24	2	

Table 8. (concl.)

Antisera	Phages	Experiment Number	Gradient Column Number	Antiserum Neutralization Index
Anti-T3C	T3B	11	1	1
	T3Bhe	13	1	1
	DF32m	15	1	1
		17	2	
	T7(ATCC)	10	1	1
Anti-T7 (ATCC)	T3Bhe	20	3	1
	T3C	14	1	0
	DF32m	17	1	1
		21	3	

Table 9. List of serological reactions involving both head and tail antigens and antibodies (positive reactions only)

Antiserum	Phages	Experiment Number	Gradient Column Number	Antiserum Neutralization Index
Anti-T3B	T3B	1	1	1
		8	1	
	T7(ATCC)	1	2	1
		8	2	
Anti-T3Bhe	T3Bhe	12	1	1
		19	1	
		24	1	
	T3B	19	2	1
		21	1	
	T7(ATCC)	20	1	1
	DF32m	18	1	1
		24	2	
Anti-T3C	T3C		1	1
		[illegible]	1	
Anti-T7(ATCC)				
	T3B	1	2	1
		8	2	
		22	1	
	T7(ATCC)	2	1	1
		6	1	

Table 10. Serological differentiation of the phage populations

Criterion	Antiserum				
	T3B	T3Bhe	T3C	DF32m	T7(ATCC)
Reacted with head antigens of DF32m phages	-	+	-	-	-
Formed precipitate with T3C phages	+	-	+	+	-
Reacted with head antigens of at least one phage	+	+	+	-	+
Reacted with T3B head antigens	+	+	-	-	+

T7-COP

Contrary to expectations the T7(ATCC) phages behaved very much like the T3 phages. They were more closely related antigenically than had been anticipated from reports in the literature. It was decided, therefore, to perform a few experiments with a different T7 phage, the T7-cop phage. Table 11 shows neutralization constants of the anti-T7-cop serum for the T7 and T3 phages tested. Table 12 tabulates the results of the density gradient experiments.

Evidently the T7(ATCC) phages and T7-cop phages are almost unrelated to each other, and while the T7(ATCC) phages are closely related to the T3 phages, the antigenic relationship of the T7-cop phages to the T3 phages is very weak.

Table 11. Neutralization constants of T7-cop antiserum

Bacteriophages	Neutralization constant
T7-cop	70
T3B	5
T3C	0
DF32m	5
T7(ATCC)	5

Table 12. Density gradient experiments with T7-cop and anti-T7-cop serum

Experiment Number	Gradient Number	Sample: Phage + Antiserum	Distance in mm of visible zone from bottom of gradient column at termination of centrifugation
1	1	T7-cop + H_2O	18
	2	T7-cop + anti-T7-cop	38
	3	T7-cop + anti-T3B	18
2	1	T7-cop + anti-T3B	19
	2	T7-cop + anti-DF32m	19
	3	T7-cop + H_2O	19
3	1	T3B + anti-T7-cop	20
	2	T3Bhe + anti-T7-cop	20
	3	T3C + H_2O	19
4	1	DF32m + anti-T7-cop	20
	2	T3C + anti-T7-cop	19
	3	T3C + H_2O	19

DISCUSSION

Host Range Experiments

Eisenstark et al. (1961) reported the host ranges of the T3B, T3Bhe and T3C phages. Host range information was extended by these experiments to include the mutants in the T7(ATCC) phage population.

Neutralization Experiments

Results of the neutralization experiments indicated that phages T3B, T3Bhe, DF32m and T7(ATCC) possessed neutralizable antigens which were very similar, though possibly not identical. Whether these reactions depended on similar or identical basic protein structure, or only on similar or identical surface chemical groups, could not be determined. Both their homologous and their heterologous ANI's, converted to the nearest unit of 5 were 10, a property ordinarily found only in homologous serological reactions (see Table 5).

None of the antisera against these four phages neutralized the T3C phages. Eisenstark et al. (1961) had previously reported that the anti-T3B serum failed to neutralize T3C, but that the anti-T3C serum neutralized the T3B phages. It was found in these experiments that the anti-T3C serum also neutralized the T3Bhe, DF32m and T7(ATCC) phages, but none of the antisera against these phages neutralized the T3C phages.

Positive neutralization reactions served to give a quantitative measure of the similarity of the antigens of the phages and indicated that tail antigens and antibodies were reacting.

Density Gradient Centrifugation

Reed and Eisenstark (1961) reported that the reaction of phage head antigens and antibodies produced complexes markedly less dense than unreacted viruses, or phage-antibody complexes formed by reaction of tail antigens and antibodies. The differences in the densities of these particles were detected by isopycnic cesium chloride density gradient centrifugation. They verified the results of this technique by complement fixation tests.

