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# Fast responses of bacterial membranes to virus adsorption: A fluorescence study*

(bacteriophage infection/timing of cell surface walk/membrane de-energization/membrane fluorescence)

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**ABSTRACT** **After collision with their host cells, virus particles may remain mobile on cell surfaces until they become attached at firm binding sites. We propose that a virion will arrive within a typical median time at such a site, generating a membrane signal such as an increased membrane fluorescence in cells labeled with the voltage-sensitive dyes 8-anilino-1-naphthalene-sulfonate (Mg-salt) (ANS), *N*-phenylnaphthylamine (NPA), or 3, 3′-dipentyl-2, 2′-oxacarbocyanine (di-O-$C_5$[3]). We found that the time span between virus adsorption and fluorescence response varies widely among phages and also depends on bacterial strain, metabolic state, and type of dye. di-O-$C_5$[3]-labeled cells react within 1 sec to uncouplers such as carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). Cells labeled with ANS and NPA react to CCCP in 4–6 sec. Bacteriophages T4, T5, χ, and BF23, added to ANS-labeled cells, change the fluorescence in 9–15 sec. T-even ghosts cause a response at identical times. Baseplate-defective phage mutant $T4^{12-}$ and isolated adsorption organelles of smaller viruses fail to cause an effect. di-O-$C_5$[3]-labeled cells respond to T4 at a multiplicity of infection ≥40 within 1 sec. A longer time (8 sec) is required at lower multiplicities. The receptor-degrading phages ε15, ε34, c341, and K29 need the longest time (1 min for ANS) to cause a fluorescence increase. We suggest that the delayed fluorescence response is concomitant with the surface "walk" of the virion, which is terminated at an injection site. T4 tail sheath contraction coincides with the onset of the membrane fluorescence response.**

The infection process starts with virus–cell collision and terminates with the permeation of nucleic acid into the host bacterium. It was postulated earlier that a one-hit collision event would suffice to produce an infected cell (1, 2). However, more recent evidence shows greater complexity: receptor-containing cell surfaces (3) require sufficient density of receptor molecules or patches (4), and critical levels of membrane energization (5, 6) are needed for infection (7, 8) and for development of the virion (6). Mathematical models (1, 2, 9) consider the entire cell surface as being available for collision and capture with a virion. However, the well-documented positioning of infecting phage particles at discrete membrane-adhesion sites (10, 11) strengthens the hypothesis that a phage is translocated after the initial collision event, either by multiple successive collisions (4, 12) or by a two-dimensional surface walk along the receptor coat (11, 13, 14).

We describe here the response of cell membranes to phage attachment. To measure changes in the electric potential of cell membranes, we used fluorescence dyes that probe the hydrophobic regions of membranes, probably the vicinity of membrane proteins (15, 16): (*i*) 8-anilino-1-naphthalene sulfonate (ANS); (*ii*) *N*-phenylnaphthylamine (NPA); and (*iii*) 3, 3′-dipentyl-2, 2′-oxacarbocyanine (di-O-$C_5$[3]) (17, 18). Uptake of the dye depends on the membrane potential (5, 17–19), which is reduced after interaction of bacterial membranes with a number of colicins (20, 21) and bacteriophages (22, 23). Diminished energization is concomitant with increasing fluorescence (24, 25); we show here that this state of the membrane is initiated by the firm irreversible binding of phage particles. We observed great differences among a variety of phage types in the time of the fluorescence response, probably reflecting the various modes of virus–cell interaction.

## MATERIALS AND METHODS

The bacterial strains and the bacteriophages used in this study have been carried for many years in our laboratory; only those not referenced before are listed, with the laboratory of origin given in parentheses: strains—*Escherichia coli* AW405 (Adler), HB11 (Boyer), *Salmonella ado* (Uetake); phages—$T4^{12-}$ (Yanagida), BF23 (Luria), χ (Adler), MS2 (Walker), fd (Marvin). Cells were grown in nutrient broth (10, 13) or in M9 (2)/0.4% carbon source. Phages were purified from lysates (2) by CsCl density gradients and stored at 4°C after dialysis against either nutrient broth or M9/glucose. Cells were grown at 37°C to densities of 2 or 3 × $10^8$ in culture tubes bubbled with air or $N_2$, sedimented at 25°C, 2000 × *g* for 3 min, and suspended in M9 medium to 1 × $10^9$/ml. 1.5-ml suspensions were placed in the quartz cuvette of an Aminco Bowman Fluorimeter, and kept at 37 ± 0.5°C, unless otherwise noted. Ten microliters of 6 mM ANS in M9 was injected into the cuvette and quickly stirred by bubbling with $N_2$ or air. di-O-$C_5$[3] was used at final concentrations of 0.1–0.5 μM. Fluorescence was measured at the maxima 480, 425, and 510 nm for ANS, NPA, and di-O-$C_5$[3], respectively; the corresponding excitations were 360, 325, and 470 nm. Rapid phage adsorption was studied by mixing with cells and virus for 1 sec, diluting the mixtures (0.1 ml) into cold medium to 1/1000, pelleting at 6000 × *g* for 10 min, resuspending the cells in 200 ml of cold medium, and centrifuging as above. Cells of the pellet were negatively stained at 4°C in 0.05% uranyl acetate (21, 27), and the adherent phages were counted. To estimate the percentage of infected cells after adsorption times of 0.8–1 sec, we subjected [$^{-14}$C]lysine-labeled cells to the same treatment but avoided staining. Plating of these cells on host cell lawns provided the number of infected cells. Assaying their radioactivity enabled us to correct for loss of cells during washing procedures. ANS and NPA were from Eastman; di-O-$C_5$[3] was a gift of A. Waggoner, Amherst College.

