KERNFORSCHUNGSZENTRUM

KARLSRUHE

Januar 1972

KFK 1551

Institut für Strahlenbiologie

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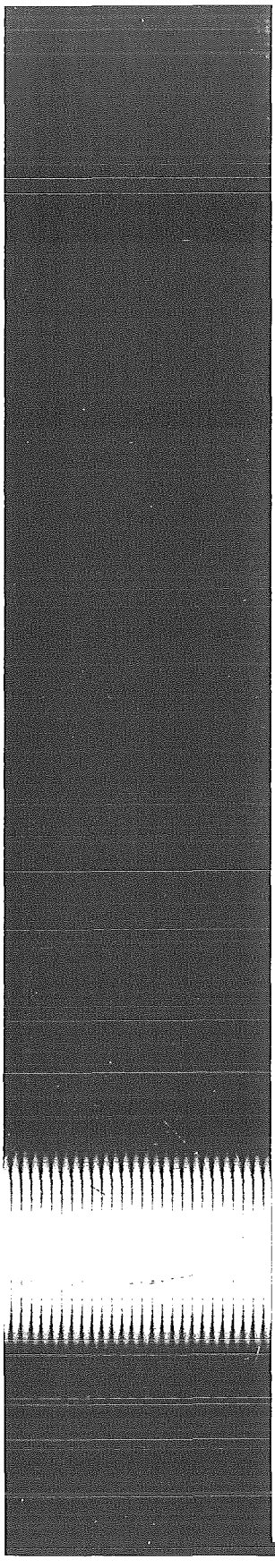

INT. J. RADIAT. BIOL., 1972, VOL. 21, NO. 1, 31–41

# Loss of adsorption and injection abilities in γ-irradiated phage T1

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(*Received* 9 *August* 1971; *accepted* 8 *October* 1971)

γ-irradiation of phage T1, suspended in 0·1 M histidine, inactivates some capacities of phage and blocks the early steps of infection. Five per cent of the inactivated phages have lesions in the protein coat, either in the head and leading to rupture and release of DNA, or in the tail and affecting adsorption. The host-killing ability is destroyed in 38 per cent of the inactivated phages. The amount of phage DNA injected into the host cell was measured and compared with the loss of plaque-forming ability after γ-irradiation. The chance of the genome becoming injected per lethal hit decreases to 31 per cent.

The amount of injected DNA is of the same order as the fraction of phages remaining without double-strand lesions in the DNA, i.e. without double-strand breaks or cross-links. This agreement suggests that only the DNA molecules with intact double strands are injected: a view confirmed by the localization of the major part of double breaks in the non-injected DNA. A partial injection of the damaged phage genome seems less probable for phage T1.

## 1. Introduction

The number of breaks in DNA produced by ionizing radiation has been determined in various biological objects, in bacteriophages, bacteria and mammalian cells. For bacteriophage T1, irradiated in 0·1 M histidine, it was shown (Bohne, Coquerelle and Hagen 1970), that about 17 per cent of the inactivated phages carry double-strand breaks and 9 per cent cross-links, either between DNA and the coat protein or intramolecular DNA–DNA cross-links. This means that 26 per cent of the inactivated phages contain such lesions affecting the structure of the double helix and, for brevity, called ' double-strand lesions ' in the subsequent sections.

In this paper the effect of this kind of damage in the double-strand structure on the injection of the phage DNA into the host cell was investigated. The injection of the phage genome may be inhibited in different ways. (I) The phages with double-strand lesions have lost their ability to inject their DNA, whereas the phages without such lesions inject all their DNA. This mechanism can conveniently be described as ' all-or-none injection model '. (II) Only part of the DNA molecule is injected, i.e. the piece from the end normally injected first to the nearest lesion. This second possibility, which we will refer to as ' partial injection model ', has already been discussed by Harm (1958) for T4 and more recently for bacteriophage λ by Sharp and Freifelder (1971). (III) A third possibility is that the inhibition occurs quite independently of double-strand lesions and is due to other unknown mechanisms. To decide between these three hypotheses, the amount of DNA injected after γ-irradiation was investigated, and the number of double-strand breaks in the non-injected DNA measured. Furthermore, the contribution of protein damage in irradiated phages was estimated by studying the adsorption and the fraction of ruptured phages in comparison with the inactivation of the plaque-forming ability.

## 2. Materials and methods

### 2.1. *Strains*

Coliphage T1 wild type, the prototroph *E. coli* B and the mutant *E. coli* CR 34/C 416 thy$^-$ were obtained from the Institut für Genetik, Cologne and from California Institute of Technology.

