MECHANISM OF CELL WALL PENETRATION BY VIRUSES*

I. An Increase in Host Cell Permeability Induced by Bacteriophage Infection‡

By THEODORE T. PUCK, Ph.D., and HOWARD H. LEE

(From the Department of Biophysics, Florence R. Sabin Laboratories, University of Colorado Medical Center, Denver)

(Received for publication, January 30, 1954)

The current status of delineation of the first phases of the invasive cycle of T2 bacteriophage is as follows: (*a*) Cell attachment is effected by binding of viral amino groups to complementarily spaced carboxyl groups on the cell surface (1, 2). (*b*) As a result of the establishment of these electrostatic bonds, the virus splits into its protein and DNA components (3, 2). (*c*) Only the DNA moiety penetrates into the cell, all or most of the protein remaining at the cell surface and apparently playing no further role in the subsequent reproductive steps (4).

The mechanism of the reaction by which the DNA is injected into the cell is still obscure. Mere splitting of the virus attached to the cell surface is not sufficient to cause DNA penetration. Thus, when cell enzymes are inactivated by previous heating to 70°C., virus attachment and splitting still occur, but most of the DNA is liberated in free form into the medium instead of penetrating into the cell (5). Hence, the injection reaction appears to require action of cellular enzymes. Moreover, by manipulation of the salt concentration and the temperature, cell-attached virus can be induced to eject its DNA into the medium. Such virus does not contribute to cell killing (5). Since the cell-killing function has been shown to reside in the protein moiety alone (6), the conclusion seems warranted that DNA injection requires a triggering of cellular surface enzymes by the protein component of the virus, and that successful triggering is sufficient to cause death of the cell, even if the DNA does not afterwards enter. Finally, penetration is complete within 3 to 5 minutes at 37°C. because new specific synthesis of virus DNA inside the cell begins within this period (7). Hence, the penetration mechanism appears to be initiated by the protein component of the virus; its first steps are purely electrostatic, but it thereafter requires participation of enzymes in the cell surface; it leads to

* This work is part of a research program carried out under Contract No. AT-(11-1)-269 with the Division of Biology and Medicine of the Atomic Energy Commission.

‡ Contribution No. 23.

death of the cell within 15 seconds, and is complete within a time less than 5 minutes at 37°C.

It has been suggested (5) that the reaction evoked by the viral amino groups is a lytic process in the cell wall which increases its permeability sufficiently to admit the macromolecular DNA. Experiments suggesed by this concept are herewith described.

Methods and Materials

Labelled Cells.—Cells of *Escherichia coli* B and its mutants were shaken at 37°C. (*a*) in a medium consisting of ½ per cent bacto-peptone (Difco), ½ per cent NaCl, and 0.1 per cent dextrose, to which was added $P^{32}O_4$, or (*b*) glycerol lactate medium (4) plus $S^{35}O_4$, the amounts of each isotope corresponding to 0.7 μc./cm.^{3} or 7 μc./cm.^{3}, respectively. The cultures were seeded with a washed inoculum of cells (10^8/cm.^{3}), and grown for an additional 2 hours after which the cells were harvested, washed 5 times to remove supernatant radioactivity, and resuspended in nutrient broth (Difco), which contained ½ per cent added NaCl in all the experiments here reported. Such cells could be stored at 0°C. for more than a week when labelled with S^{35}, but the P^{32}-labelled cultures deteriorated after 4 to 5 days. The final suspensions which were always washed again just before each experiment contained approximately 10^9 bacteria/cm.^{3} and displayed counting rates of 1000 to 7000 counts/cm.^{3}sec. when measured with a G.M. tube containing an end-window of thickness equal to 2 mg./cm^2. Radioactive assays were carried out in multiplicate on aliquots diluted to yield counting rates of 5 to 50 counts per second, and each assay included at least 2048 counts. The precision of duplicate sample determinations was approximately ± 3 to 5 per cent. Because of its soft radiation, particular care was exercised with S^{35} to insure a constant thickness of absorbing material on the planchets in all radioactivity measurements. All centrifugations (except those involved in purification of virus stocks when ultracentrifugation is necessary) were performed in a refrigerated, angle head International centrifuge, (12 cm. radius) operating at 3000 R.P.M. All the present experiments involved the viruses T2r$^+$ and T1, suspensions of which were prepared by methods which are now standard (8) and purified by 3 alternating cycles of low speed and high speed centrifugation (4). Titration of virus particles of these suspensions by plaque formation and by ability to kill host cells (8) usually gave values agreeing within 30 to 40 per cent.