The density gradient experiments described in this thesis were designed so as to parallel those of Reed and Eisenstark, and the results obtained were essentially the same as theirs. However, whereas they created their density gradients by overnight centrifugation while simultaneously sedimenting the viruses and virus-antibody complexes, in these experiments artificial density gradients were employed, requiring only one hour of centrifugation.

To show that the artificial density gradients were as effective when centrifuged for one hour as the gradients created by overnight centrifugation Experiments 1, 2 and 3 were performed according to the method of Reed and Eisenstark. Parallel to these Experiments 6, 7 and 8 were performed using the artificial density gradients centrifuged for one hour. Identical results were obtained by both methods.

Unreacted viruses formed a characteristic bright bluish band in the cesium chloride density gradient columns at density 1.50. Virus-antibody complexes involving only tail antigens

and antibodies sedimented to positions in the columns within 2 mm of the unreacted viruses. Phage head antigen-antibody complexes migrated to positions 10 mm or more above the unreacted virus bands. No virus-antibody complex assumed an intermediate position in the gradient columns. All of the phage-antibody complexes were white, granular precipitates, whether they were formed by reaction of only tail antigens and antibodies or by reaction of both head and tail antigens and antibodies.

Evidence that head antigens and antibodies had combined was the marked decrease in the density of the virus-antibody complex as determined by density gradient centrifugation. Positive neutralization tests showed that tail antigens and antibodies had reacted. Virus-antibody complexes in the heterologous reactions of the T3C phages in which neither neutralization nor significant decrease in density were observed involved non-neutralizable tail antigens (Reed and Eisenstark, 1961).

Pure cultures of the DF32m phages produced a very heterogeneous population of plaque types when grown on E. coli, as shown in Figure 3. Fraser (1957) studied the DF32 phages from which DF32m was isolated and suggested that their unusual behavior might be attributed to the mating of the bacteriophages with the host genome during multiplication. To account for the failure of the anti-DF32m serum to react with the head antigens of the DF32m phages it may be suggested that the DF32m head antigen population was very heterogeneous. The broad