Abbreviations: ANS, 8-anilino-1-naphthalenesulfonate (Mg salt); NPA, *N*-phenylnaphthylamine; di-O-$C_5$[3], 3,3-dipentyl-2,2′-oxacarbocyanine; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; moi, multiplicity of infection (in this paper, the term should be understood as the number of infectious phages offered to a cell).

* Parts of this work were reported at the Annual Meetings of the American Society for Microbiology (1978) abstr. #S51; (1979) abstr. #S247; and (1980) abstr. #J1.

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## RESULTS

The membrane fluorescence of dye-labeled *E. coli* and *Salmonella* was measured as it responded to bacteriophage infection. The fluorescence intensity showed sensitivity to $O_2$ (by quenching) and caused de-quenching with uncouplers of oxidative phosphorylation such as carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), as well as with the majority of phages. Unless indicated otherwise, the fluorimeter cuvette was kept open to air, providing a condition in which injection of 0.5 ml of $O_2$ or air with either glucose or lactate present caused both the ANS and the di-O-$C_5$[3] fluorescences to decrease within 1 sec, indicating an increased membrane potential (ref. 28; Fig. 1). Without an extra supply of $O_2$, the fluorescence yield returned to the previous level in 60–80 sec. With glucose as the carbon source, CCCP caused a sharp fluorescence increase in 7–9 sec (due to reduction of membrane potential); addition of T4D at a multiplicity of infection of 5–10 had a similar effect (see below). These responses reached a plateau within 2 min. In M9/glucose, the effect of CCCP could be partially reversed by forcing 0.5 ml of air through the cuvette. However, once the fluorescence plateau had been reached, T4-infected cells showed no response. With lactate as the carbon source, bubbling of 0.2 ml air into the cuvette caused a steeper decrease (quenching) of fluorescence but otherwise the responses were similar to those in glucose-grown cultures. CCCP (0.5 $\mu$M) increased the fluorescence in lactate-grown *E. coli* B in 21.9 ± 6.2 sec (24 experiments); maximum effect was reached in 2 min. After addition of CCCP, addition of T4D had no measurable effect (Fig. 1). di-O-$C_5$[3] responded more rapidly: addition of either CCCP or T4 (of ≥40) led to a fluorescence increase within 1 sec. However, this dye did not fluoresce measurably in our *Salmonella* strains or in capsulated *E. coli*.

**ANS Fluorescence Response to Virus Attack.** The outcome of these experiments was greatly affected by the following:

*Cell Density and moi.* To obtain sufficiently strong fluorescence signals, cell densities of 1 or 2 × $10^9$/ml were used. Calculations (29) show that, at a moi of 1, virus–cell collisions (mean) will occur within 5.4 sec for $\varepsilon$ phages or ≈9 sec for T4. A decrease in cell density from 5 × $10^8$/ml to 5 × $10^7$/ml delayed the fluorescence response 16–60 sec. The onset of the fluorescence response, as well as its intensity, depended on the moi of the virus. Multiplicities of 100 T4 showed the shortest reaction times (9 sec with ANS); however, a moi >50 was avoided. With ANS- and NPA-labeled cells ($10^9$/ml), the average timing for the response to T4 was ≈13 sec.

*Temperature.* At 21°C, *Salmonella* phage $\varepsilon$15 (moi 20–80) failed to elicit a fluorescence response even 4 min after phage addition (Table 1). At 23°C and a moi of 10 or 20, a delayed response (85–90 sec) was observed. In contrast, T4D elicited a strong fluorescence at 20–21°C, but showed a time delay of up to 18 sec.