The following chemical procedures were carried out on various cell fractions obtained in the course of this study:—

Determination of trichloroacetic acid (TCA) solubility:—

To the sample maintained at 0°C., bovine plasma albumin was added to a concentration of 1 mg./cc., followed by TCA to a final concentration of 5 per cent. The sample was kept at 0°C. for 5 minutes to insure completeness of precipitation, then centrifuged for 8 minutes at 0° at a speed of 3000 R.P.M. The clear supernatant was decanted and its radioisotope content determined.

Determination of fractions sensitive to desoxy-ribo-nuclease (DNA-ase):

To one portion of the sample at 0°C. was added crystallized DNA-ase[1] to a final concentration of 0.10 mg./cc. in the presence of 3×10^{-3} M Mg^{++}, while to another, maintained under identical conditions, an equal amount of saline without enzyme was added. Both tubes were incubated for 25 minutes at 37°C., then returned to the ice bath and their total P^{32} radioactivity titrated. Albumin and TCA were added to each, as described above; the tubes

[1] Worthington Biochemical Laboratories, Freehold, New Jersey.

were centrifuged and the clear supernatants titrated again for radioactivity. Any increased P^{32} activity in the supernatants of the tube which had been treated with the enzyme represents P^{32} which had been present as DNA. In studies with DNA obtained from T2 virus, approximately 70 per cent of the DNA–P^{32} was solubilized by this procedure (3).

Throughout this paper, the terms, non-reproductive lysis, abortive lysis, or lysis-from-without, shall be used to indicate the cell destruction unaccompanied by new virus synthesis, which occurs as a result of attachment of a large excess of bacteriophage to normal host cells in nutrient medium (9). The term multiplicity denotes the virus:cell ratio of a suspension.

TABLE I

Typical Experimental Data Indicating the Effect of T2 Infection in Causing an Increased Transfer of P^{32} and S^{35} from Labelled Cells to the Medium

Cell label	Infection multiplicity	Radioactivity released into medium	Initial cellular radioactivity released	
			Gross	Net
	virus particles/cell	*counts/cm.²sec.*	*per cent*	*per cent*
(*A*) P^{32}	0	124.6	5.71	—
	3	354	16.2	11.5
(*B*) S^{35}	0	2.35	0.94	—
	9	22.7	9.20	8.26

Young cultures of *E. coli* B (3–4 × 10^8/cm.³) respectively labelled with P^{32} (*A*) and S^{35} (*B*) were suspended in nutrient broth and infected with T2 in the multiplicities shown, at 0°C. After standing about 4 minutes to permit attachment, the tubes were transferred to a 37°C. bath for 3 minutes, then returned to the 0°C. bath. The radioactivities of the total suspensions and the supernatants were measured.

EXPERIMENTAL RESULTS

1. Demonstration That Virus Infection Induces Leakage of Cellular Constituents

Washed cells respectively labelled with P^{32} and S^{35} were suspended in nutrient broth at 0°C., and inoculated with T2 bacteriophage. The tubes were then incubated for 4 minutes at 37°C. after which they were chilled, centrifuged in the cold, and the radioisotope content of the supernatants assayed and compared to that of control tubes treated in an identical manner except that they received no virus.

As the data of Table I indicate, 16.0 per cent of the total P^{32} and 9.2 per cent of the S^{35} content of the cells were liberated into the medium, amounts which far exceed those released from the uninfected cells carried through an identical procedure. Although the absolute amount of radioactivity liberated either from infected or uninfected cells varied with the age and history of each culture, the presence of a distinct virus-induced increase in isotope content of the supernatant medium was completely reproduceable in a series of more than fifty experiments.

2. Demonstration That Each Infected Cell Participates in the Leakage Reaction

Data like those of Table I do not prove that irreversible attachment of T2 virus alters the permeability of the host cell. The same results would have been obtained if a few per cent of the cells had lysed, discharging all of their contained radioisotope in non-sedimentable form, a possibility particularly to be considered in view of the known propensity of T2 virus to lyse cells abortively. In these experiments the optical density of the infected cultures does not fall appreciably below that of the control, and the plaque count achieves the theoretically expected value (Table II). However, these measurements are not sufficiently sensitive to rule out the possibility that 3 per cent or even 15 per cent of the infected cells had completely lysed. Two lines of evidence are herewith presented to show that the observed liberation of cell

TABLE II

Demonstration That the Per Cent Virus Attachment, Plaque Count, and Cell Survivors in These Experiments Achieve the Theoretically Expected Values

Cell concentration	Virus multiplicity	Virus attached to cells	Total plaque count (infective centers)		Cells remaining viable		P³²liberated into medium
			Expected	Found	Expected	Found	
		per cent			*per cent*	*per cent*	*per cent*
1.9×10^8	2.8	99	1.9×10^8	2.0×10^8	6.9	6.0	6.0
1.9×10^8	0						3.2

The procedure was as in Table I. The fact that theoretical number of infective centers is obtained indicates that no gross cell destruction has occurred.

constituents in the medium is not due to complete disintegration of a small fraction of the infected cell population.

