Bacteriophage Conjugates: The Effect of Coupling Reaction and Specific Antibodies on the Kinetics of Inactivation and Reactivation

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The reaction of bacteriophage T4 with 1-fluoro-2,4-dinitrobenzene resulted in a covalent binding of 2,4-dinitrophenyl (DNP) determinants to the phage. From the kinetics of inactivation reflecting the coupling process it is concluded that attachment of more than one DNP group to the critical site(s) of the phage is required for inactivation (multi-hit reaction). Contrary to this the neutralization of DNP-T4 by anti-DNP antibody turned out to be a first order reaction, until 80 % neutralization fitting one-hit kinetics.

If compared with native T4, the susceptibility of DNP-T4 to neutralization by anti-T4 antibody is considerably higher, indicating that attachment of DNP groups to T4 amplifies the sensitivity to neutralization by anti-T4. Comparing neutralization kinetics of DNP-T4 and native T4 by anti-DNP-T4 antibody it is suggested that native determinants and DNP groups, as well as determinants resulting from alteration due to the coupling process, all together may contribute as targets for neutralization. Three characteristics strengthen the view that the velocity of T4 conjugates in infecting the host strain is markedly decreased if compared with that of native T4: (a) considerable discrepancy between direct plating and decision technique (b) increasing variety of plaque size and (c) decreased velocity of the first step of reproduction. The kinetics of neutralization observed can be reconciled with a model proposed by KRUMMEL and UHR. The kinetics of reactivation of neutralized DNP-T4 by the presence of DNP-BSA has been investigated and the problems involved in the reaction are discussed.

1. Introduction

The neutralization of bacteriophages is among the most sensitive methods for the detection and quantitation of very small amounts of antibodies and has for this reason been used extensively in immunological studies [1–5]. However, this assay is limited to the detection of antibodies directed against bacteriophages. To extend the sensitivity of this method to other antigens, attempts have been made to attach a series of haptens and proteins to bacteriophages [6–10]. Hapten conjugated baceriophages were shown to be an efficient means of detecting antibodies to a wide range of specificities on a nanogram to picogram scale.

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Abbreviations: T4, Bacteriophage T4; DNP, 2,4-dinitrophenyl-groups; DNP-T4, 2,4-dinitrophenyl-bacteriophage T4 conjugates; DNFB, 1-fluoro-2,4-dinitrobenzene; DNBS, 2,4-dinitrobenzene sulfonate; DP, direct plating; DT, decision technique.

Extensive studies have been carried out to analyze the kinetics and the mechanisms of neutralization of bacteriophage by anti-bacteriophage antibodies [11, 12]. However, relatively little is known about the events taking place during the coupling process.

The present study deals with the conjugation of DNP groups to bacteriophage T4 (DNP-T4) using 1-fluoro-2,4-dinitrobenzene as the coupling reagent. For comparison conjugates of T4 with *p*-azobenzene arsonic acid, lysozyme and rabbit IgG were prepared and tested. At first, attention has been directed to the inactivation kinetics of the phage conjugates resulting from the coupling reaction. Our findings lend support to the conclusion that the inactivation of DNP-T4 due to the coupling process behaved as a multi-hit reaction. In contradistinction to this the neutralization of DNP-T4 by anti-DNP antibody, exhibited consistently one hit neutralization kinetics. Moreover, the results present evidence that the velocity of infective activity of DNP-T4, surviving the coupling process, is considerably decreased if compared to that of native T4. Additionally, antibodies produced in guinea

pigs by immunization with DNP-T4 were analyzed with respect to the activity of anti-DNP and anti-T4 antibodies. Finally, the reactivation kinetics of DNP-T4, neutralized with anti-DNP antibody will be presented and discussed.

2. Materials and Methods

2.1. Buffers

1. PBS: phosphate buffered saline (0.14 M NaCl in 0.01 M phosphate buffer pH 7.2).
2. TGB: Tris-gelatine buffer (12.1 g Tris, 5.0 g NaCl, 1.0 g NH_4Cl and 20 mg gelatine per liter) adjusted with 2 N HCl to pH 7.3-7.6.
3. Carbonate buffer (0.3 M $NaHCO_3$, pH 8.2).

2.2. Culture media

M9a [13] and nutrient broth (Merck, Standard I) were used as liquid media. Agar was used as solid (1.5 %) and as soft (0.6 %) medium according to LOEB [14].