*Growth Conditions and Cell Strain.* Stationary *E. coli* B from "overnight" glucose-nutrient agar plates, washed and resuspended in M9/glucose at 37°C, needed 70 sec to respond to phage T4D in ANS (Table 1). *E. coli* B, with a generation time of 55 min in M9/0.4% lactate, responded to T4D after ≈40 sec (see Table 2), whereas *E. coli* B growing in M9/glucose had a generation time of 25 min and responded to T4 in 13 sec. Significant differences were also found between cell strains: *E. coli* M72, growing aerobically in M9/glucose with a 25-min generation time, responded to T4D after 28 ± 4.0 sec, twice the time for *E. coli* B under identical conditions.

**Comparison of Fluorescence Response Among Different Phages.** The fluorescence response times (comparable culture conditions) varied considerably among bacteriophages (Table 2). T4, T5, and BF23 averaged 13, 13, and 11.5 sec, respectively; the flagellotropic phage $\chi$ needed 15 sec. Phage T2, however, required 48 sec with *E. coli* B as host (Fig. 2) and 60 sec with *E. coli* Cla as host. $\varepsilon$ and K phages elicited the signal in 54–66 sec (Fig. 3). The small phage $\phi$X174 required an unexpectedly long time span (42 sec) with *E. coli* Cla as host cell (Fig. 3). Phage T5, adsorbed to *E. coli* B under glycolytic conditions, showed an initial response after 13 sec. We did not study the later response, occurring after 4–6 min, reported previously (22, 23, 33). Phages MS2 and fd, requiring the F pilus for adsorption, caused very little increase in fluorescence activity, measurable only at a moi of 25–100.

Anaerobic conditions led to long response times: 39 ± 10 sec for T4D and 30 ± 6 sec for T5. In contrast, the receptor-degrading phage $\varepsilon$15 showed no prolonged response time under either glycolytic or aerobic conditions. Fluorescence of cells maintained under $N_2$ in M9/lactate did not respond to addition of phage T4.

**Defective Phage and Ghosts.** Empty capsids (ghosts) obtained by osmotic shock treatment of phages T2 and T4 (34) showed a fluorescence timing indistinguishable from that of the infectious virus preparations (Table 2). Baseplate-defective mutant $T4^{12^-}$ (26) falls off the cell during tail contraction (35, 36). Accordingly, $T4^{12^-}$ failed to cause a measurable effect (moi, 3–50) (Fig. 2; Table 2).

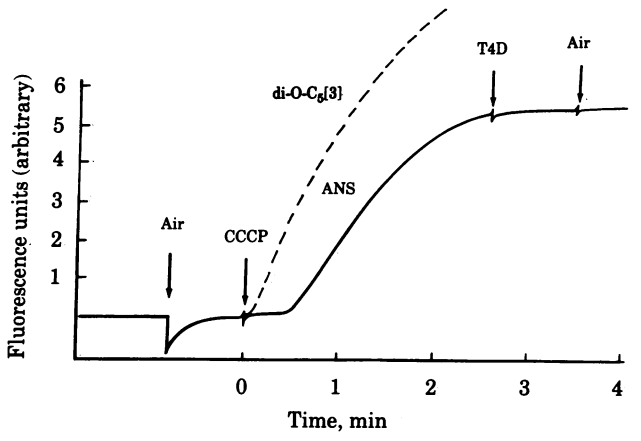


FIG. 1. Fluorescence responses of ANS-labeled *E. coli* B after injection of air (0.5 ml), CCCP (2 $\mu$M), and T4D (moi = 5). The small spikes in the curve are caused by the injection process.

Table 1. Temperature dependence of fluorescence response in ANS

| Phage | moi | Cells | Temperature, °C | Onset of response, sec |
|---|---|---|---|---|
| $\varepsilon$15 | 20 | *S. anatum* | 41 | 60 |
| $\varepsilon$15 | 20 | *S. anatum* | 37 | 60 |
| $\varepsilon$15 | 10 | *S. anatum* | 23 | 90 |
| $\varepsilon$15 | 20 | *S. anatum* | 23 | 85 |
| $\varepsilon$15 | 20 | *S. anatum* | 21 | None* |
| $\varepsilon$15 | 80 | *S. anatum* | 21 | None* |
| T4D | 4 | *E. coli* B | 20 | 18 |
| T4D | 4 | *E. coli* B | 21.5 | 18 |
| T4D | 5 | *E. coli* B† | 37 | 18 |
| T4D | 5 | *E. coli* B | 37 | 10 |
| T4D | 5 | *E. coli* B‡ | 37 | 70 |

Response of cells ($10^9$/ml unless otherwise noted) in the logarithmic phase of growth in M9/glucose was measured in the presence of air after addition of phage at $t = 0$.

* No increase after 4 min.

† Cells at 2 × $10^8$/ml.

‡ Cells from overnight L-broth plate suspended in M9/glucose.