### 2.2. *Preparation of radioactive phages*

2-$^{14}$C-Thymine (spec. activity 17 $\mu$Ci/mg) was synthesized by Dr. K. H. Schweer, Institut für Strahlenchemie, Kernforschungszentrum Karlsruhe. *E. coli* CR 34/C 416 were grown in minimal medium (Stahl, Crasemann, Okun, Fox and Laird 1961) plus 2-$^{14}$C-thymine (20 $\mu$g/ml) to $2 \times 10^8$ cells/ml and infected with T1 phages at a multiplicity of 0·1. After incubating for 3 hours, about 10 ml chloroform were added per litre of phage suspension. The phages were precipitated by 50 per cent saturation with ammonium sulphate, concentrated, and separated from bacterial debris by differential centrifugation. A high-purity phage concentrate with a titre of about $10^{13}$ phages/ml was obtained by banding in CsCl. Finally the phages were dialysed against 0·8 per cent nutrient broth (Difco) (N.B.).

### 2.3. *γ-irradiation of phages and preparation of DNA samples*

The phages (titre $10^{12}$/ml) suspended in 0·165 M NaCl + 0·1 M histidine, adjusted to pH 7, were saturated with $N_2$ for 10 min, sealed in glass-stoppered tubes and irradiated at 0°c in a $^{60}$Co-gamma source (Gammacell 220, Atomic Energy of Canada, Ltd., dose-rate about 0·9 Mrad/hour). The phages were dialysed against NCE buffer (0·179 M NaCl, 0·02 M Na citrate, 0·001 M EDTA). From this suspension DNA was extracted by duponol and water-saturated distilled phenol, following the method of Mandell and Hershey (1960).

### 2.4. *Adsorption and injection of T1 phage*

A culture of *E. coli* B grown in nutrient broth in the exponential phase was centrifuged and concentrated to $10^9$ cells/ml in 1 : 10 diluted adsorption buffer (Hershey and Chase 1952). Chloramphenicol was added to a final concentration of 25 $\mu$g/ml. The bacteria were infected with $^{14}$C-T1 phages with a multiplicity (m.o.i.) of 3 to 10. After incubation for 15 min at 37°c the infected complexes were centrifuged at 5000 rev./min for 10 min and resuspended in N.B. The radioactivity was measured in the supernatant fluid and in the pellet. The relative amount of radioactivity in the pellet corresponds to the fraction of adsorbed phages.

To promote injection, the complexes were washed, resuspended to a concentration of $10^9$/ml and incubated again for 5 min at 37°c, then chloramphenicol was added. The phage–bacteria complexes were sheared for 5 min at 15 000 rev./min with a rotating scissor (Ultraturrax, Jancke and Kunkel) and centrifuged for 30 min at 5000 rev./min. From the amount of radioactivity in the supernatant fluid and in the pellet, the per cent of injected DNA was calculated. To measure radioactivity, a constant amount was pipetted directly onto glass fibre disks in scintillation vials. After drying the discs were immersed without further washing in 10 ml of toluene scintillation mixture (0·1 g POPOP and 4·0 g PPO per litre toluene) and measured in a scintillation counter (Mark I, Nuclear Chicago). With this analysis of the total activity in

the various fractions also the acid-soluble DNA is measured, which may be present in highly degraded DNA samples.

2.5. *Assay of ' bacterial killing ' titre of a phage suspension*

According to the method of Delbrück and Luria (1942), *E. coli* B were infected with a fixed number of phages particles (m.o.i. of 1 to 10). After 10 min at 37°C, the infected suspension was diluted and a sample of 0·1 ml was spread on agar plates plus anti-T1 serum. The number of colonies was compared with a control using uninfected bacteria.

2.6. *Sucrose density gradient sedimentation and measurement of the molecular weight of DNA*

0·2 ml of DNA solution (about 20 μg/ml) was layered on top of a 5 to 20 per cent linear sucrose gradient in 1 M NaCl, 0·01 M tris buffer, pH 7·0, 0·001 M EDTA, and spun for 3·5 hours in a SW 50 L swinging-bucket rotor at 20°C

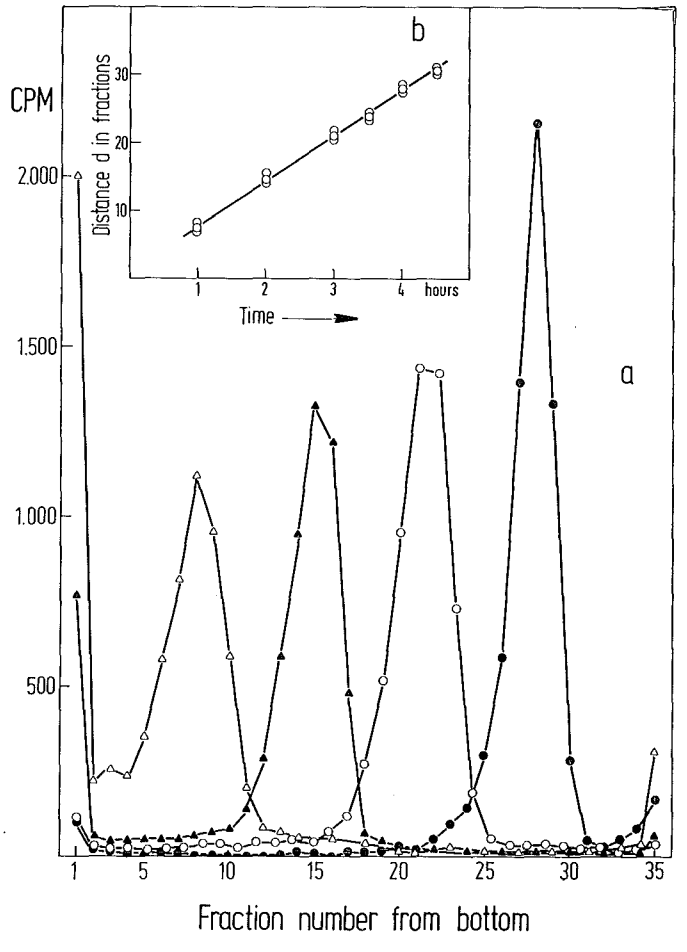


Figure 1. (*a*) Sedimentation patterns of $^{14}C$-thymine-labelled, native T1 DNA in a neutral sucrose gradient (5 to 20 per cent w/v). Centrifugation was carried out at 20°C, 35 000 rev./min, during 1 hour (●–●–●); 2 hours (○–○–○); 3 hours (▲–▲–▲); and 4 hours (△–△–△). (*b*) Distance sedimented by native T1 DNA in a neutral sucrose gradient as a function of time.

and 35 000 rev./min (Model L2–65 K Spinco Ultracentrifuge, Beckman Instr.). After centrifugation the tubes were pierced at the bottom and the fractions were collected dropwise.

The molecular weight $M_i$ of a DNA sample can be calculated from the expression given by Burgi and Hershey (1963)

$$M_i = \left[\frac{d_i}{d_0}\right]^{2\cdot 8} \cdot M_0 , \qquad (1)$$

where $d_i$ is the distance covered by the DNA molecules of fraction $i$ from the meniscus to a given position in the sucrose gradient, and $d_0$ the distance migrated by a molecule of known molecular weight $M_0$. Equation (1) is, however, valid only if $d_i$ is proportional to the sedimentation coefficient $s_i$ of the particles investigated. For sucrose gradients, this assumption is correct within certain limits (van der Schans, Aten and Blok 1969). To show this supposition holds under our experimental conditions, unirradiated T1 DNA was centrifuged for 1 to 4 hours (figure 1 *a*). As shown in figure 1 (*b*), the distance $d$ increases linearly with the time of centrifugation; thus $d_i$ is proportional to $s_i$. Therefore it is possible to calculate the molecular weight of DNA for each fraction $i$ from equation (1) and to derive the molecular weight distribution of a given DNA sample as well as the number of breaks (see Coquerelle, Bohne, Hagen and Merkwitz 1969).

## 3. Results

### 3.1. *Inactivation of the ability to adsorb on the bacterial host*

The ability of phages to adsorb on the bacterial host after $\gamma$-irradiation in 0·1 M histidine decreases exponentially with dose, the $D_{37}$ being approximately 1750 krads. In comparison, the $D_{37}$ for the inactivation of the plaque-forming ability (PFA) amounts to 90 krads (figure 2). This means, that 5 per cent of the inactivated phages are unable to adsorb to the host bacterium.

The ability to adsorb is lost by lesions occurring in the tail protein. In the fraction of the non-adsorbed phages, not only the radioactivity of phages with a lesion in the tail is measured but also the radioactivity of DNA which is released from ruptured phages. This rupture of the phage may be due to lesion in the protein coat of the head. Such release of DNA induces an increase of the viscosity in the phage suspension (Luthjens and Blok 1969). To determine the contribution of this fraction of ruptured phages to the measured fraction of non-adsorbed phages, suspensions of T1 phages containing $2 \times 10^{12}$ particles per ml were irradiated in 0·1 M histidine. The specific viscosity (see Coquerelle *et al.* 1969) increases with dose and reaches a plateau at about 1·2 Mrads. This plateau may be due to a simultaneous degradation of the liberated DNA by attack of the radiation-induced radicals. The extent of this degradation was measured by studying the loss of viscosity of a DNA solution, $\gamma$-irradiated under the same conditions as in the phage suspension, i.e. in 0·1 M histidine. There is a distinct decrease in this viscosity (figure 3, upper curve), in the dose-range investigated.

To calculate the percentage of ruptured phages, the increase of the viscosity in the irradiated phage suspension was compared with the viscosity of the DNA solution the concentration of which (98 $\mu$g/ml) corresponds to the DNA content of $2 \times 10^{12}$ phages per ml. Two values are obtained. One by relating the

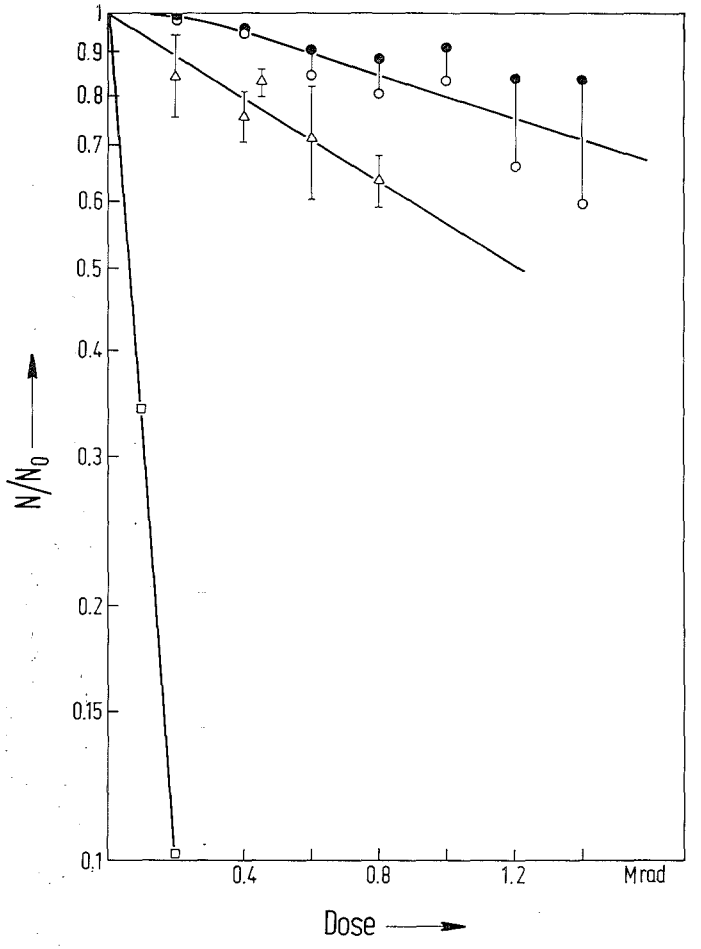


Figure 2. Survival of the plaque-forming ability of T1 (□), of the ability to adsorb to the host bacteria (△) and fraction of phages with intact head membrane (*a*) assuming that the liberated DNA is not degraded (●), (*b*) assuming that the liberated DNA is degraded (○) (cf. § 3.1).

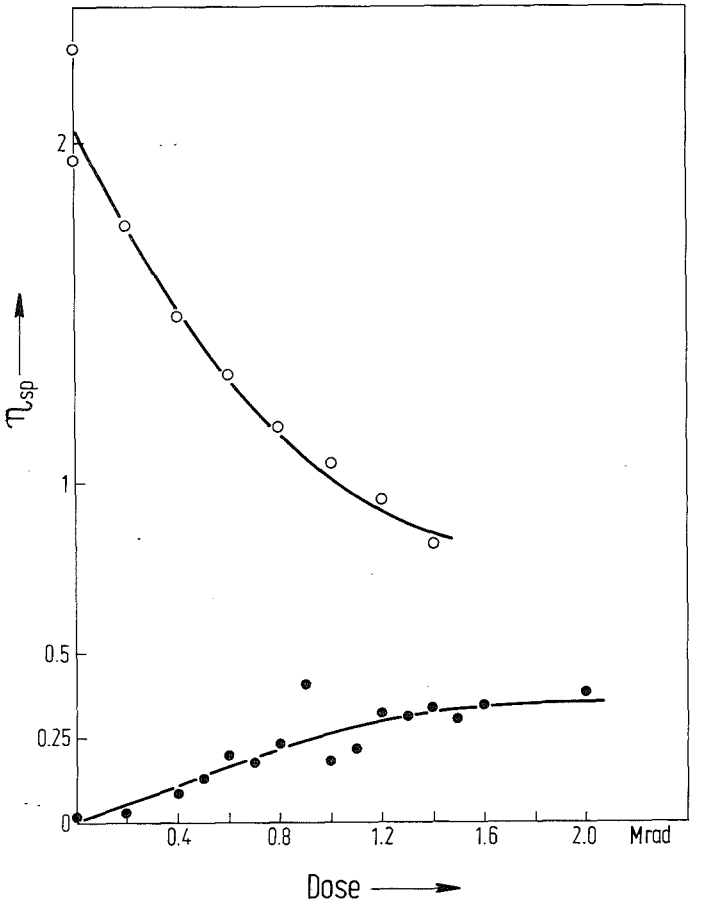


Figure 3. Specific viscosity ($\eta_{sp}$) of a suspension of T1 phages irradiated in 0·1 M histidine with γ-rays (●, lower curve). The initial titre was $2 \times 10^{12}$/ml. Decrease of the specific viscosity of DNA isolated from the phage and γ-irradiated in 0·1 M histidine (○, upper curve). The DNA content of 98 μg/ml corresponds to that of a phage suspension with a titre of $2 \times 10^{12}$/ml.

viscosity of the phage suspension to that of unirradiated DNA solution neglecting the degradation of liberated DNA (full circles in figure 2). The second value for the percentage of ruptured phages is obtained relating the viscosity of phage suspension to the viscosity of the irradiated DNA solution, assuming the liberated DNA to be degraded in the same way as the DNA in free solution (open circles in figure 2). The latter value may lead to an overestimation of the fraction of ruptured phages, as the conformation of the DNA molecules still attached to the ghosts may be more strongly coiled than in free solution and the DNA may be damaged to a less extent. The average of both values leads to a dose–effect curve with a $D_{37}$ of about 3500 krads. This means that 2·5 per cent of the phages with a lethal hit are inactivated by rupture of the protein coat.

3.2. *Inactivation of the killing ability*

We performed the experiment with m.o.i. varying from 1 to 10 to be sure that the loss of the colony formation was not due to the ' lysis from without ' (Delbrück 1940). The killing ability of T1 phage is destroyed exponentially with dose (figure 4). The slope of this curve is smaller by a factor of 0·38 than that of PFA, indicating that 38 per cent of the inactivated phages have lost the ability to kill the host bacteria.

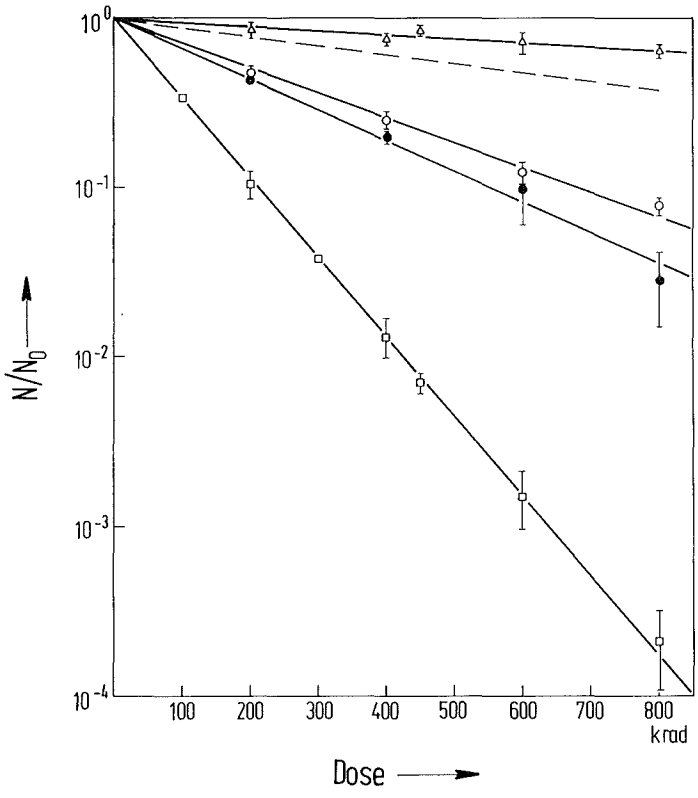


Figure 4. Survival of the plaque-forming ability of T1 phages (□), of the abilities to adsorb (△), to inject the DNA into the host cell (○), and to kill *E. coli* B (●). Dashed line represents the theoretical curve for the partial injection following equation (3) (see discussion).

3.3. *Inactivation of the ability to inject DNA*

The amount of DNA injected from the phages into the bacterial host cell was measured by the amount of radioactivity remaining bound to the bacteria after shearing and centrifugation. As shown in figure 4, the ability to inject the DNA is destroyed exponentially, the $D_{37}$ being 290 krads. Related to the $D_{37}$ of the PFA of 90 krads, this means that 31 per cent of the inactivated phage DNA is not injected.

3.4. *Double-strand breaks in non-injected DNA*

To determine the number of double-strand breaks in the non-injected DNA the phage heads remaining in the supernatant fluid after shearing and centrifugation were concentrated in NCE by centrifugation at 20 000 rev./min for 2 hours. The DNA was extracted following the usual method and sedimented in a sucrose gradient. In figure 5 sedimentation patterns are shown to be obtained for DNA isolated from irradiated whole sample phages (150 krads) as well as for DNA isolated from non-injecting phage heads. The frequency of double-strand breaks per nucleotide pair ($B_2$) was calculated for both DNA preparations and plotted against the dose (figure 6). A good agreement is found between $B_2{}^t$, estimated for phage DNA, by sedimentation in the sucrose gradient and our earlier measurements with the analytical ultracentrifuge. The frequency of double-strand breaks in the non-injected DNA, however, is higher than that

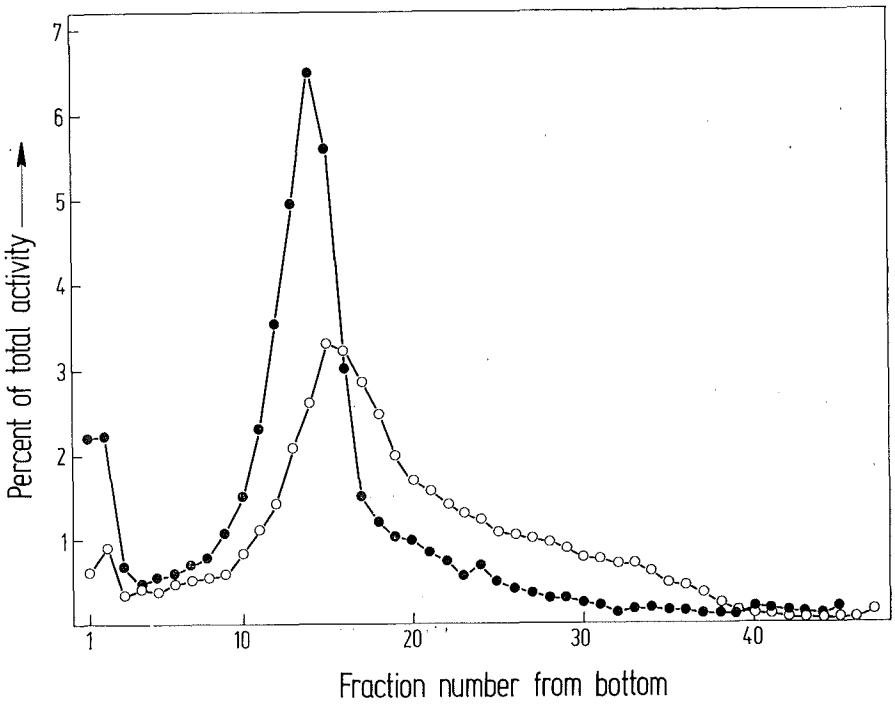


Figure 5. Sedimentation patterns of $^{14}$C-thymine-labelled native T1 DNA in neutral sucrose gradient under standard conditions (3·5 hours at 35 000 rev./min). The DNA was isolated from phages irradiated with a dose of 150 krads (●) and from non-injecting phage heads for the same dose (○).

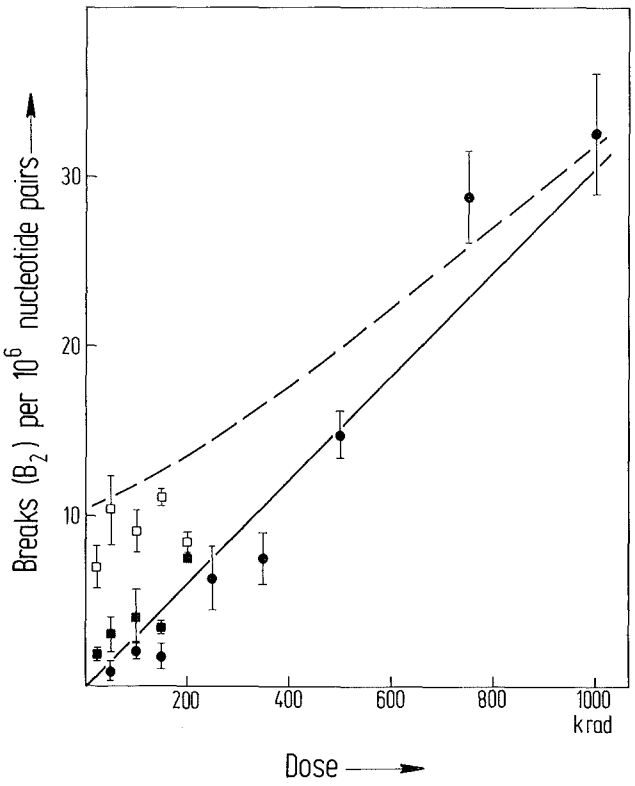


Figure 6. Frequency of double breaks per nucleotide pair ($B_2$) in the DNA of irradiated T1 as a function of the dose. The breaks are estimated by sedimentation of the DNA in the analytical centrifuge (●) (Bohne *et al.* 1970) and by sedimentation in a neutral sucrose gradient (■), ($B_2^t$). Breaks in the non-injected DNA are estimated in a neutral sucrose gradient (□). The dashed line is the theoretical curve calculated for the frequency of double breaks ($B_2^*$) in the not injected DNA under the assumption that all the breaks remain in the phage heads (see discussion).

in the complete sample of irradiated phage. This means that, in the fraction of adsorbed phages which cannot inject, the degradation of DNA is more pronounced than in the total population of irradiated phages.

## 4. Discussion

It is known from earlier work that the ability to adsorb is not appreciably reduced by direct effects of ionizing radiation (Watson 1950, Lytle and Ginoza 1969, Hradečná 1965, Sharp and Freifelder 1971). This was confirmed in the present paper: it was shown that only 5 per cent of inactivated T1 do not adsorb and that half of this fraction of non-adsorbed phages are ruptured. The loss of adsorption ability expresses a protein damage. Damage of the coat protein resulting in a rupture of the head occurs with about the same probability as damage to the tail.

The ability of phages to prevent colony formation may be related to the ability to stop host DNA synthesis (Lytle and Ginoza 1969). Phage DNA has to be injected to disturb the DNA synthesis of the host. This suggests that

the loss of killing ability results from the prevention of injection. It was shown that 38 per cent of the inactivated phage have lost the ability to kill the host bacteria. Similarly, 31 per cent of the lethal effect is due to inactivation of injection. Our observation concerning the killing ability is in good agreement with the results of Watson (1950) who found that approximately 40 per cent of x-irradiated T1 do not kill the host cells.

To explain the inhibition of injection, several models were mentioned in an earlier section, in which the contribution of lesions in the double-strand structure was emphasized. Our results allow us to discriminate between two of the models: the all-or-none injection model (I) and the partial injection model (II). A comparison between the amount of injected DNA and the fraction of DNA molecules without ' double-strand lesions ', i.e. without double-strand breaks or cross-links, is given in figure 7, in relation to the loss of PFA. The number of double-strand lesions in phage T1 was determined by Bohne *et al.* (1970) under the same irradiation conditions as used in this study. The $D_{37}$ of this dose-effect curve was 345 krads (figure 7) which is near to that of the injected DNA ($D_{37}=290$ krads).

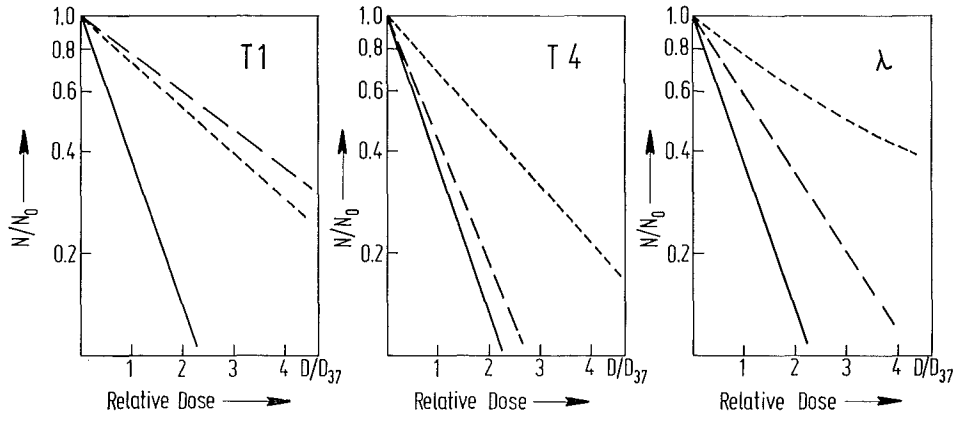


Figure 7. Fraction of DNA with intact double strands (—— ——), fraction of injected DNA (– – – –) and fraction of phages able to form plaques (————) in relation to the $D_{37}$ of the plaque-forming ability. For T1, present paper. For T4, data from Harm (1958) and Freifelder (1968). For λ, data from Sharp and Freifelder (1971).

Our results differ from corresponding observations on other bacteriophages, e.g. T4 or λ (figure 7). There is a fair agreement as to the inactivation of injection measured in T4 by means of marker rescue experiments (Harm 1958) or in λ by autoradiography of the injected DNA (Sharp and Freifelder 1971). Differences are found, however, in the fraction of intact double-strand molecules remaining after irradiation. In T1 far fewer double-strand lesions were found in relation to the PFA than were observed by Freifelder (1968) in T4 and by Sharp and Freifelder (1971) in λ. Concerning the mechanism of inhibition of injection in T1, we therefore reach conclusions differing from those of earlier authors. The similarity of the number of intact double strands to the amount of injected DNA suggests that, in T1, undamaged double-strand molecules only are injected. This favours the all-or-none injection model (I).

A further support of the model I is the observation, that the frequency of the double-strand breaks in the non-injected DNA is far higher than in the total fraction of irradiated phages (figure 6). The experimental points lie near to a theoretical curve which can be calculated for the frequency of double-strand breaks in the non-injected DNA ($B_2^*$) (dashed line in figure 6), provided that all of the breaks remain in the non-injecting phages

$$B_2^* = B_2^t \cdot [(N_A/N_0) - (N_I/N_0)]^{-1}, \quad (2)$$

where $B_2^t$ is the frequency of double-strand breaks per nucleotide pair measured in the total fraction of irradiated DNA. $N_A/N_0$ represents the fraction of adsorbed phages and $N_I/N_0$ the fraction of injecting phages. $[(N_A/N_0) - (N_I/N_0)]$ represents the fraction of phages able to adsorb but not to inject their genome, i.e. the fraction in which $B_2^*$ is measured. Equation (2) does not hold, of course, for values of $D \to 0$.

On the other hand, model (II), the partial injection model, seems less probable in our experiments with T1 phages. From the frequency of ' double-strand lesions ' per DNA molecule per rad ($k$), the amount of DNA can be calculated which is injected into the bacterial host assuming the injection stops on the first double-strand lesion of the genome. The interval $L$ between the free end of DNA and the lesion on the double strand follows the equation (Bresler, Kalinin and Perumov 1964, Sharp and Freifelder 1971),

$$L/L_0 = 1 - \exp(kD)/kD, \quad (3)$$

where $D$ is the absorbed dose in rads, and $L_0$ the total length of the DNA molecule. $L/L_0$ was calculated using $k = 1{\cdot}5 \times 10^{-6}$ as previously determined for the frequency of double-strand lesions per rad (Bohne *et al.* 1970) and plotted against the dose. As shown in figure 4, the calculated amount for the injected DNA, which corresponds to $L/L_0$ in the case of a partial injection model, does not fit at all the inhibition of injection, measured in our experiments. Consequently, the double-strand lesions, i.e. double breaks or cross-links, are most significant for the inhibition of injection, and the experimental results favour the ' all-or-none injection model ' for phage T1.

ACKNOWLEDGMENTS

We should like to thank Drs. Hotz, Jung and Zimmer for their helpful criticism and discussion in the preparation of the manuscript. We appreciate the skilful assistance of Mrs. G. Schütz.

L'irradiation par les rayons $\gamma$ du phage T1, en suspension dans une solution d'histidine 0·1 molaire, entraîne l'inactivation de certaines propriétés du phage et bloque les stades précoces de l'infection. 5 pour cent des phages irradiés présentent des lésions du manteau protéique, soit au niveau de la tête ce qui provoque l'éclatement du phage, soit au niveau de la queue ce qui entrave l'adsorption sur la bactérie. 38 pour cent des phages inactivés perdent la propriété de tuer les bactéries. La quantité de DNA injecté dans la bactérie est mesurée et comparée à la perte de la capacité de former des plages après irradiation. La chance du génome d'être injecté diminue de 31 pour cent par événement léthal.

Le taux de DNA injecté est sensiblement le même que la fraction de phages qui possèdent encore une molécule de DNA avec une double hélice intacte, c'est-à-dire sans cassures ni branchements. Cela suggère que seules les molécules de DNA intactes sont injectées. La localisation de la plus grande partie des doubles cassures dans le DNA non-injecté confirme cette hypothèse. Il semble moins probable que le phage T1 injecte une partie de son DNA lésé.

Nach γ-Bestrahlung einer Suspension von T1 Phagen in 0·1 m Histidin werden verschiedene Eigenschaften der Phagen verändert und auch die frühen Schritte der Infektion gestört. Bei 5 Prozent der inaktivierten Phagen ereignen sich Läsionen im Proteinanteil des Phagen, die entweder zum Platzen der Kopfhülle führen oder die Adsorption des Schwanzes verhindern. Die Fähigkeit, die Wirtszelle abzutöten, geht bei 38 Prozent der inaktivierten Phagen verloren. Weiter wurde die Menge der Phagen-DNS gemessen, die in die Wirtszelle injiziert wird und mit der Inaktivierung der Plaquebildungsfähigkeit verglichen. Die Wahrscheinlichkeit des Phagengenoms, injiziert zu werden, nimmt pro lethalem Ereignis auf 31 Prozent der Kontrolle ab.

Die Menge der injizierten DNS ist ähnlich der Fraktion der Phagen, in deren DNS sich keine Doppelstrangläsionen ereignet haben, weder Doppelstrangbrüche oder Vernetzungen. Dies macht wahrscheinlich, daß nur DNS-Moleküle mit intaktem Doppelstrang injiziert werden. Die Lokalisierung der meisten der Doppelstrangbrüche in der nicht injizierten DNS unterstützt diese Vorstellung. Eine partielle Injektion des geschädigten Phagengenoms scheint dagegen für T1 Phagen wenig wahrscheinlich.

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