2.3. Preparation of bacteriophage

Bacteriophage T4Br$^+$ and its host bacterium *Escherichia coli* B were grown in M9a according to the procedure of ADAMS [15]. The lysate was concentrated by negative pressure ultrafiltration using 8/32 Visking tubing. Then the suspension was treated with deoxyribonuclease (I lyophilized pure, Serva Heidelberg, W. Germany) for 60 min at 37°C and dialyzed subsequently against PBS. For further purification concentrated T4 were subjected to repeated differential centrifugation as described by PUTNAM *et al.* [16]. The final preparation was shown to be pure by analytical ultracentrifugation as well as by electron microscopy. The optical density of the purified T4 preparation at 260 nm was determined to be 1.0×10^{11} PFU/ml at 1 cm path length. Phage suspensions with a final concentration of 1×10^{13} PFU were stored at 4°C with chloroform as preservative until used.

2.4. Preparation of bacteriophage conjugates

2.4.1. DNP-T4 conjugate

T4 was suspended in 2.8 ml of 0.3 M carbonate buffer (pH 8.2) at a concentration of $2\text{-}3 \times 10^{11}$ PFU/ml and 0.25 mg 1-fluoro-2,4-dinitrobenzene (Fluka, Buchs, Switzerland) dissolved in 0.2 ml distilled dioxan was added within 3 min. The reaction was allowed to proceed at 22.5 °C with continuous stirring. Aliquots (0.2 ml) of the suspension were removed at appropriate intervals, immediately diluted two hundredfold in TGB and the inactivation of the phage conjugate was tested by direct plating as well as by the decision technique as described in section 2.5. Finally the unreacted hapten was removed by exhaustive dialysis against TGB. DNP-T4 was used for immunization as well as for kinetic studies by the neutralization assay.

2.4.2. Arsanil-T4 conjugate

Phage suspension (1×10^{13} PFU/ml) was reacted with p-azobenzene arsonic acid tyrosine [17] using Tolylene-2,4-diisocynate (practical; Fluka Switzerland) as bifunctional reagent.

2.4.3. Protein-T4 conjugates

Egg white lysozyme (3 times crystallized) was obtained from Serva, Heidelberg, Germany. RgG was prepared using QAE-Sephadex A-50 [18]. The conjugation of lysozyme and RgG was effected by glutaraldehyde and Tolylene-2,4-diisocyanate respectively as described by HAIMOVICH *et al.* [10].

2.5. Phage assay

Phage titration was performed by the agar double layer technique described by ADAMS [15], referred to as direct plating method (DP). In addition the decision technique (DT) was applied for phage assay slightly modified from that of JERNE and AVEGNO [19]. A portion of 0.2 ml T4 was added to 0.2 ml of a newly grown suspension of *E.coli* in nutrient broth. The mixture was incubated for 10 min at 37 °C. Then 0.1 ml of bovine anti-T4 antiserum ($K = 3500$ min^{-1}) diluted one hundred fold in nutrient broth was added, mixed thoroughly and incubated a further 5 min at 37 °C. This step was followed by the addition of 2.4 ml of soft agar, and the mixture was poured on base agar and incubated for 16 hrs. at 37°.

2.6. Velocity of T4 reproduction

A suspension of T4 or DNP-T4 in nutrient broth containing 1×10^3 PFU/ml was preincubated at 37 °C for 30 min and added to an equal volume of a newly grown culture of *E.coli* containing approximately 2×10^9 bacteria. The resulting suspension was mixed thoroughly and incubated at 37 °C. The number of PFU was determined at appropriate time intervals by DP.

2.7. Haptenated proteins

BSA prepared as described by MICHAEL [20] and Edestin (Serva, Heidelberg, Germany) were both used as carrier proteins. These were conjugated with DNP according to the method of EISEN [21] using 2,4-dinitrobenzene sufonate (Eastman Organic Chemicals, Rochester, N.Y.) to yield complexes of DNP_{33}-BSA and DNP_{40}-Edestin where the subscript indicates the number of haptenic residues per molecule of protein.

2.8. Antisera

2.8.1 Anti-T4 antiserum

Anti-T4 antisera were prepared in two pregnant Simmentaler cows 4 and 7 years of age and with an average weight of 600 kg. They were immunized with

purified T4 in incomplete Freund's adjuvant containing 24 x 10^{12} PFU. A total of 14.4 ml of emulsion was administered in 1.2 to 3.0 ml doses injected into multiple sites both intramuscularly and subcutaneously three months prior to delivery. After two months rest the cows were reinjected in the same manner. The animals were bled one week after the second immunization and the sera obtained were stored at –20 °C without preservatives until used. The inactivation constants (K) were calculated as described in section 2.9. K for the above antisera was 1 300 min^{-1} and 3 500 min^{-1}, respectively.

2.8.2. Antiserum to DNP-T4

Antisera to DNP-T4 were prepared in albino guinea pigs of the Rockefeller strain (300 – 400 g) using a conjugate which became inactivated to 92 % by the coupling reaction.