Table 2. ANS-fluorescence response of host cells to bacteriophage adsorption/infection

| Phage | moi | Host cell | Phage receptor* | Fluorescence response time, sec |
|---|---|---|---|---|
| T4D | 1–80 | *E. coli* B | LPS-C (30) | 13.6 ± 1.8 |
| T4D | 10–30 | *E. coli* B | LPS-C | 11 ± 1 |
| T4D | 5–25 | *E. coli* B† | LPS-C | 55.3 ± 5.2 |
| T4D | 1–20 | *E. coli* B‡ | LPS-C | 39 ± 10.7 |
| T4D | 1–20 | *E. coli* B†‡ | LPS-C | None |
| T4D | 1–20 | *E. coli* M72 | LPS-C | 28 ± 4 |
| $T4^{12-}$ | 3–50 | *E. coli* B | LPS-C | None |
| T4 ghosts | 1 | *E. coli* B | LPS-C | 15 ± 3 |
| T2 | 5–10 | *E. coli* B | Protein Ia (31) | 48.3 ± 9.0 |
| T2 | 3 | *E. coli* Cla | | 60 ± 4 |
| T2 ghosts | 2 | *E. coli* B | | 60 ± 5 |
| K29 | 10–100 | *E. coli* Bil46 (K29) | Capsule polysaccharide (14) | 66.8 ± 8.5 |
| ε15 | 2–100 | *S. anatum* [3, 10] | LPS-O (14, 32) | 62.8 ± 10.1 |
| ε15 | 5–30 | *S. anatum*† | LPS-O | 65.5 ± 9.5 |
| ε34 | 10–60 | *S. anatum* [3, 15] | LPS-O (14) | 54.1 ± 5.3 |
| c341 | 10–30 | *S. anatum* [3, 10] | LPS-O | 60 ± 8 |
| φX174 | 5–80 | *E. coli* Cla | LPS-C (14) | 42.5 ± 10.2 |
| φX174 (spikes only) | | *E. coli* Cla | LPS-C | None |
| φX174 (spikes, then virus) | | *E. coli* Cla | LPS-C | None |
| T7 | 5–20 | *E. coli* B | LPS-C (3) | 44.5 ± 5.6 |
| T5 | 5–20 | *E. coli* B | Protein tonA (8, 52) | 13 ± 2 |
| T5 | 5–20 | *E. coli* B† | Protein tonA | 30 ± 6 |
| BF23 | 4–5 | *E. coli* B | Protein bfe (52) | 11.5 ± 1.5 |
| χ | 20–50 | *E. coli* AW405§ | Flagellum (3) | 15 ± 1 |
| MS2 | 25–200 | *E. coli* HB11¶ | F pilus | 85 ± 10 |
| fd | 50–100 | *E. coli* HB11¶ | F pilus | 67.4 ± 18.5 |

Response of cells ($10^9$/ml) was measured in M9/glucose and in presence of air unless otherwise noted.
* LPS-C and LPS-O, lipopolysaccharide core and O antigens, respectively. Values in brackets represent *S. anatum* O-antigen types.
† $N_2$ purged.
‡ Response measured in M9/lactate.
§ Response measured in M9/galactose.
¶ Response measured in M9/lactose.

Isolated adsorption organelles of phages $\varepsilon_{15}$ and φX174 (27, 37) did not change the host cell fluorescence (Table 2; Fig. 3); however, their addition to host cells blocked subsequently added complete virus from generating the fluorescence signal.

**Superinfection.** During infections that are followed within a few minutes by a second infection of homologous virus, the genetic information of the superinfecting phage is excluded (38–40). Homologous superinfecting phages T2, T4 (Fig. 2), ε15, ε34, and K29 failed to affect the fluorescence yield unless superinfection occurred before maximum fluorescence from the first infection had been reached; although a steeper increase in the intensity was produced, the maximal fluorescence intensity was identical to that of a singly infected culture.

**di-O-$C_5$[3] Fluorescence Response to Virus Attack.** di-O-$C_5$[3], a sensitive probe for membrane potentials (24, 25), is rapidly incorporated into the membranes of *E. coli* B, CIA, and HB11. Addition of $10^9$ uninfected cells to the dye (0.1 μM in M9/glucose) caused a 10-fold higher fluorescence intensity than the analogous procedure using 6 μM ANS. However, capsular *E. coli* K29 and "smooth" lipopolysaccharide-containing *Salmonella anatum* failed to show a significant increase with the cyanine dye, possibly due to interference in penetration of the charged-surface polysaccharides of these cell strains; other membrane components (proteins, pores) might also be responsible. In contrast, the ANS label allowed us to compare a wide range of cell strains including the cyanine-impermeable cells. However, the response with di-O-$C_5$[3] is considerably faster than that with ANS. Addition of T4D to labeled *E. coli* B (moi, 40–70) produced a steep fluorescence increase within 1 sec; at a moi of 6–10, the fluorescence signal occurred after 8 sec (Fig. 4). The response to CCCP required 1 sec with cyanine and 7–9 sec with ANS. Similarly, the fluorescence response to other phages, such as T2 (moi, 8) (48.3 sec with ANS-labeled cells), was 13 sec with the cyanine dye, that of φX174 (42 sec in ANS) was 30 sec in di-O-$C_5$[3]. A fluorescence plateau was reached after 3 or 4 min in all experiments and persisted until measurement was stopped 3 or 4 min later.