(*a*) *Differences between Conditions Eliciting the Leakage Reaction and Those Necessary For Lysis.*—

The first of these sets of experiments will demonstrate that the discharge of cellular material as a result of virus infection occurs under conditions where lysis of cell populations by T2 virus does not ordinarily occur. Moreover, the kinetics of the reaction follows a pattern distinctly different from that characteristic of non-reproductive lysis.

Lysis-from-without exhibits the following reaction characteristics: (*a*) Normal cells in nutrient broth require a high multiplicity of infection by T2 for abortive lysis. No noticeable effect occurs in multiplicities less than 20, and 50 per cent of the cells are not lysed until a ratio of 100 phage particles per bacterium has been achieved (9, 10).[2] (*b*) The extent of lysis always increases

[2] The experiments of Visconti (10), using ultraviolet irradiated T2 as a lysing phage, were repeated and found to give the same results with the virus and cell stocks employed in this study.

progressively with multiplicity until cell destruction is complete. (*c*) No lysis occurs at 0°C., regardless of the multiplicity (Table III). (*d*) Lysis from without is observed with certain viruses only. Thus, whereas T2, T4, and T6 are active, T1 phage does not produce abortive lysis of *E. coli* B in any infection multiplicity.

In contrast, the reaction by which isotope leaks into the medium displays the following behavior: (*a*) The reaction readily occurs under conditions in which practically none of the cells is infected with more than a single virus. (*b*) Moreover, after all the cells have been infected, further increase of virus multiplicity causes no additional isotope release (as long as the lysis-from-without threshold is not exceeded). Both of these effects are illustrated in the

TABLE III

Demonstration That Lysis-from-Without Does Not Occur at 0°C.

A 3 hour culture of *E. coli* B (9.6×10^8/cm.^{3}) in nutrient broth was divided into 2 portions at 0°C. Each was infected with a multiplicity of 68 T2 particles and allowed to stand for 5 minutes during which complete cell attachment is attained. One tube was then placed in a 37°C. bath for 10 minutes, after which it was restored to 0°C. Optical densities were determined at 490 mμ in a Beckman spectrophotometer, on both tubes and on 2 control tubes treated in a fashion identical to that of the test tubes, except that they received no virus.

Multiplicity	Temperature	Optical density
	C°.	*cm.*$^{-1}$
68	37	0.18
0 (control)	37	0.60*
68	0	0.58*
0 (control)	0	0.62*

* These three figures are experimentally indistinguishable.

data of Fig. 1, in which the behavior of cells labelled with P^{32} is presented. Similar results were obtained with S^{35}-labelled B. The data of Fig. 1 show that definite isotope leakage occurs at multiplicities as low as 0.26, when an additional 3.25 per cent of the cellular P^{32} was liberated beyond that released from control cells.

The following calculation is illuminating: If only those cells which had received 2 or more virus particles participate in this release of radioisotope, the observed effect must have arisen from 100 $(1-1.26e^{-0.26}) = 2.7$ per cent of the population. Hence, when a multiplicity of 4.23 was employed, 100 $(1-5.23e^{-4.23}) = 92.4$ per cent of the population had received 2 or more virus particles, so that an isotope liberation of $92.4/2.7 \times 3.25$ per cent, which is more than 100 per cent, would have been expected. Actually the net release was only 14.9 per cent. Similarly, if a calculation is made on the assumption that net isotope liberation requires at least 3 virus particles on each cell, an even greater discrepancy is obtained.

Moreover, Fig. 1 demonstrates the existence of a plateau in isotope liberation, such that increase in multiplicity beyond 3, a point at which most of

the cells are infected with at least one virus, produces no further isotope liberation.

This relationship is made clearer in Fig. 2 in which the data from a similar experiment have been plotted against the per cent of cells infected with at least one virus, as calculated from the Poisson distribution formula. These data permit the conclusion that, in the region of multiplicity below 10, infection with a single virus particle is sufficient to evoke maximum isotope liberation from labelled cells under the stated experimental conditions.