2 x 10^{12} DNP-T4 particles in 0.5 ml 0.15 M NaCl were incorporated in 0.5 ml complete Freund's adjuvant (Difco Laboratories, Inc. Detroit). Each animal received per injection site 0.1 ml of this suspension in the foot pads (four sites) intramuscularly in the legs (four sites) and subcutaneously (two sites). The same dosage was repeated 7 weeks later again subcutaneously and intramuscularly. The animals were exsanguinated by cardiac puncture 14 days after booster injection. The sera were harvested, pooled and stored at –20 °C until used. The K value of the same antiserum was found to be 10 600 min^{-1} when tested with DNP-T4.

2.8.3. Anti-DNP antiserum

An anti-DNP antiserum was prepared by immunizing a cow with 125 mg DNP_{40}-edestin made up in incomplete Freund's adjuvant three months prior to delivery. A total volume of 12.0 ml of the mixture was distributed among several sites as described above. After an interval of two months the cow was reinjected using the same dosage of the antigen. A blood sample was taken one week after the booster injection.

The antibody content of the antiserum used for neutralization of DNP-bacteriophage was 1.5 mg/ml as determined by quantitative precipitation with DNP_{33}-BSA [22].

2.9. Kinetics of phage neutralization

These were performed in nutrient broth as described by ADAMS [15]. Inhibition experiments were performed using anti-DNP antiserum at a dilution of 1:8000 by preincubation with 50 μg DNP-BSA/ml.

2.10. Phage reactivation

The rate of reactivation of neutralized DNP-T4 was determined by adding 1.0 ml bovine anti-DNP antiserum to 4.0 ml DNP-T4 (5 x 10^4 PFU/ml). The mixture was incubated for 2 hr at 37 °C after which the number of PFU had been decreased to about 75 PFU/ml as assayed by DT. Then at zero time of reactivation a sample of 1.9 ml was removed from the incubation mixture. Different concentrations of DNP-BSA dissolved in 0.1 ml saline were added and further incubated at 37 °C. Duplicate 0.2 ml samples were removed at appropriate time intervals and assayed for PFU by DT.

3. Results

3.1. Inactivation kinetics of DNP-T4 during coupling reaction

As illustrated in Fig. 1 the fraction of DNP-T4 surviving the coupling process was inversely related to the incubation time of T4 with DNFB. The shape of the curve demonstrates clearly that there is a definite time lag before exponential inactivation begins. The highest sensitivity of DNP-T4 survivors to anti-DNP antibodies was found to be in the range of 93 to 98 per cent inactivation (DP). Furthermore increasing discrepancies could be demonstrated between DP and DT during the inactivation process as depicted in Fig. 1. In the insert, the difference of log % PFU is plotted as a function of time.

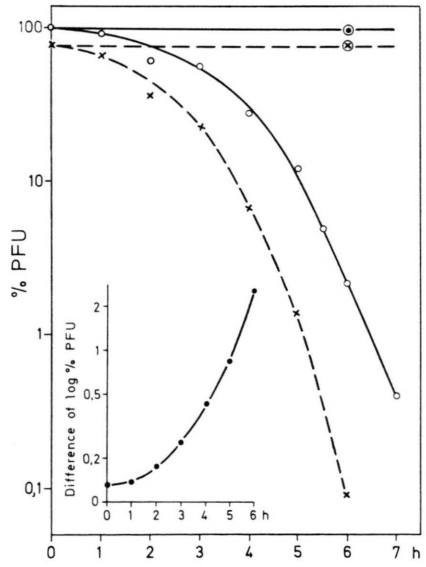

Fig. 1. O Directplating (DP), X Decision technique (DT), ⊙ DP control, ⊗ DT control. Effect of the reaction time between T4 and DNFB on the number of surviving phages as assayed by DP and DT. In the insert, the difference of log % PFU of DP and DT is plotted as a function of time

Additionally, in a series of tests, T4 preparations conjugated with p-azobenzene arsonic acid, lysozyme or RgG exhibited similar discrepancies between DP and DT.

Furthermore, when assaying a representative sample of a conjugated phage by DP a great variability in terms of the plaque size was observed (Fig. 2*). There was obviously an increase in variability of the plaque size as coupling time proceeded. In contrast the plaques induced by unmodified phage were quite homogenous in size. On the other hand, when phage conjugates and unmodified phage respectively were assayed by DT the plaques produced were found to be homogenous in size as illustrated in Fig. 3. In this context, however, it should be mentioned that anti-DNP antiserum was used instead of anti-T4 antiserum as outlined in section 2.5.

3.2. *Velocity of phage reproduction*

The velocity of the first step of reproduction was taken as further parameter to investigate the effect of the coupling process on the infectivity of DNP-T4.