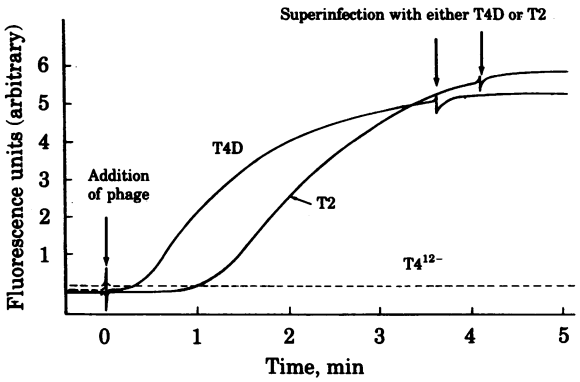


FIG. 2. Increase in fluorescence of ANS-labeled *E. coli* B ($1 \times 10^9$ cells/ml) after addition of phages T4, $T4^{12-}$, and T2 at moi = 5.

**Tail Contraction.** Tail sheath contraction of phage T4 appears to correlate with the fluorescence response of the cell. When 0.1 ml of cells at 37°C were squirted into a small virus-containing dish (starting adsorption, T4 at moi = 40) and the dish then tossed into chilled medium to dilute the virus-cell mixture 1/2000 and thereby stop further adsorption, adsorption times of 0.8–1.3 sec were regularly obtained. Inspection of these cells in the electron microscope for adsorbed virus showed that, of 53 cells inspected, 7 had one T4 particle adsorbed, with each virion showing a contracted tail (and an empty head). One cell had two phages attached, one with an extended and one with a contracted sheath. Plating of the washed cells on host cell

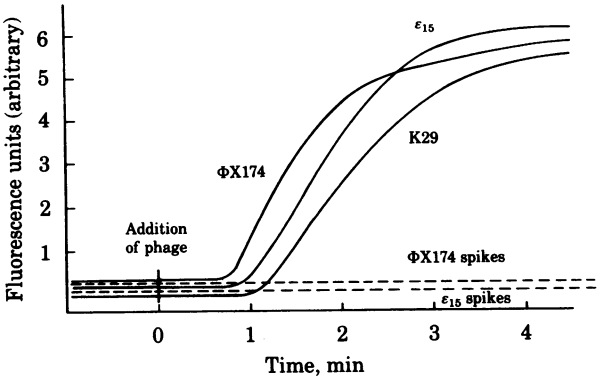


FIG. 3. Increase in ANS fluorescence in response to *Salmonella* phage ε15, to capsule-specific phage K29, to φX174, and to isolated adsorption organelles ("spikes") of φX174 and ε15.

lawns showed that the number of infectious centers increased with the moi (Table 3). T4-resistant cells were lacking adsorbed virus particle, whereas their infectious-center counts (after a 1.4-sec adsorption time) represented the experiment's background (trapped virions). These data suggest that tail contraction of phage T4 is concomitant with (*i*) infection of cells and (*ii*) the fluorescence increase in di-O-$C_5$[3]. Therefore, tail sheath contraction or analogous capsid interactions appear to affect the electrogenic processes of host cell membranes rapidly (5, 6, 20, 22, 28).

## DISCUSSION

We describe measurement of the time span between initial virus–cell collision and irreversible attachment of the virion. This time period is thought to represent a continuing or rapidly renewing cell contact of adsorption organelles and cell—i.e., a cell surface "walk." Infectious virions can still be desorbed during this period (2, 9, 27). Virus adsorption kinetics is measured by the decreasing amount of unadsorbed virus in the virus–cell mixture and the increasing number of infected cells in the mixture (13, 34). In such experiments, the fastest adsorption rates follow one-hit kinetics with the speed of adsorption being limited by the diffusion of the virus particle (11). Table 4 shows the computed time of virus–cell collision at a moi of 1. At higher moi, the time of first collision is proportionally shorter (S. Litwin, personal communication). After collision, a virus walk may come about in several ways. (*i*) The virion may stay in the neighborhood of the cell surface (4, 12); it will hit the cell repeatedly with a high probability and, eventually, attach to a site at which injection of nucleic acid is triggered. (*ii*) The "true" walk is combined with receptor penetration as, for example, in capsule-specific phage K29 and lipopolysaccharide-specific phages (13). Electron microscope studies showed that these virions maintain receptor contact as they "burrow" a path through or "browse" along the polysaccharide surface layer without releasing their nucleic acid (13, 27); for the viral DNA to be ejected, further specific interactions of virus and cell surface are necessary (7, 8, 32, 41). We demonstrated here that T4D may cause a membrane response within 1 sec (Fig. 4), that tail sheath contraction also occurs at that time, and that >10% of the host cells are "infected" (Table 3). A short pre-infectious