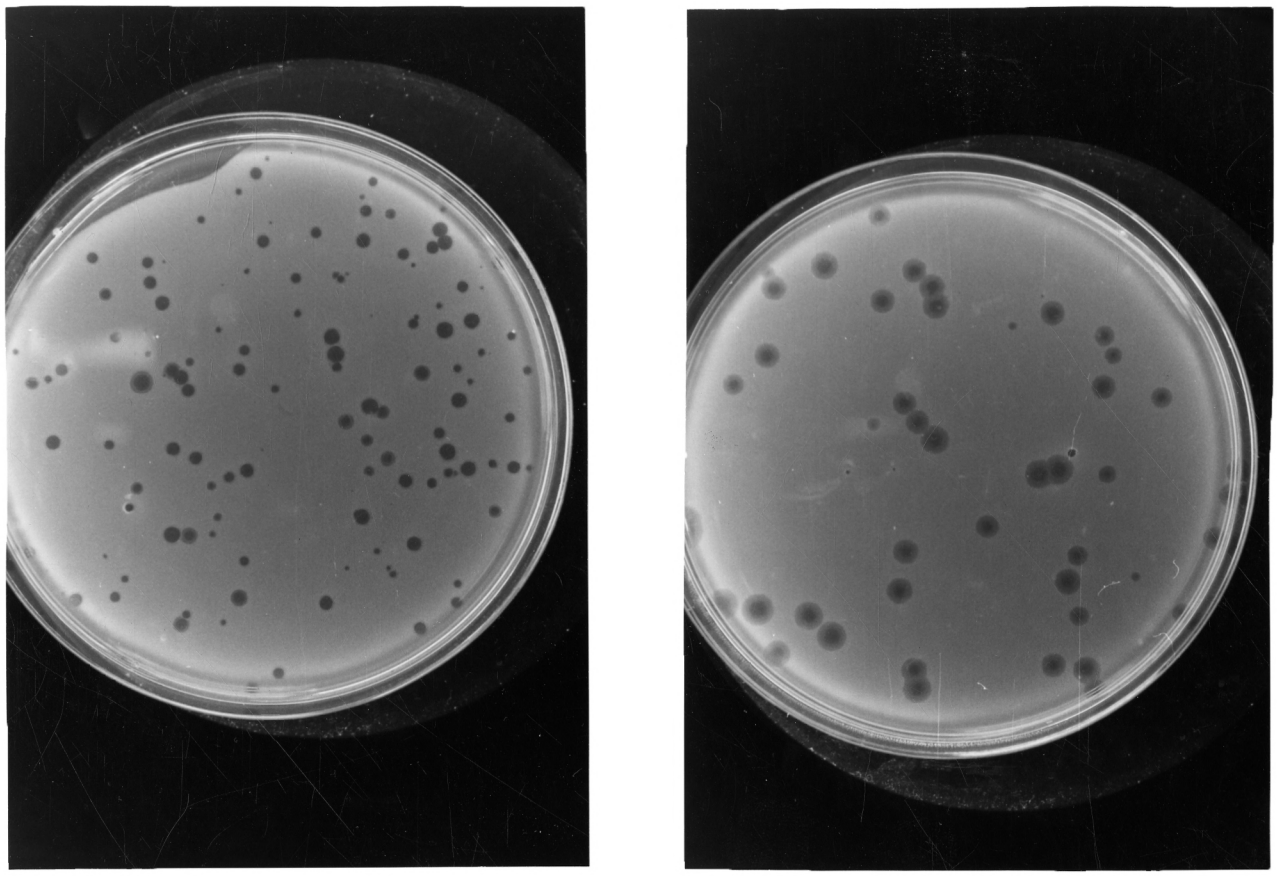

Figure 3. Photographs of petri dishes containing growing cultures of bacteriophages. Dish 1 (left) contains plaques of the DF32m phages. Dish 2 (right) shows the plaque morphology typical of all of the other phages. Notice that the sizes of the DF32m plaques vary widely, whereas the plaques of the other phages vary but little.

spectrum neutralizing anti-T3Bhe serum only was capable of reacting with the head antigens of the DF32m phages. It is most probable that the DF32m phage population consisted of an insufficient number of a single head protein type to stimulate antibody production in the rabbit. If there were equal numbers of ten different head protein types the number of particles of each type injected into the rabbit at each inoculation would have been approximately 2×10^{10}. It has been found in this laboratory that injecting fewer than 10^{11} infectious units per inoculation resulted in little or no antibody production. It may be suggested that perhaps the rabbit was incapable of producing antibodies against the head antigens. However, this is not very probable because he produced an excellent, broad spectrum antiserum capable of neutralizing the T3B, T3Bhe, T7(ATCC) and DF32m phages.

The absence of many reciprocal serological reactions was also of interest. The anti-T3C serum neutralized and precipitated the T3B, T3Bhe, DF32m and T7(ATCC) phages, but was neutralized by none of the antisera against these phages. The work of Eisenstark et al. (1961) and Reed and Eisenstark (1961) suggested that the T3C phages possessed a non-neutralizable tail antigen which stimulated antibodies capable of neutralizing the T3B phages. Consistent with this suggestion, the anti-DF32m serum precipitated the T3C phages as well as the anti-T3B serum, but the anti-T3Bhe and anti-T7(ATCC) sera did not precipitate the T3C phages. The anti-T3Bhe serum combined with the head antigens of the T3B, T3Bhe, DF32m and T7(ATCC) phages, but none of the antisera against these phages combined with the head

antigens of the T3Bhe phages.

Results of these experiments suggest that phages have at least two distinct antigens, one on the head and one on the tail, as has already been reported. Some phages, for example the T3C phage, perhaps have three surface antigens, a head antigen, a neutralizable tail antigen, and a non-neutralizable tail antigen. It is not possible at this time to determine the exact relationship between the protein subunits of the phage coats and the antigen-antibody reactions of the phages.

CONCLUSIONS

The Antiserum Neutralization Index, introduced in this thesis, gives a convenient means of quantitatively comparing the homologous neutralization constants of any antiserum. Such comparisons suggest an idea of the basic similarity of the neutralizable antigens of the phages.

Only by serological findings were each of the phage populations distinguishable from all of the others being studied.

The equilibrium zonal centrifugation technique employing the artificial density gradient identified the phage-antibody complexes in which head antigens and antibodies had reacted as well as the isopycnic density gradient technique, with the great advantage that less time was required for the former.

Results of the serological experiments with the long tailed T3C phage suggested that this phage had three distinct surface antigens: a head antigen, a neutralizable tail antigen, and a non-neutralizable tail antigen.

The T3 phages were shown to be very closely related antigenically to each other, and to the T7(ATCC) phages.

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DENSITY GRADIENT STUDIES OF SOME T3 AND T7 BACTERIOPHAGES AND THEIR ANTIBODY COMPLEXES

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MAURICE VAN MITCHELL, JR.

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Major Instructor

This thesis reports results of experiments designed to discover the antigenic relationships among the T3B, T3Bhe, T3C, DF32m and T7(ATCC) phages, describes the methods of executing these experiments, and introduces the Antiserum Neutralization Index.

The Antiserum Neutralization Index, computed from neutralization constants, is a comparison of the homologous and heterologous rates of neutralization by any antiserum.

The equilibrium zonal centrifugation technique employing an artificial density gradient column is shown to be effective in detecting phage head antigen-antibody complexes.

The neutralizable antigens of all of these phages appear to be very similar if not identical, with the exception of T3C which evidently has non-neutralizable tail antigens similar to neutralizable tail antigens of several of the other phages. There is considerable variation among the head antigens of these phages.

The absence of many reciprocal serological reactions cannot be explained at this time.