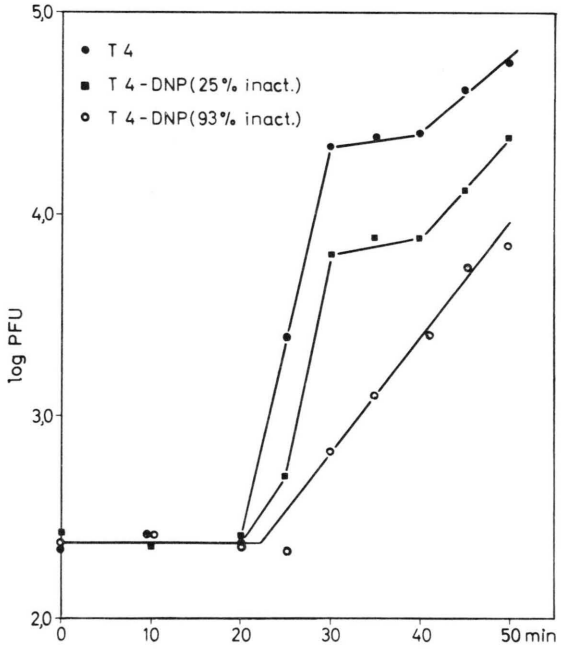

Fig. 4. Comparison of the first step of reproduction of T4 and DNP-T4 showing 25 % and 93 % inactivation, respectively.

DNP-T4 fractions were removed from the reaction mixture after an incubation period sufficient to inactivate 25 and 93 per cent of the phage preparation, respectively, and compared with native T4 following the method described in section 2.6. The results of a typical experiment are shown in Fig. 4. As can be seen, the velocity of the first step of reproduction is related to the degree of inactivation, which is dependent upon the extent of conjugation. As expected, native T4 as well as T4 conjugates exhibited no differences in further steps of reproduction.

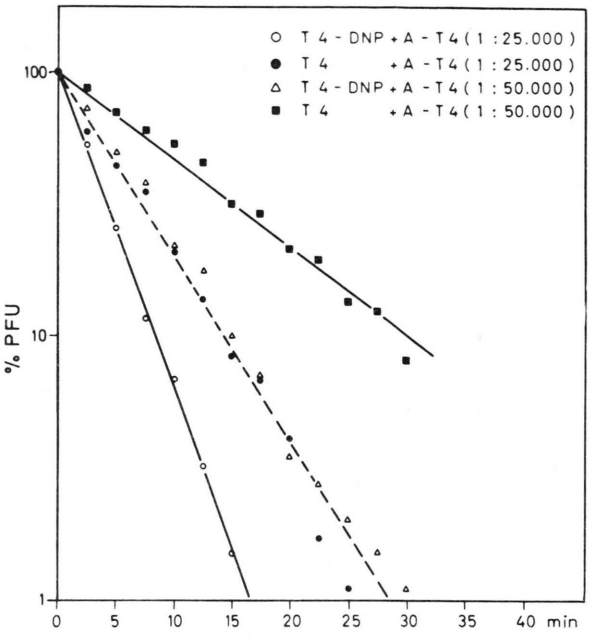

Fig. 5. Comparison of the neutralization kinetics between T4 and DNP-T4 effected by anti-T4 antibody at two different concentrations.

3.3. *Neutralization of T4 and DNP-T4 by anti-T4 antibody*

The rate of neutralization was determined by utilizing T4 and DNP-T4, the latter being inactivated during the coupling process to an extent of 93 to 98 per cent. The phage preparations were reacted with bovine anti-T4 antiserum ($K = 3.500\ \text{min}^{-1}$) at 1:25,000 and 1:50,000 dilution, respectively. While the neutralization of DNP-T4 by anti-T4 antiserum follows first order kinetics, there is, possibly a short time lag before T4 is exponentially neutralized by the same antiserum. The neutralization kinetics of T4 and DNP-T4 by equal concentrations of anti-T4 antibody is compared in Fig. 5.

* Figs 2 and 3 see Table page 88a.

It is apparent that the neutralization velocity of DNP-T4 was twice as much as that found for T4 when tested at the same dilution of anti-T4 antiserum. This finding points to a significant increase in suspectibility of DNP-T4 to neutralization by anti-T4 antibody although it must be taken into account that some antigenic determinants of the phage are not accessible because of the attachment of DNP-groups.