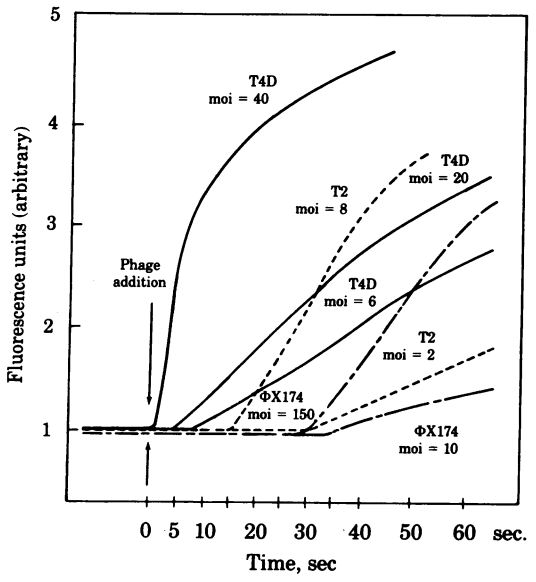


FIG. 4. Dependence of fluorescence response of di-O-$C_5$[3]-labeled cells on phage and moi. Note the generally faster response compared with ANS fluorescence.

Table 3. Effect of moi on T4 infection at adsorption times of 1 ± 0.1 sec

| moi | Adsorption time, sec | Cells infected, % |
|---|---|---|
| 1 | 1.1 | 0.1 |
| 10 | 0.9 | 1.8 |
| 15 | 0.8 | 2.1 |
| 41 | 0.9 | 4.8 |
| 70 | 1.0 | 13.2 |
| 46* | 1.4 | 0.3 |

*E. coli* B cells at $2 \times 10^9$ were washed and suspended in 1 ml of M9 medium. Cell suspensions provided infectious centers after plating on *E. coli* B lawns.
* Experiment using T4-resistant cells (*E. coli* B/3, 4, 7).

period was also concluded from an elegant set of experiments in which the infection of T4 was terminated by osmotic shock; plating of the shock mixture showed infected cells 10–15 seconds after virus adsorption (34). DNA packaging in the phage head, when measured with electron spin resonance, showed a relaxation within 15 sec after mixing of virus and host (42). The relatively slow relaxation of the phage DNA packing may be due to slow release of the DNA or to differences in cell concentration and growth conditions. Furthermore, one phage is able to generate a strong fluorescence due to the amplification of the fluorescence over the entire cell membrane (43). Phage $T4^{12-}$, which failed to produce a fluorescence response (Fig. 2; Table 2), also showed a rather weak DNA uncoiling in the presence of host bacteria (42), probably due to shedding of phage DNA into the medium after abortive cell contact (44). Our results show that the surface contact by tail fibers and other adsorption organelles alone does not evoke a fluorescence signal, whereas the effect of ghosts of T4 or T2 is equivalent to that of complete phage in which the fluorescence response parallels tail sheath contraction of adsorbed virions. Other phages exhibiting different DNA-ejection mechanisms (14) also trigger the fluorescence response of voltage-sensitive dyes, expressing membrane de-energization (15, 17, 24, 25, 28, 45). ANS-labeled cells seem to measure membrane events at another level or compartment of the cell surface; such labeled cells respond to air in 1 sec (or less), while CCCP-induced de-energization becomes visible in ≈7 sec. In contrast, cyanine dye-labeled cells respond to both conditions in 1 sec. However, ANS can interact with the membranes of all of our cell strains, making the comparative studies possible. Another dye, 3,3-dimethylindodicarbocyamine iodide, does not fluoresce in our cells but seems to require EDTA-pretreated cells (46). A decreased fluorescence shortly after infection (47) was not observed with our cyanine dye.