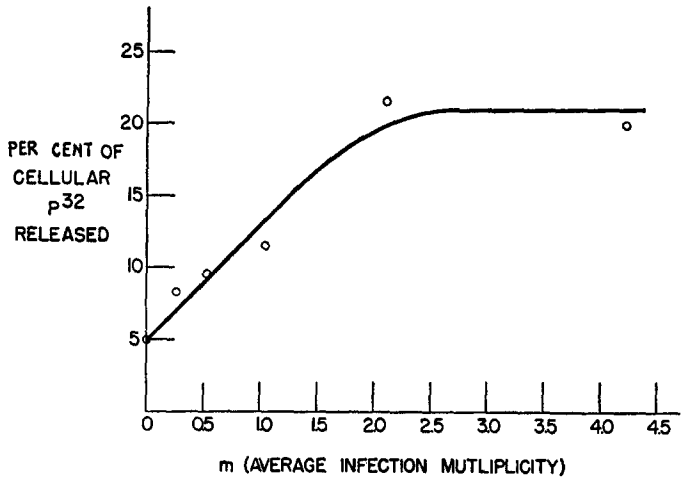

FIG. 1. To 6 tubes containing a P^{32}-labelled suspension of *E. coli* B (1.6×10^9; 2400 counts/cm.^{2}sec.) at 0° was added T2 virus in multiplicities of 0, 0.26, 0.52, 1.05, 2.1, and 4.2, respectively. After 4 minutes, the tubes were placed in a 37°C. bath for 10 minutes and then returned to 0°C. The radioactivity of the entire suspension and of the supernatants obtained after centrifugation were measured.

(*c*) In addition, the leakage phenomenon occurs at 0°C., though its extent is less than that at 37°C. As shown by the data of Table IV, leakage of radioisotope at low temperature is reduced in both infected and normal cells, but is still definitely greater in the infected cells.

(*d*) Finally, the leakage reaction is readily demonstrable with T1 virus and, just as in the case of T2, the amount of isotope liberated remains constant as the multiplicity is increased, once all the cells have become infected. Since T1 does not produce lysis-from-without, there is no need in these experiments to keep the multiplicity low. It was found that isotope liberation by T1 is definite, but smaller in amount than that obtained with T2. Experimental results are illustrated in Table V.

The data so far presented constitute strong evidence, though not proof, that the isotope liberation which occurs as a result of virus action, is one in which every infected cell participates, rather than a complete lysis of a much

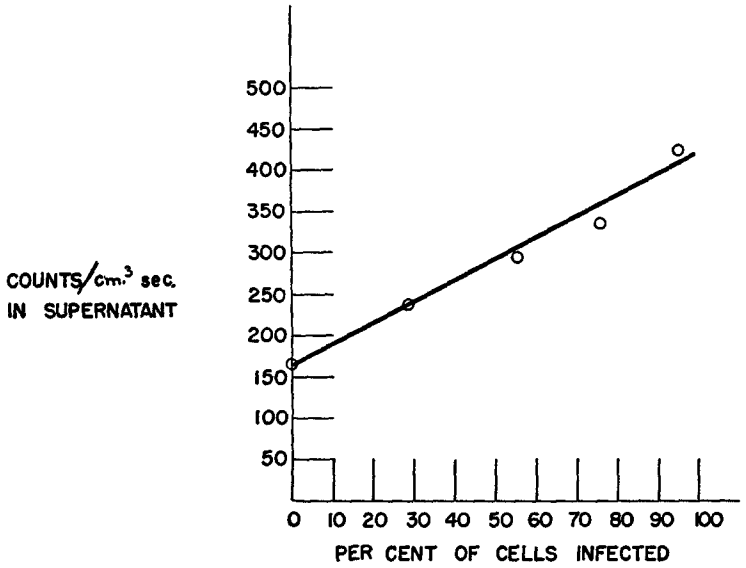

FIG. 2. A P^{32}-labelled suspension of *E. coli* B (8.3×10^8 cells/cc.; 9000 counts/cm.³sec.) was infected in 5 separate tubes at 0° with T2 virus multiplicities (*m*) of 0, 0.33, 0.81, 1.44, and 3.05, respectively. The fraction of cells infected in each tube is $1\text{-}e^{-m}$. 2 minutes after the virus addition, the tubes were placed in a 37°C. bath for 3 minutes, then restored to 0°C., centrifuged, and the supernatants titrated for radioactivity.