3.4. Neutralization of DNP-T4 or T4 by anti-DNP-T4 antiserum

In this experiment pooled anti-DNP-T4 antiserum generated in guinea pigs was capable of neutralizing DNP-T4 with an average $K = 10.600\ min^{-1}$ and unmodified phage with $K = 1.400\ min^{-1}$ (values calculated from five separate determinations). As already outlined in section 2.9, 50 μg DNP-BSA was found to be sufficient to inhibit the neutralization of DNP-T4 by anti-DNP antibody at a dilution of 1:8000 completely. The same dose of DNP-BSA inhibited the neutralization of DNP-T4 by guinea pig anti-DNP-T4 antibody to about 50 percent as reflected by the reduction of inactivation constants from $K = 10.600\ min^{-1}$ to $K = 5.680\ min^{-1}$. In appropriate controls it could be demonstrated that DNP-BSA did not affect the inactivation of unmodified phage by anti-DNP-T4 antibody.

3.5. Reactivation of DNP-T4

The dissociation of anti-DNP antibody from the critical site of neutralized DNP-T4 was studied kinetically in the presence of adequate concentrations of DNP-BSA. The details of the procedure employed have been outlined in section 2.10. The neutralization and reactivation curve of DNP-T4 is shown in Fig. 6. As can be seen, the time required to recover 60 % of the original number of PFU was determined to be 40 min.

Furthermore, it is apparent that the reactivation kinetics depend to some extent on the concentration of DNP-BSA present in the reaction mixture. The recovery of PFU is plotted on a logarithmic scale (Fig. 7) as well as on a non-logarithmic scale (Fig. 8) as a function of the incubation time. However, in studying reactivation kinetics the question arises whether reactivation might take place during decision assay. To test this possibility, duplicate samples were removed from the reaction mixture after 2 hrs. of

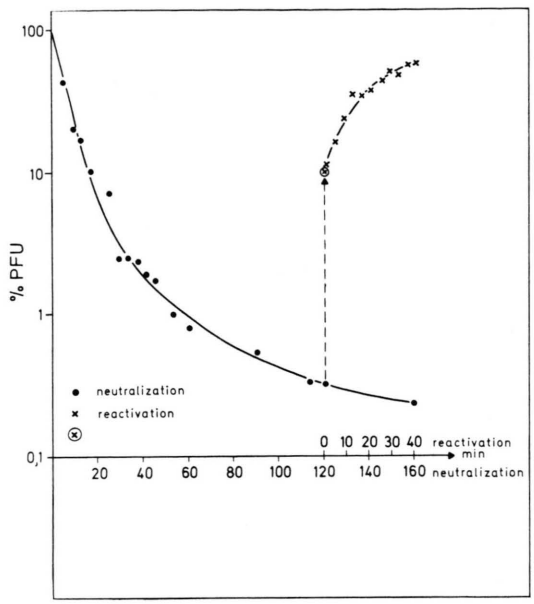

Fig. 6. Neutralization kinetics of DNP-T4 by anti-DNP antibody and reactivation of neutralized DNP-T4 by DNP-BSA (50 μg/ml) assayed by decision technique (DT). The arrow demonstrates reactivation taking place during the decision assay.

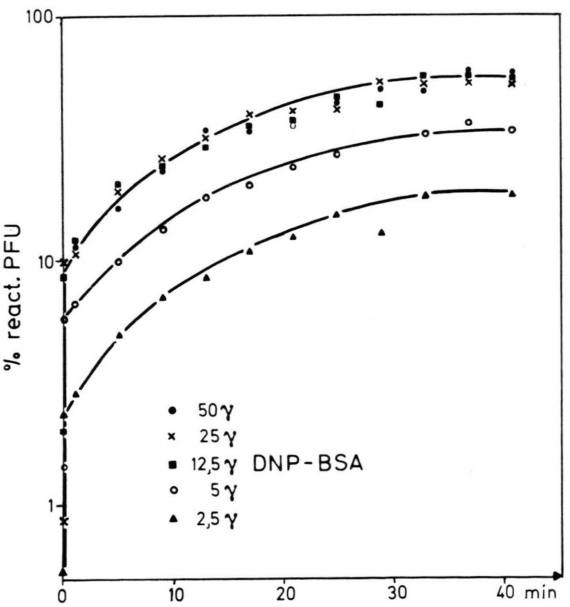

Fig. 7. Reactivation of neutralized DNP-T4 by DNP-BSA at different concentrations. The neutralization and the reactivation taking place during decision assay are indicated on the ordinate. The zero point of the curve represents the reactivation taking place during the decision assay.