In conclusion: (*i*) The diffusion constant of the virion does not permit prediction of the timing of the fluorescence signal; the largest virus particle tested (T4) showed the most rapid cell response. However, the slow actions of phage T2, which has the same adsorption rate constant as T4, and of a "receptor" protein

Table 4. Comparison of time required for virus–cell collision and onset of fluorescence

| Phage | $K_D$, cm²/sec | Collision time, sec | | Fluorescence response time,* sec | |
|---|---|---|---|---|---|
| | | Mean | Median | ANS | di-O-$C_5$[3] |
| T4D | $3.2 \times 10^{-8}$ | 13 | 9 | 13.6 ± 1.8 | 8 ± 1.5 |
| T2 | $3.3 \times 10^{-8}$ | 13 | 9 | 48.3 ± 9 | 31 ± 2 |
| φX174 | $1.9 \times 10^{-7}$ | 2.2 | 1.6 | 42.5 ± 10.2 | 32 ± 2 |
| ε15 | $9 \times 10^{-8}$ | 7.4 | 5.4 | 62.8 ± 10.1 | |

Collision times were calculated for $1 \times 10^9$ cells/ml and moi = 1; at higher moi, for example, 5, the median time at which a cell is hit by a virion is reduced to one-fifth of that at moi = 1.
* moi = 5.

(Ia; ref. 31), which constitutes an abundant outer membrane protein in *E. coli* B, remain unclear.

(*ii*) The fluorescence response time is independent of the chemical class of receptor: of the phages specific for "rough" lipopolysaccharide (30), $\phi$X174, with its small size and fast adsorption rate (11, 46), has a fluorescence-response time 3 times that of T4. In contrast, capsule-degrading *E. coli* phage K29 and O-antigen-degrading *Salmonella* phages $\varepsilon$15, $\varepsilon$34, and c341 cause a fluorescence increase in 55 and 65 sec, respectively, independent of the moi (moi $\geqq$ 1).

(*iii*) Phage $\varepsilon$15 failed to elicit a fluorescence response at 21°C or below (Table 1), at which infection is blocked (32).

(*iv*) The difference in T4-induced fluorescence between *E. coli* B and M72 may be due to differences in adsorption rate constants ($4.06 \times 10^{-10}$ ml/min for *E. coli* B vs. $1.85 \times 10^{-10}$ ml/min for M72). Also, slower adsorption rates of T4 ($k = 3 \times 10^{-10}$ ml/min) were found with *E. coli* B grown in lactic acid; the fluorescence onset was delayed in comparison with that of cells in glucose. We hypothesize that fluorescence timing may reflect the concentration of "triggering" sites or different walking mechanisms affecting the efficiency among virus types: T-even viruses need their long tail fibers for adsorption (48), phages T5 and $\lambda$ require small tail fibers for efficient adsorption (49), and phages such as P22 (50) depend for infection on functional proteins of their adsorption organelles, including the ability to hydrolyze the receptor molecule (51).

As bacteriophages infect the cells at the areas of adhesion (11), most of the virus–cell collisions occur outside these areas. A high moi will elicit more direct hits at the adhesion site, an increased rate of infected cells, and fast fluorescence responses. A low moi necessitates a time-consuming search for the prospective injection site. We hypothesize that virus migration stops on encounter with a cell surface site capable of triggering tail sheath contraction (in T-even phages) or unplugging of the capsid (in $\phi$X174), or induction of analogous responses (52).

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1. Schlesinger, M. (1932) *Z. Hyg. Infektionskrankh.* **114**, 136–159.
2. Adams, M. H. (1959) in *Bacteriophages*, ed. Adams, M. (Interscience, Wiley, New York), pp. 138.
3. Lindberg, A. A. (1973) *Annu. Rev. Microbiol.* **27**, 205–241.
4. Berg, H. & Purcell, E. M. (1977) *Biophys. J.* **20**, 195–219.
5. Harold, F. M. (1977) *Curr. Top. Bioenerg.* **6**, 83–149.
6. Wagner, E. F., Ponta, H. & Schweiger, M. (1980) *J. Biol. Chem.* **255**, 534–539.
7. Labedan, B. & Goldberg, E. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4669–4673.
8. Hancock, R. E. W. & Braun, V. (1976) *J. Bacteriol.* **125**, 409–415.
9. Stent, G. S. (1963) in *Molecular Biology of Bacterial Viruses* (Freeman, San Francisco), pp. 88–96.
10. Bayer, M. E. (1968) *J. Gen. Microbiol.* **53**, 395–404.
11. Bayer, M. E. & Starkey, T. W. (1972) *Virology* **49**, 236–256.
12. Schwartz, M. (1976) *J. Mol. Biol.* **103**, 521–536.
13. Bayer, M. E., Thurow, H. & Bayer, M. H. (1979) *Virology* **94**, 95–118.
14. Lindberg, A. (1977) in *Surface Carbohydrates of the Prokaryotic Cell*, ed. Sutherland, I. W. (Academic, New York), pp. 289–356.
15. Gulik-Krzywicki, T., Schechter, E., Iwatsubo, M., Ranck, J. L. & Luzzati, V. (1970) *Biochim. Biophys. Acta* **219**, 1–10.
16. Nieva-Gomez, D. & Gennis, R. B. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1811–1815.
17. Waggoner, A. (1976) *J. Membr. Biol.* **27**, 317–334.
18. Sims, P. J., Waggoner, A. S., Wang, C.-H. & Hoffman, J. F. (1974) *Biochemistry* **13**, 3315–3330.
19. Padan, E., Zilberstein, D. & Rottenberg, H. (1976) *Eur. J. Biochem.* **63**, 533–541.
20. Weiss, M. & Luria, S. E. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2483–2487.
21. Konisky, J. (1978) in *The Bacteria*, eds. Ornston, L. N. & Sokatch, J. R. (Academic, New York), Vol. 6, pp. 71–136.
22. Hantke, K. & Braun, V. (1974) *Virology* **58**, 310–312.
23. Oldmixon, E. & Braun, V. (1979) *J. Supramolec. Struct.* **10**, 329–347.
24. Brewer, G. J. (1976) *Biochemistry* **15**, 1387–1392.
25. Seligmann, B. E., Gallin, E. K., Martin, D. L., Shain, W. & Gallin, J. I. (1980) *J. Membr. Biol.* **52**, 257–272.
26. Crowther, R. A., Kikuchi, Y., Lenk, E. V. & King, J. (1977) *J. Mol. Biol.* **116**, 489–523.
27. Bayer, M. E., Takeda, K. & Uetake, H. (1980) *Virology* **105**, 328–337.
28. Griniuviene, B., Dzheia, P. & Grinius, L. (1975) *Biochem. Biophys. Res. Commun.* **64**, 790–796.
29. Litwin, S. (1980) *Biophys. J.* **31**, 271–277.
30. Prehm, P., Jann, B., Jann, K., Schmidt, G. & Stirm, S. (1976) *J. Mol. Biol.* **101**, 277–281.
31. Hantke, K. (1978) *Mol. Gen. Genet.* **164**, 131–135.
32. McConnell, M., Reznick, A. & Wright, A. (1979) *Virology* **94**, 10–23.
33. Glenn, J. & Duckworth, D. H. (1980) *J. Virol.* **33**, 553–556.
34. Anderson, T. F. (1953) *Cold Spring Harbor Symp. Quant. Biol.* **18**, 197–203.
35. Simon, L. D., Swan, J. G. & Flatgaard, J. E. (1970) *Virology* **41**, 77–90.
36. King, J. (1968) *J. Mol. Biol.* **32**, 231–262.
37. Edgell, M. H., Hutchinson, C. A. & Sinsheimer, R. L. (1969) *J. Mol. Biol.* **42**, 547–557.
38. Graham, A. (1953) *Ann. Inst. Pasteur* **84**, 90–94.
39. Hershey, A. D., Garen, A., Fraser, D. K. & Hudis, J. D. (1954) *Carnegie Inst. Wash. Yearb.* **53**, 210–212.
40. Hirsch-Kauffmann, M., Pfennig-Yeh, M.-L., Ponta, H., Herrlich, P. & Schweiger, M. (1976) *Mol. Gen. Genet.* **149**, 243–249.
41. Furukawa, H., Yamada, H. & Mizushima, S. (1979) *J. Bacteriol.* **140**, 1071–1080.
42. Maeda, T., Ohnishi, S.-I. & Yanagida, M. (1978) *FEBS Letts.* **89**, 29–32.
43. Luria, S. (1973) in *Bacterial Membranes and Walls*, ed. Leive, L. (Dekker, New York), pp. 293–320.
44. Benz, W. C. & Goldberg, E. B. (1973) *Virology* **53**, 225–235.
45. Reeves, J. P., Lombardi, F. J. & Kaback, R. H. (1972) *J. Biol. Chem.* **247**, 6204–6211.
46. Labedan, B. & Letellier, L. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 215–219.
47. Bayer, M. E. & DeBlois, R. W. (1974) *J. Virol.* **14**, 975–980.
48. Crawford, J. T. & Goldberg, E. B. (1980) *J. Mol. Biol.* **139**, 679–690.
49. Heller, K. & Braun, V. (1979) *J. Bacteriol.* **139**, 32–38.
50. MacKay, D. & Bode, V. (1976) *Virology* **72**, 154–166.
51. Berget, P. B. & Poteete, A. R. (1980) *J. Virol.* **34**, 234–243.
52. DiRienzo, J. M., Nakamura, K. & Inouye, M. (1978) *Annu. Rev. Biochem.* **47**, 481–532.