TABLE IV

Demonstration That at 0°C., Radioisotope Leakage Is Decreased Both in Normal and Infected Cells, but Is Still Demonstrably Greater in the Latter

Temperature	Cellular radioactivity liberated into supernatant	
	Control: virus absent	Test: virus present
°C.	*counts/cm.³ sec.*	*counts/cm.³ sec.*
(*A*) 0 (throughout)	38.0	72.8
(*B*) 37 (for 3 minutes)	124.6	354.0

Cells of *E. coli* B (3.6×10^9/cm.³) labelled with P^{32} (2180 counts/cm.³sec.) were infected with virus at 0°C. in both cases. In (*B*) only, the cells were given a 3 minute sojourn at 37°C. during which the (*A*) cells continued their stay at 0°C. At both temperatures the amount of radioisotope leaking from infected cells was unaffected by variation in virus multiplicity between the range of 3.4 to 13.

smaller fraction of the cell population. The latter hypothesis would demand that anywhere from 2 to 15 per cent of the cells of different cultures be different from the rest of the population in such a manner as to lyse completely upon contact with a single virus particle. The remainder of the population

would be immune to lysis under conditions of low multiplicity, but would again begin to become susceptible when a large number of virus particles had attached to each cell. Such a bimodal distribution curve is improbable, but not impossible.

(b) Demonstration of Differences in Chemical Composition between the Material Leached Out of Cells under Conditions of Low Infection Multiplicity, and That Resulting from Lysis.—

If the observed isotope leakage results from complete lysis of a part of the cells, the distribution of isotope among the various chemical fractions should be the same as that observed when all of the cells are lysed; whereas, if low virus multiplicity causes all the infected cells to discharge a small part of their

TABLE V

Demonstration of Production of Leakage Reaction from P^{32}-Labelled coli B Cells by T1 Bacteriophage Which Cannot Produce Non-Reproductive Cell Lysis

T1 virus in a variety of multiplicities between 4.6 and 50 was added to a suspension of P^{32}-labelled cells in nutrient broth at 0°C. The cells were then placed in a 37°C. bath for 9 minutes, after which they were returned to 0°C. The total suspensions and their supernatant were titrated for radioactivity.

Cell label liberated into medium	
Control: virus absent	Test: virus present in multiplicities varying from 5 to 50 particles per cell
per cent	*per cent*
3.34	5.01
3.34	5.32
2.9	5.20
2.9	5.30

sulfur and phosphorous, it would be expected that the chemical composition of the liberated material would not mirror that of the total cell contents. It might be expected that a small increase in permeability of each cell would cause small molecular weight components to predominate in the escaping material. One might also expect[3] that DNA, which is confined to the nucleus, would be completely absent. In Table VI are presented experimental results substantiating these effects. These data show: (*a*) The fraction of the liberated P^{32} which is in small molecular form (TCA-soluble) is more than three times greater in the case of low infection multiplicity than in multiplicities high enough to produce visible lysis. (*b*) No DNA is liberated from cells infected with a low multiplicity, whereas lysed cultures release significant amounts of this material. Inspection of the data reveals that the distribution of P^{32} leaked

[3] Suggested by Dr. Leo Szilard.

from cells with a small multiplicity of infection resembles that which arises from uninfected cells under the same conditions.

TABLE VI

Results of 3 Experiments Analyzing the Chemical Composition of P^{32} Obtained from Labelled B Cells under Conditions of Low Multiplicity of Virus Infection; Infection Multiplicity Great Enough to Produce Lysis-from-Without, and No Virus Present

(*A*) Per cent of total cellular P^{32} liberated			(*B*) Amount of liberated P^{32} which is TCA-soluble			(*C*) Amount of liberated P^{32} which is sensitive to DNA-ase		
Low multiplicity	High multiplicity	Control (no virus)	Low	High	Control (no virus)	Low	High	Control (no virus)
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
I. 22.8	92.6	6.2	71.4	19.0	72.0	0	9.6	≤4*
II. 24.2	87.4	6.5	60.6	20.1	78.0	0.7	9.4	≤4*
III. 19.8	93.5	5.4	35.5	10.1	34.0	0.7	7.4	≤4*

* The amount of radioactivity was too small for accurate analysis, but the amount was definitely less than 4 per cent.