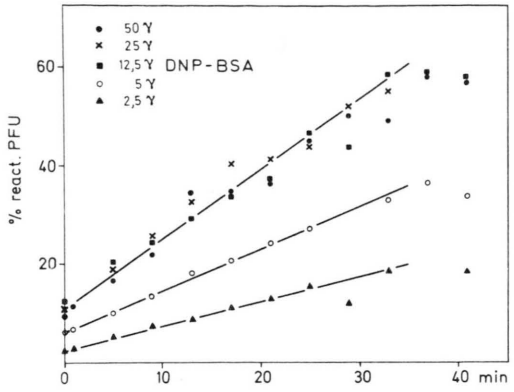

Fig. 8. Reactivation of neutralized DNP-T4 by DNP-BSA at different concentrations. The zero point of the curve represents the reactivation taking place during the decision assay. Note that PFU are plotted at a linear scale.

neutralization, *i. e.* zero point of reactivation, and mixed with *E. coli* B to which DNP-BSA had been added in an amount corresponding to that used for reactivation. By adding DNP-BSA to *E.coli* B a considerable increase in PFU was obtained if compared to that elicited in the absence of DNP-BSA as referred to in Figs. 7 and 8. From these results the conclusion can be drawn that reactivation takes place to some extent during decision assay. Taking this into account it was impossible to determine the dependence of reactivation on the time required from the start of reactivation to the reactivation taking place during the decision assay. Therefore the extrapolation to zero time appears to be rather difficult. For the same reason, further reactivation in the test tube could not be determined precisely.

4. Discussion

The neutralization of haptenated bacteriophages has proven to be an extremely sensitive assay for detection of antibodies to a chemically defined group [6–10]. Little, however, is known about the events taking place during coupling reaction and particularly about the properties of coupled phage [7, 23]. In the present study the conjugation of the DNP residue to T4 was effected by reacting bacteriophage with DNFB, differing from that described by CARTER *et al.* [9] who conjugated DNP groups to T2 phage by reaction with DNBS. Using DNFB, however, maximum efficiency of surviving DNP-T4 was achieved in a shorter reaction time and at a lower pH value than that required for the reaction with DNBS.

That T4 became inactivated with an extensive lag phase during the coupling process may appear as surprising. From the law of mass action it would be rather expected that the chemical attachment of DNP groups to T4 should proceed without any lag phase. Therefore, it is reasonable to assume that coupling of more than one DNP residue to the critical site(s) is required to neutralize T4 (multihit reaction). A comparable lag phase has been found with respect to the neutralization of T4 by monomeric Fab fragments, indicating that attachment of a single Fab fragment to the critical site(s) was not sufficient to render the bacteriophage inactive [24, 25]. On the contrary, the neutralization of DNP-T4 by anti-DNP antibody proceeds according to a first order reaction up to 80 % neutralization (Fig. 6) providing confidence for a single hit reaction.

Furthermore, it would be expected that the coupling of DNP groups to T4 blocks partially the susceptible region(s) of the bacteriophage reducing the neutralization rate by anti-T4 antibody. In contrast to this the present data show that the susceptibility of DNP-T4 to neutralization by anti-T4 was found to be twice as much as that of T4. The data strengthen the view that those DNP groups chemically attached to or near the critical site rather amplify the sensitivity to neutralization by anti-T4 antibody. These findings substantiate work reported by BARBER and RITTENBERG [26].

We have tested neutralization of DNP-T4 and of unmodified T4 by anti-DNP-T4 antiserum and its inhibition by DNP-BSA. As inferred from the inactivation curves, anti-DNP-T4 antibody was capable of neutralizing DNP-T4 with an average $K = 10.600$ min^{-1} whereas unmodified T4 was inactivated with a $K = 1.400$ min^{-1} by the same antiserum.

Neutralization of DNP-T4, but not of unmodified T4, by anti-DNP-T4 antibody was competitively inhibited to about 50 % ($K = 5.680$ min^{-1}) by the presence of DNP-BSA at a concentration sufficient to block anti-DNP-activity of an anti-DNP antiserum completely at a dilution showing the same rate of neutralization.Taking into consideration that the neutralization rate of DNP-T4 by anti-T4 antibodies is about twice that of native T4, the difference in the neutralization rate between unmodified T4 and DNP-T4 by anti-DNP-T4 antiserum provides evidence that the antiserum contains a considerable amount of antibodies against antigenic determinants resulting from alterations due to the coupling process. The present