In these experiments a 6- to 9-fold larger number of cells was employed in the low multiplicity tubes, in order to achieve a counting rate in the supernatant large enough to make the tests for TCA solubility and DNA-ase sensitivity significant. In the low multiplicity cases, a value of 3 to 7 virus particles per cell was employed, while in the high multiplicity tubes the virus was present in an excess of several hundredfold. Virus and cells in nutrient broth were mixed at 0° and allowed to stand 4 minutes to complete attachment. The tubes were then transferred to a 37°C. bath for 10 minutes, after which they were returned to 0°C., centrifuged, and the supernatants analyzed for total radioactivity, per cent radioactivity which is TCA-soluble and the per cent of radioactivity which becomes TCA-soluble only after treatment with the enzyme DNA-ase. The suspensions with high multiplicity exhibited extensive clearing, while no change was visible, either in the controls or the tubes with low virus:cell ratio. That the differences in DNA-ase sensitivity (column C) between the low and high multiplicity cases is real, is shown by the following set of experimental data:—

Low multiplicity		High multiplicity	
Counts/cm.^{3} sec. of supernatants		Counts/cm.^{3} sec. of supernatants	
With DNA-ase	Without DNA-ase	With DNA-ase	Without DNA-ase
522	511	227	131.8
581	576	249	169
486	486	227.5	151

In Table VII are presented analytical results on the P^{32} obtained from labelled cells by grinding with powdered quartz at low temperatures (11). It is apparent from a comparison of this data with that of Table VI, that the phosphorous liberated from cells which have undergone lysis-from-without resembles that obtained by thorough grinding of whole cells in (*a*) the small

proportion of TCA-soluble P present, and (*b*) the presence of appreciable quantities of DNA. Since cellular DNA is confined almost entirely to nuclear bodies, it may be concluded that during the initial stages of the normal cycle of infection by T2, cell permeability is increased but not to an extent which permits escape of chromosomal material.

TABLE VII

Experiments in Which the P^{32} from Labelled Cells Was Liberated by Grinding in the Cold, and Analyzed by the Procedures of Table VI

(*A*) Total cellular P^{32} liberated	(*B*) Liberated P^{32} which was TCA-soluble	(*C*) Liberated P^{32} which is TCA-soluble only after DNA-ase treatment
per cent	*per cent*	*per cent*
I. 65	11	17
II. 62	14	13
III. 77	10	18

Labelled B cells were ground at 0° with 400 mesh-powdered quartz, and extracted with nutrient broth. The material was centrifuged and the supernatant collected and analyzed, as in Table VI, for total radioactivity, TCA-soluble radioactivity, and radioactivity which becomes TCA-soluble only after treatment with the enzyme, DNA-ase.

TABLE VIII

Demonstration That Isotope Leakage from Infected Cells Remains Approximately Constant after 3 Minutes at 37°C.

P^{32}-labelled *coli* B cells (3.6×10^8/cm.^{3}) were infected with T2 in a multiplicity of 3.4 at 0°C. After 4 minutes to permit virus attachment, the tubes were placed in a 37°C. bath. Samples were removed after 0, 3, and 10 minutes incubation at 37°C., and the amount of radioactivity which had escaped from the cells determined.

Incubation time at 37°	Amount of cellular P^{32} liberated	
	Control: virus absent	Test: virus present
min.	*per cent*	*per cent*
0	1.74	3.34
3	5.71	16.2
10	6.10	14.6

Results essentially identical were obtained with a virus multiplicity of 6.8 instead of 3.4.

3. Other Characteristics of the Reaction Causing Isotope Release

(*a*) *Time Course at 37°C.*—

The reaction leading to the release of cell-bound constituents is extremely rapid at 37°C. Definite increase in isotope liberation from virus infected cells is demonstrable within 20 seconds after the mixture is placed at 37°C. Often the reaction appears to be complete within 2 to 3 minutes in the sense that no further isotope release is observed between the interval of 3 to 15 min-

utes, as illustrated by the data of Table VIII. However, some cell preparations manifest continued isotope liberation for 15 minutes or more but at a rate less than the initial one. It is evident that initation of the leakage reaction occurs within the time limits of T2 virus penetration.

(*b*) *Salt Effects: Suppression of Leakage by Mg^{++}.*—

The leakage reaction does not require any organic metabolites. Cells suspended in a 0.10 M NaCl buffered at pH 7 by 10^{-4} M PO_4 exhibit almost exactly the same behavior as that observed in nutrient broth. However, 0.025 M Mg^{++} strongly suppresses this reaction, as illustrated in the data of Table IX. Control tests demonstrate that this effect is not a simple inhibition of virus attachment.

TABLE IX

Typical Experiments Demonstrating the Effect of Mg^{++} in Suppressing the T2-Induced Leakage Reaction

T2 in a multiplicity of 0.5 was added at 0°C. to S^{35}-labelled B suspensions in (*A*) 0.1 M NaCl, and (*B*) 0.10 M NaCl + 0.025 M $MgCl_2$. Both tubes were placed in a 37°C. bath for 5 minutes, then returned to 0°C. Assay of the plaque-forming particles in the supernatant revealed that more than 90 per cent of the virus had become cell-attached in each case. Analysis of the radioactive contents of the total suspensions and the supernatants, as well as of a control tube containing no virus, was performed. The total radioactivity assay of the cell suspension was 376 counts/cm.^{2}sec.

Virus multiplicity	$[Mg^{++}]$	Cellular S^{35} in supernatant
		counts/cm.^{2} sec.
0.5	0	7.55
0.5	0.025 M	4.12
0 (control)	0	3.65

DISCUSSION

Hotchkiss (12), Gale and Taylor (13), and Salton (14) showed that when amino-containing compounds bind to *E. coli* bacteria, a leakage of cellular constituents into the medium occurs. Since it has now been shown that the attachment of T2 bacteriophage to its host cell involves binding by viral amino groups (2), the virus-induced leakage reaction appears to be an expression of a general cellular mechanism which can be triggered by such electrostatic bond formation.

It is convenient to designate as the virus penetration reaction, the sequence of events beginning with the first irreversible reactions after the initial cell attachment, and ending when the DNA finds its target site inside the cell (15). At least some of these steps are non-enzymatic in character, since T2 virus-splitting and cell-killing occur readily at 0°C. (5). The increase in cell permeability here described requires 37°C. for its full expression, although, as indicated, a small but definite liberation of cellular constituents occurs at the low

temperature. The main difference between the penetration of T1 and T2 viruses appears to lie in these non-enzymatic steps, since T1 virus does not readily carry out irreversible attachment or host cell killing at 0°C., as does T2 (16). The fact that in the course of their individual penetration reactions the two viruses produce a similar increase in host cell permeability makes it appear likely that the two sequences are similar in principle, though differing in detail.

The present considerations also suggest an explanation for lysis-from-without in the economy of virus-host interaction.

It was suggested by Watson that this reaction is not an expression of phage invasion of bacteria, because he found that x-irradiated T2 phage was not capable of killing host cells in single infection, and yet was able to produce cell lysis in multiplicities lower than that required by normal virus (17). Further, the fact that phages like T1 do not exhibit this non-reproductive lysis, though their life cycle bears clear resemblance to that of T2, appears to support this interpretation. On the other hand, the parallelism between the conditions needed for lysis-from-without by T2 and those required for successful penetration of its host (5) (15) seems too imposing to be coincidental. It seems permissible to regard lysis-from-without as the unchecked and hence destructive operation of a lytic mechanism which, in limited form, is part of the normal sequence of virus penetration. Both T1 and T2 viruses produce an increased cell permeability. In normal invasion, this lytic reaction is soon halted (presumably after the DNA injection) and the leakage of cell constituents falls. These processes which normally check this reaction might themselves be inhibited by the attachment of an overwhelming dose of virus particles to the same cell, or by disturbance of the cell's metabolism by poisons (18, 19), or nutrient deprivation (20). Heavily x-rayed phage may have suffered in its ability to carry out both the lytic and the subsequent lysis-inhibiting function, but if the latter were more readily damaged by irradiation, Watson's observations would be accounted for. This interpretation is supported by Visconti's observation (10) that bacteria infected by T2 develop a resistance against lysis-from-without by as much as 1000 superinfecting virus particles, and that some resistance is already demonstrable within 2 minutes after the initial infection. By this picture, T1 would differ from T2 either in its lesser capacity to trigger the lytic reaction or its more rapid evocation of the inhibiting phase. Either action would explain the smaller cell leakage produced by T1 infection, as well as its failure to induce abortive lysis. The leakage reaction described in the present communication would then be a result of the temporary, increased cell permeability effected by this transient action of cellular autolytic enzymes. The fact that these enzymes can be activated by synthetic polyamino compounds to produce a lytic reaction with kinetics similar to that of T2-induced lysis (5) fits into this conception.

The similarity in chemical composition between the P fraction which leaks out of the cells as a result of virus infection, and that which leaches in lesser

amounts from normal cells suggests that the mechanism triggered by the virus may be one which operates normally in the transport of certain types of materials across the cell wall.

It is of interest that a similar virus-induced increase in cell permeability has been demonstrated in mammalian viruses. Influenza and Newcastle's disease virus, after attaching to chick red cells, can initiate a reaction in which the hemoglobin completely leaches out of the cell without destroying cellular morphology. In the case of Newcastle's disease virus, the nuclei are retained inside the cells after the hemoglobin has leaked out, and certain aspects of the reaction kinetics resemble those described in the experiments of the present paper (21). Other analogies between the action of influenza virus on red cells and the phenomenon of lysis-from-without have been recently described (22).

T. F. Anderson (23) has briefly noted that an ultraviolet-absorbing substance is released from cells infected with T2 phage, and while the present paper was in proof, kindly sent us a dissertation (24) by C. D. Prater describing experiments which were reported at a meeting in 1951, but have not been published.

SUMMARY

Treatment of radioactively labelled host cells with T1 or T2 bacteriophages induces a leakage of cellular P and S into the medium.

Evidence is presented showing that this increased cell permeability is not the result of complete lysis of a small fraction of the cells, but rather is made up of contributions from all or most of the infected population.

This leakage of cellular constituents exhibits the following characteristics: (*a*) Infection of a cell with a single virus suffices to evoke the reaction; (*b*) Increasing the multiplicity up to 7 to 8 virus particles per cell does not affect the extent of leakage produced; (*c*) Some leakage does occur at 0°C., but much less than at 37°C.; (*d*) Infection by T1 virus results in a smaller amount of leakage than in the case of T2, but the pattern of response to varying virus multiplicity is the same; (*e*) The P resulting from such leakage contains no DNA and chemically resembles that which elutes in smaller amounts from uninfected cells; (*f*) At 37°C. the virus-induced leakage reaction appears within a matter of seconds, and usually decreases after 2 to 3 minutes; (*g*) The reaction is inhibited by 0.025 M Mg^{++}.

Theoretical considerations are presented suggesting the place of this reaction in the sequence of events constituting the virus penetration reaction; its relationship to the phenomenon of lysis-from-without; and its resemblance to the leakage reaction produced by electrostatic binding of ionized compounds to cell surfaces. The existence of similar effects in avian-mammalian virus systems is noted.

BIBLIOGRAPHY

1. Tolmach, L., and Puck, T., *J. Am. Chem. Soc.*, 1952, **74**, 5551.
2. Puck, T., and Tolmach, L., *Arch. Biochem. and Biophysics*, in press.
3. Puck, T., and Sagik, B., *J. Exp. Med.*, 1953, **97**, 807.
4. Hershey, A. D., and Chase, M., *J. Gen. Physiol.*, 1952, **36**, 39.
5. Puck, T., *Cold Spring Harbor Symp. Quant. Biol.*, 1953, **18**, 149.
6. Herriott, R. M., *J. Bact.*, 1952, **61**, 752.
7. Hershey, A. D., Dixon, J., and Chase, M., *J. Gen. Physiol.*, 1953, **36**, 777.
8. Adams, M., *Methods in Medical Research*, 1950, **2**, 1.
9. Viruses 1950, (M. Delbrück, editor), Pasadena, Division of Biology of the California Institute of Technology, 1950, 142.
10. Visconti, N., *J. Bact.*, 1953, **66**, 248.
11. Gandelman, B., Zamenhof, S., and Chargaff, E., *Biochim. et Biophysic. Acta*, 1952, **9**, 399.
12. Hotchkiss, R., *Ann. New York Acad. Sc.*, 1946, **46**, 479.
13. Gale, E. F., and Taylor, E. S., *J. Gen. Microbiol.*, 1947, **1**, 77.
14. Salton, M. R. J., *J. Gen. Microbiol.*, 1951, **5**, 391.
15. Puck, T., *in* The Dynamics of Virus and Rickettsial Infections, (F. W. Hartman, F. L. Horsfall, and J. G. Kidd, editors) New York, The Blakiston Co., 1954, 16.
16. Garen, A., and Puck, T., *J. Exp. Med.*, 1951, **94**, 177.
17. Watson, J. D., *J. Bact.*, 1950, **60**, 697.
18. Anderson, T. F., *J. Cell. and Comp. Physiol.*, 1945, **25**, 1.
19. Cohen, S. S., *Bact. Rev.*, 1949, **13**, 1.
20. Heagy, F. C., *J. Bact.*, 1950, **59**, 367.
21. Sagik, B., and Levine, S., data to be published.
22. Sagik, B., Puck, T., and Levine, S., *J. Exp. Med.*, 1954, **99**, 251.
23. Anderson, T. F., *Bot. Rev.*, 1949, **15**, 464.
24. Prater, C. D., Dissertation presented to the Faculty of the Graduate School of the University of Pennsylvania, 1951.