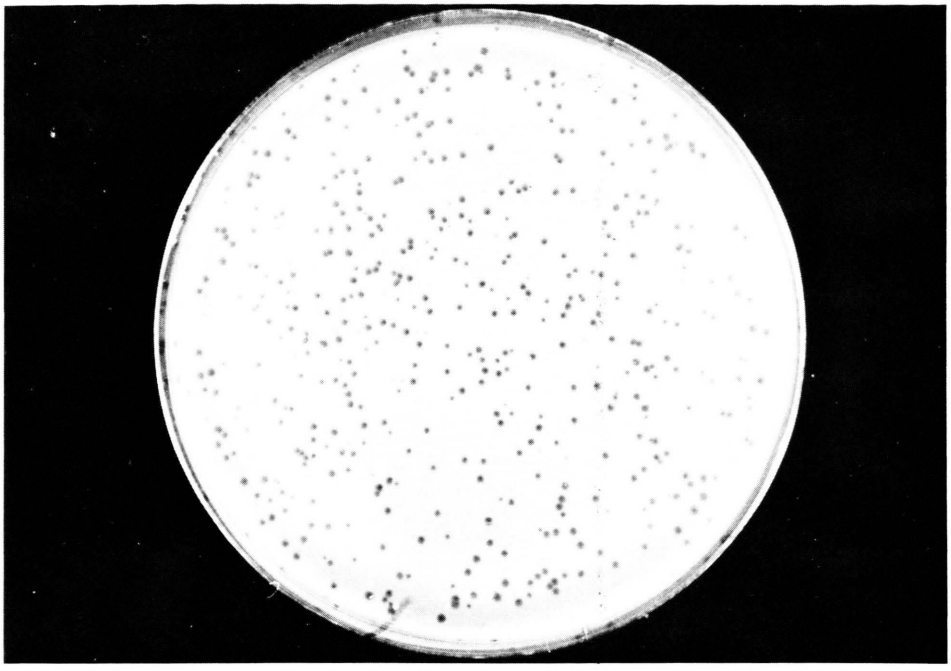

Fig. 2. The effect of the coupling process on the plaque size of conjugated phages as tested by direct plating (DP).

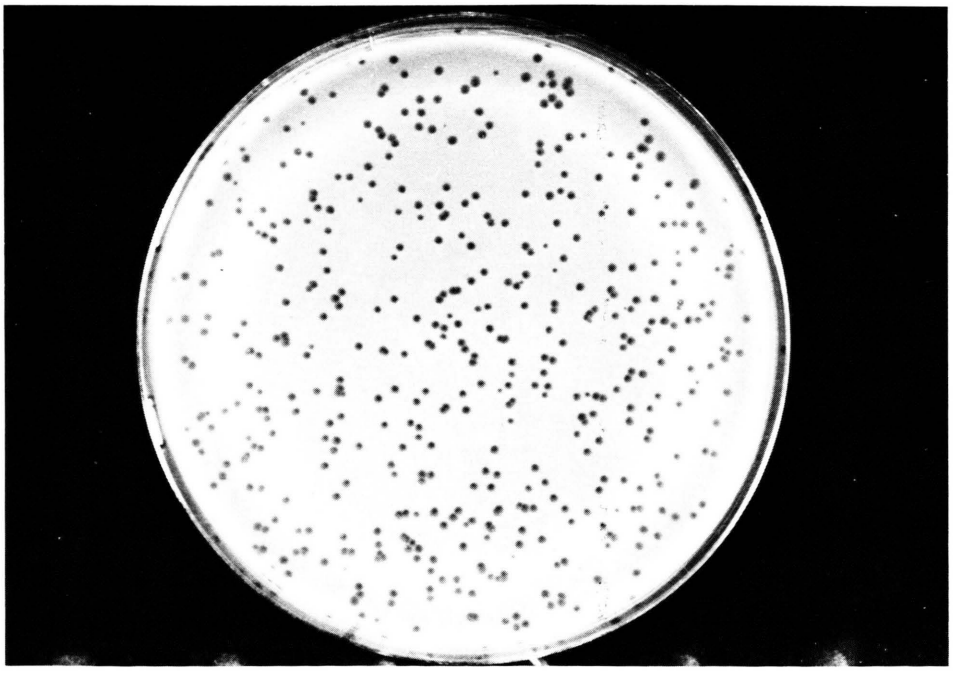

Fig. 3. The effect of the coupling process on the plaque size of conjugated phages as tested by decision technique (DT) conducted by anti-hapten antiserum.

findings further suggest that DNP-T4, which could be neutralized up to 99.9 per cent by anti-DNP antibody exhibited native determinants in the critical site(s) as shown by the immune response of guinea pigs and by the neutralization of DNP-T4 with anti-T4 antibody.

Furthermore, a substantial variety of plaque sizes was observed with DNP-T4, appearing increasingly small as coupling time proceeded. This suggested a decrease in the velocity of infective activity of the coupled phages. On the other hand, T4 assayed by the same method (DP) formed plaques of identical size. In contrast, when the assay was conducted by DT, DNP-T4 as well as T4 developed plaques of identical size. Conjugation of T4 with *p*-azobenzene arsonic acid, lysozyme or RgG behaved similarly to DNP-T4. These data confirm and extend previous results on the assay of poly-DL-alanyl-T4 [7].

Additionally, a comparison of fractions surviving the coupling process scored by DP and by DT demonstrated that the extent of inactivation was increasingly higher utilizing DT (Fig. 1). Therefore, it may be concluded that the velocity of infective activity of DNP-T4 decreased during the coupling process. Additional support for this conclusion derives from the observation that the velocity of the first step of reproduction of DNP-T4 was found to be dependent on the extent of conjugation (Fig. 4). Conjugates of T4 with *p*-azobenzene arsonic acid, lysozyme and RgG behaved in the same manner.

The decrease in infective activity of DNP-T4 seems to be a reasonable explanation for the marked discrepancies between DP and DT because reactivation of coupled phage by dissociation of covalently linked hapten or protein would be unlikely.

In addition we have obtained evidence that partially neutralized native T4 by anti-T4 antibody behaved similarly as indicated by (a) variety of plaque size, (b) discrepancies between DP and DT and (c) decreased velocity of the first step of reproduction [27]. These parameters became even more pronounced as neutralization proceeded and it seemed as if the slight reactivation by dissociation of anti-T4 antibody could not account for this. These findings are consistent with data from others, which demonstrated that the negligible dissociation of ϕX 174-anti-ϕX 174 complexes could not account for the differences in the inactivation rate obtained by utilizing DP or DT respectively [28].

This study supports the assumption that there is a decrease in the velocity of reproduction of neutralized ϕX 174 directly related to the number of anti-ϕX 174 molecules bound to the phage. The authors present a mathematical model based on a system of n differential equations and an infectivity function for the phage antibody complexes. This model can be taken to explain our experimental results.

Furthermore reactivation kinetics of neutralized DNP-T4 were studied in order to test whether dissociation of a single antibody molecule from the critical site results in reactivation. Thus HORNICK and KARUSH [29, 30] reported that dissociation of anti-DNP antibody occurred from the critical site of neutralized DNP-ϕX 174 by the presence of DNP-lysine. Since the reactivation curve did not show a significant lag it was concluded that there is only one critical site for neutralization. In our experiments it was found that the time required for the recovery of the maximum of PFU was found to be independent of the concentration of DNP-BSA used (range of 2,5-50 μg/ml). Further interpretation of the present results, however, seems rather difficult taking into account that a considerable reactivation could be demonstrated during the course of the decision assay. Therefore we have been unable to extrapolate precisely to zero time *i. e.* the point where reactivation begins. Considering this it cannot be said with certainty if detachment of a single anti-DNP molecule from the critical site would result in reactivation of DNP-T4.

From the results it is clear that the properties of phage conjugates differ markedly from those of native phage with respect to the fact that the velocity of infection is markedly decreased depending on the coupling process. Further neutralization kinetics of T4 and DNP-T4 points to problems concerning the mechanism of neutralization.

Because of the high sensitivity DNP-T4 conjugates were used to pursue the sequence and kinetics of antibody formation in cattle immunized with DNP-Edestin as will be described in a forthcoming paper.

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The Lipid Composition of Rabies Virus

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The lipid composition of the Flury HEP strain of rabies virus grown in BHK 21/C 13 cells was determined. The dried purified virus preparation contains 5.5 % neutral and 19.5 % polar lipids. Cholesterol was found to be the major neutral lipid. Phopholipids constitute 11.2 %; and glycolipids represent 4.6 % of the virus mass. The residual 3.7 % of extracted polar lipid material could not be accounted for by any known lipids. Phosphatidylethanolamine, phosphatidylcholine and sphingomyelin are the main constituents of the viral phospholipids. Hematoside is the only ganglioside and the main glycolipid present in the virus. The glycolipids of the host cell and the virus are identical. The molar ratio of sphingolipids to glycerophospholipids is 0.8.

Introduction

Electronmicrographs of rabies virus show bullet-shaped particles of complex morphology [1]. Treatment of rabies virus with ionic detergents results in inactivation of infectivity, in the release of the ribonucleocapsid and the solubilization of viral lipids and envelope proteins [2]. The sensitivity of virus infectivity to organic solvents [3] and to phospholipase C [4] provides strong evndence for the presence of lipids as essential components of the virus structure.

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The knowledge of the quality and quantity of the viral constituents is a prerequisite for the understanding of their cooperative interactions which stabilize the physical structure of the virus particle, and of their biological functions. The objective of the present communication is to describe the lipid composition of of the Flury HEP strain of rabies virus.

Materials and Methods

Virus: The Flury HEP strain of rabies virus was propagated in BHK 21/C 13 cells and extracellular virus was purified as described recently [5].

Lipid extraction: Lipids were extracted at 20 °C from the dialyzed, lyophilized virus with chloroform: