NUCLEIC ACID TRANSFER FROM PARENTAL TO PROGENY BACTERIOPHAGE

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INTRODUCTION

In recent years several investigators have used isotopic markers to determine the transfer of P or N atoms from parental to progeny virus particles. PUTNAM AND KOZLOFF[1] using the bacteriophage T6 labelled with ^{32}P, found that 20–40% of the label appeared in material identified as progeny phage by differential centrifugation. LESLIE *et al.*[2] found similar, or lower, values with ^{32}P-labelled T2 bacteriophage. KOZLOFF[3] extended his original observations by studying P and N transfer from normal as well as radiation damaged phage particles. All these experiments show incomplete transfer varying greatly from one experiment to the other.

To determine the true transfer values, two technical problems must be solved: (1) adsorption of the labelled phage must be complete within about 2 minutes because later adsorbing particles are broken down before entering the cells (LESLIE *et al.*[2]), (2) means must be found to prevent progeny particles from adsorbing onto bacterial debris or unlysed cells. Failure to control either of these processes will result in underestimation of the transfer values.

In our first experiments (MAALØE AND WATSON[4]), although the second factor was well controlled, the first was not, and our ^{32}P transfer values of about 30% were, therefore, like those of PUTNAM AND KOZLOFF[1], LESLIE *et al.*[2], and KOZLOFF[3], too low. In this paper, we present experiments in which both factors are controlled, and which indicate that T2 and T4 phages transfer about 50%, T3 phages about 40% of their phosphorus to the progeny. Identical values are found when, instead of ^{32}P, ^{14}C-labelled adenine is used to label the parental phage. The ^{32}P experiments show that the transferred material goes predominantly to the early formed phages, and that the transfer values of about 50% are maximum values. Our finding that the early completed phages receive most of the parental material confirms similar observations by DOERMANN (personal communication) and WEED AND COHEN[5] (unpublished).

In agreement with KOZLOFF[3], we have also demonstrated that considerable amounts of ^{32}P may be transferred from labelled particles which do not participate in reproduction, either because of radiation damage or because they are excluded by another phage. These "abnormal" cases show that infecting particles may be broken down

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extensively before[3] their constituents are used for synthesis of new phage particles. We do not, however, think that this evidence excludes the possibility that under normal conditions the infecting and reproducing particle remains essentially unbroken. We shall return to this important point in the discussion.

MATERIALS AND METHODS

The phages T2, T3 and T4, and their common host *E. coli*, strain B/1, have been used: the latter because contamination of cultures with phage T1 occurs in our laboratory. Most of the techniques used in this study have been described in detail by ADAMS[6]. All cultures were grown at 37° C: centrifugations were done in a Servall Angle centrifuge at 10° C.

Media. The nutrient broth is an aqueous extract of minced meat enriched with 1% peptone and containing 0.02% Tween 80 and 0.5% NaCl: pH is adjusted to 7.4. For experiments with T3, the concentration of NaCl was reduced to 0.05% to obtain rapid adsorption. In this medium, at 37° C the latent period of T4r is 22–23 minutes, and the burst size about 150. ^{32}P-labelled phage was prepared using the synthetic g-medium described earlier (MAALØE AND WATSON[4]).

Anti-sera. Rabbit anti-phage sera were prepared, using highly purified and concentrated phage suspensions as antigens. All sera had k-values (ADAMS[6]) of 500–1000 when tested against phage suspended in broth (*cf.* JERNE[7]). These sera showed no agglutination of *E. coli*, strain B/1, in dilution 1 to 10. Samples of anti-phage sera were absorbed with large amounts of live B/1, and used in parallel with unabsorbed serum for phage precipitation: no evidence for the presence of antibacterial antibodies was found. Serum against *E. coli*, strain B/1, was obtained after series of subcutaneous and intravenous injections of heat-killed and subsequently of live cultures. This serum had no anti-phage activity.

X-ray technique. The X-ray source was a Holbeck-Beaudouin tube operating at 33 kv and 36 mA. A cooled molybdenum target produced radiation with an average wave-length of 0.9 A. On the surface of the samples the intensity of radiation was 66,000 r.p.m. The irradiation was done by Dr R. LATARJET on samples sent to Paris by airmail. No decrease in titer was observed in control samples as a result of the shipment.

Isotope technique. Carrier-free orthophosphoric acid was obtained from the Isotope Division of the Oak Ridge National Laboratory, United States Atomic Energy Commission. Adenine labelled with ^{14}C in position 8 was also used: this preparation was synthesized by CLARK AND KALCKAR[8], and had a specific activity of 0.8 mC per mMole. All samples containing ^{14}C were evaporated to dryness and self-absorption due to solids in the suspension medium was made uniform by diluting into nutrient broth before counting. Variation between counts on duplicate ^{14}C samples was less than 10%. The counting equipment was the same as previously described.

Preparation of ^{32}P-labelled T4r. Washed B/1 bacteria from a 24-hour broth culture were inoculated into 10 ml of g-medium containing 20 μC ^{32}P. After two and a half hours of aeration, the bacterial density was about 10^8 cells per ml, and the culture was then infected with about 10 T4r particles per cell. Aeration was continued, and 2–3 minutes before the onset of lysis 0.5 ml of undiluted antibacterial serum was added. If antibacterial serum is not added, the titer of a crude T4r lysate will drop appreciably during the first 24 hours and a fraction of the remaining phages will adsorb slowly. Both effects are presumably due to phage particles absorbing on bacterial debris (MAALØE AND STENT[9]). The antibacterial serum proved completely effective in blocking adsorption of T3 and T4 on B/1, but was not fully effective for T2r$^+$. T2r$^+$ stocks may, therefore contain inactive as well as slowly adsorbing particles even when antibacterial serum is used. It is possible to restore infectivity and full adsorbability to these particles by diluting into distilled water for several hours at 37° C: presumably because adsorbed particles dissociate from the debris at low salt concentrations (PUCK, GAREN, AND CLINE[10] and HERSHEY, personal communication). This treatment was first used by BERTANI (personal communication) to raise the titer of T2r$^+$ broth lysates.

The crude radioactive lysates were centrifuged at 5000 g for 5 minutes to remove bacterial debris and at 12,000 g for one hour to sediment the phage. Three cycles of low and high speed centrifugation reduced the concentration of inorganic ^{32}P by a factor of about 10^5. Further purification was achieved by adding heat-killed resistant bacteria (B/3, 4, 7 heated to 58° C for 1 hour) at a concentration of $5 \cdot 10^8$ per ml. After 30 minutes at 37° C, about 5% of the radioactivity had adsorbed to the resistant cells, which were removed by centrifugation. A similar number of sensitive B/1 cells adsorbed 95–98% of the activity. An additional test of the purity of the virus stock was obtained by precipitation with antiphage serum, as previously described; with anti-T4 serum, 94% of the radioactivity was precipitated, while in a control sample in which T3 phage was precipitated with anti-T3 serum, the precipitate contained less than 2% of the activity.

Assuming that about 95% of the ^{32}P in the final preparation was present as phage phosphorus, the initial specific activity was 10^{-6} counts per minute per particle. From this we can calculate

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that each virus particle contained an average of 0.25 ^{32}P atoms. Since the inactivation efficiency of nuclear decay is only 1/12 (HERSHEY, KAMEN, KENNEDY AND GEST[11]), the fraction of labelled particles which will lose infectivity during an experiment is negligible.

With minor variation indicated by adsorption requirements, etc., the procedure just described was also used to prepare ^{32}P-labelled stocks of the phages T2r$^+$, T3, and T4r$^+$. Phage labelled with ^{14}C adenine was grown on a purine requiring mutant of *E. coli*, strain B, which was obtained from Dr A. H. DOERMANN of the Oak Ridge National Laboratory. For this purpose the g-medium was supplemented with 5 γ ^{14}C adenine per ml. The specific activity of phages grown on the purine requiring strain was ten times that of phages grown similarly on B/1.

EXPERIMENTAL

The basic experimental procedure is unchanged throughout this study. It will, therefore, suffice to describe one typical and simple experiment in detail; more complex experiments can then be introduced briefly and the results summarized in tables.

Distribution of ^{32}P following infection of unlabelled B/1 with labelled T4r:

Exponentially growing bacteria from an unlabelled broth culture were collected by centrifugation and resuspensed in unlabelled broth at 37° C at a concentration of $2 \cdot 10^9$ cells per ml. ^{32}P-labelled T4r phage was added at a ratio of 4.5 particles per bacterium and one and one-half minutes allowed for adsorption. The culture was then chilled and centrifuged at 5000 *g* for 5 minutes. The supernatant was carefully siphoned off and samples for phage assay and radioactivity measurement were taken. The pellet was resuspended in broth at 37° C to give a suspension of infected bacteria with about 10^8 cells/ml; this figure was determined by a separate assay. Aeration was then started, and 25 minutes after infection one volume of undiluted antibacterial serum was added to 19 volumes of culture to prevent adsorption of progeny phage particles to bacterial debris. The cooling and centrifugation retarded phage growth by about 8 minutes. The assays and radioactivity measurements showed that in this experiment over 99.5% of the phages and 96% of the input radioactivity were adsorbed on the bacteria.

About 30 minutes after lysis, the culture was centrifuged at 5000 *g* for 5 minutes to remove bacterial debris and then at 12,000 *g* for one hour to sediment the progeny phage. The material collected during these centrifugations will be referred to as the "low speed pellet" and the "high speed pellet", respectively. The latter was resuspended in broth and again centrifuged at low speed to remove remaining bacterial debris. The

TABLE I

DISTRIBUTION OF ^{32}P AFTER INFECTION OF B/1 WITH LABELLED T4r

Growing bacteria were concentrated to $2 \cdot 10^9$ cells per ml and infected with labelled T4r at a concentration of $9 \cdot 10^9$ particles per ml. Following an adsorption period of 1½ minutes the bacteria were centrifuged for 4 minutes at 5000 *g* to remove unadsorbed phage and then resuspended in nutrient broth at a concentration of $1.1 \cdot 10^8$ cells per ml. Approximately 99.5% of the phage and 96% of ^{32}P adsorbed to the bacteria.

Material	*Phage titer/ml*	*^{32}P distribution (% of radioactivity adsorbed on bacteria)*
Crude lysate	$1.5 \cdot 10^{10}$	100%
Low Speed Pellets	$.027 \cdot 10^{10}$	8.4%
High Speed Pellet	$1.45 \cdot 10^{10}$	42.1%
High Speed Supernatant	$.11 \cdot 10^{10}$	49.5%

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two low speed pellets were resuspended in broth and all fractions assayed for ^{32}P and phage. Table I shows that 42.1% of the ^{32}P which initially adsorbed to the bacteria and about 95% of the progeny phage was recovered in the high speed pellet.

The radioactivity of the high speed pellet was characterized as belonging to phage particles by precipitation with an excess of anti-T4 serum and by adsorption to sensitive bacteria. Native as well as B/1 adsorbed serum was used and in both cases 93% of the ^{32}P was precipitated with the phage. Adsorption tests showed that over 90% of the radioactivity adsorbed on B/1 cells while less than 5% adsorbed on the resistant strain B/3, 4, 7 on which T4 does not itself adsorb. These tests show that 90–95% of the radioactivity in the high speed pellet is somehow associated with the phage particles.

Before asserting that this ^{32}P is truly incorporated into the progeny phages, the following possibilities must be considered: (1) degraded parental nucleic acids might stay attached to the surface of the progeny particles, or (2) non-infective parental particles might be adsorbed to the bacteria initially, released during lysis, and later sediment together with the progeny particles.

Both these possibilities are ruled out by the fact that the progeny particles from an experiment like the one just described transfer their ^{32}P in exactly the same way as did their uniformly labelled parents (MAALØE AND WATSON[4]). We, therefore, conclude that about 95% of the ^{32}P in the high speed pellet is incorporated into the progeny particles. Using this estimate in correcting for the phage present in the low speed pellets and in the high speed supernatant, the fraction of the parental ^{32}P which has become incorporated into the progeny particles is 45% in this experiment.

Table II shows a series of similar experiments involving different stocks of labelled T4r; it is notable that the differences in transfer are very small, usually less than 5%. These values are not changed by varying the number of infective particles from 1 to 10 (the highest number tested). The amount of ^{32}P remaining attached to bacterial debris is uniformly about 5 to 10%. If the different phage stocks had contained greatly varying fractions of adsorbing but non-infecting phages, this would have caused the low speed pellet values to fluctuate greatly.

TABLE II

EXPERIMENTAL VARIATION IN ^{32}P TRANSFER VALUES FOR T4r

Experiment	*Burst size*	*% of parental radioactivity in*		
		Low speed pellets	*Progeny phage*	*High speed supernatant*
1	122	6	47	47
2	141	7	46	47
3	150	6	45	49
4	126	3	45	52
5	160	9	43	48
6	155	10	42	48

^{32}P transfer from secondarily adsorbed phage

The experiments given in Table III show that particles which adsorb on a bacterium more than two minutes after a primary infection with T4r transfer insignificant amounts of ^{32}P. This was demonstrated by infecting bacteria with an average of 5 non-labelled phages per cell and at various times later reinfecting with labelled phage. Column 1

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of Table III further shows that with increasing intervals between primary and secondary infection, the amount of ^{32}P which stays attached to the bacteria decreases. Since the labelled phages adsorb at the normal rate, most of the ^{32}P in the supernate of the infected cells must initially have been adsorbed onto the cells. The observed increase in unadsorbed material is probably an expression of the breakdown of secondarily adsorbed particles described by LESLIE, FRENCH, GRAHAM, AND VAN ROOYEN[2]. These authors observed that a primary infection stimulates within a few minutes the infected cell in such a way that, if a new phage particle adsorbs, it is broken down extensively on the surface of the bacterium, releasing about 50% of its phosphorus into the medium in the form of material soluble in 5% trichloroacetic acid.

This stimulation phenomenon is of further importance for transfer experiments, since we must assume that early released progeny particles which adsorb on unlysed cells will be broken down and release half their phosphorus into the medium. In most of the previous work adsorption of progeny particles was not prevented and the breakdown effect presumably decreased the transfer values. Since in our experiments adsorption was blocked by antibacterial serum, the 50% transfer values can be taken as a good estimate of the transfer to *all* the progeny particles.

TABLE III

DISTRIBUTION OF ^{32}P FOLLOWING SECONDARY INFECTION OF B/1 BY LABELLED T4r

Growing bacteria concentrated to $1.3 \cdot 10^9$ cells/ml in broth were infected at $t = 0$, with an average of 5 unlabelled T4r particles. At various intervals, labelled T4r was added at a ratio of 1 particle per bacterium. Several minutes after the addition of the labelled phage, unadsorbed ^{32}P was removed by low speed centrifugation and the infected bacteria resuspended in nutrient broth at a concentration of 10^8 cells/ml.

Minutes between primary and secondary infection	*Unadsorbed* ^{32}P*	*% of parental radioactivity in*		
		Low speed pellet	*Progeny phage*	*High speed supernatant*
0	5	6	44	45
0.5	6	4	48	42
2	23	15	18	44
4	48	23	4	25
6	45	20	3	32

* This material must initially have been adsorbed but has been released again because of the breakdown effect described by LESLEY *et al.*[2].

Isotope transfer from parental to progeny T3

At the salt concentration usually employed in growth media, T3 phage adsorbs rather slowly. To ensure the necessary rapid adsorption of the labelled T3 particles, the NaCl concentration in the adsorption tube had to be lowered to 0.05%. At this low salt concentration, over 99% of the infecting particles adsorbed within two minutes. Except for this modification, the experiments were like the T4 experiments and included characterization of the progeny by serum precipitation and adsorption on sensitive bacteria. Table IV shows that about 40% of the parental ^{32}P was transferred. Since nearly simultaneous adsorption of the infecting phage particles and isolation of the entire progeny were achieved, we may consider the transfer value of about 40% as a maximum value. As in the case of the phages T2 and T4, the transfer of phosphorus is incomplete.

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TABLE IV

DISTRIBUTION OF ^{32}P FOLLOWING INFECTION OF B/1 BY LABELLED T3

Experiment	Burst size	% of parental radioactivity in		
		Low speed pellet	Progeny phage	High speed supernatant
1	195	9	46	45
2	234	8	38	54

Purine transfer from parental to progeny phage

Over 95% of the phage phosphorus is located in DNA, and it is therefore desirable to know whether other nucleic acids constituents such as the purine bases are transferred incompletely like the phosphorus. Phages T2, T3 and T4 were grown in purine requiring bacteria in the presence of adenine labelled with ^{14}C in position 8 (see page 433 *et seq.*). Paper chromatography shows that in this way both the phage adenine and guanine is labelled with ^{14}C. Table V presents a series of experiments with the purine labelled phages. They all show incomplete transfer with values not significantly different from those obtained with ^{32}P; T3 again seems to transfer a little less than do T2 and T4.

TABLE V

DISTRIBUTION OF ^{14}C FOLLOWING INFECTION OF B/1 WITH PURINE LABELLED PHAGE

Experiment	Phage	Burst size	% of parent radioactivity in		
			Low speed pellet	Progeny phage	High speed supernatant
1	T2r+	322	12	55	33
2	T2r+	238	16	54	30
3	T2r+	162	18	43	39
4	T2r+	360	14	48	38
5	T3	180	14	38	48
6	T3	276	11	38	51
7	T3	210	14	32	54
8	T4r	115	12	44	44
9	T4r	122	14	40	46

Isotope transfer as a function of burst size

In experiments with T3 and T4r spontaneous lysis always occurs when the burst size is relatively low. It is known, however, that the r+-phages behave differently, and that lysis can be delayed by a secondary infection (DOERMANN[12]). At the time of normal lysis the infected cells have not exhausted their capacity for phage production. During the last ten minutes before normal lysis, large amounts of phosphorus-containing phage material is produced in the cells which is not developed into mature phage before lysis (MAALØE AND STENT[9]). After normal lysis, this material cannot be recovered by centrifugation. The incompleteness of the phosphorus and purine transfers might, therefore, be due to our failure to detect parental material transmitted to the immature phage particles formed late in the latent period.

This hypothesis was tested by infecting bacteria with labelled T4r+-phage and inhibiting lysis artificially by reinfection with unlabelled phage. The burst size in this

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experiment was 350, or 2 or 3 times greater than in the experiment with T4r. Despite this increase in phage yield, the transfer was again about 50%, showing that the late formed particles received very little, if any, of the parental phosphorus.

The experiment reported in Table VI shows directly that the transferred ^{32}P goes predominantly to the early formed particles. In this experiment, bacteria infected with labelled T2r$^+$-phage were lysed prematurely by addition of $M/1000$ KCN and a large number of ultraviolet inactivated phage, as previously described[4]. The excess of added phage served as carrier material during centrifugation, and insured that the labelled progeny particles were effectively isolated even when the yield was low. Several identical experiments were carried out, all showing the same trend. Thus the material which an infecting particle transfers to the progeny usually goes to one or more of the early formed particles, while the later formed ones virtually never receive any of it. We therefore conclude that our transfer values of about 50% are true maximum values. Table VI also shows that bacteria lysed before the appearance of the first progeny particles contain insignificant amounts of radioactivity sedimentable at high speed. This is additional evidence that our results are not affected by spurious measurements of non-infective but still sedimentable parental particles released upon lysis.

TABLE VI

DISTRIBUTION OF PARENTAL ^{32}P AMONG PROGENY PARTICLES FROM PREMATURELY LYSED BACTERIA

Concentrated B/1 were infected with an average of 5 ^{32}P-labelled T2r$^+$ particles per bacterium. Two minutes after infection the culture was chilled and centrifuged at 5000 g for 4 minutes to remove unadsorbed radioactivity. The pellet was resuspended in broth at 37° C at a bacterial concentration of 10^8 cells/ml. The progress of phage growth was retarded about 8 minutes by the cooling and centrifugation. Ten minutes after infection ultraviolet (UV) inactivated T2r$^+$ at the average multiplicity of 5 particles was added to inhibit lysis. Samples of the infected bacteria were then broken open at various times by the addition of $M/1000$ KCN and approximately 2000 UV inactivated T2r$^+$ particles per cell. Readsorption of the progeny particles was prevented by saturation of the bacterial surface with the UV treated phage (MAALØE AND WATSON[4]).

Time of premature lysis	*Burst size*	*% of parental ^{32}P in*		
		Low speed pellet	*High speed pellet*	*High speed supernatant*
19 minutes	0	13	2	85
25	34	15	15	70
28	100	14	26	60
34	210	15	38	47
48	275	16	37	47

Isotope transfer in the absence of genetic transfer

The "second generation experiment" (MAALØE AND WATSON[4]) referred to earlier in this paper permits the conclusion that the transmitted phosphorus is distributed in the progeny particles in the same uniform way as in the parental particles. It leaves open the question whether transfer occurs via large blocks carrying biological specificity or via highly degraded material. In this study we have tried to answer a complementary question: Does isotope transfer occur under conditions where no genetic transfer is possible? *i.e.*, can material from an infecting particle be broken down to genetically unspecific structures which are then incorporated into new phage particles?

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TABLE VII

TRANSFER OF ^{32}P FROM X-RAY INACTIVATED T4r

Bacteria were mixedly infected with unlabelled T4r+ and X-ray irradiated ^{32}P-labelled T4r. The adsorption of ^{32}P to bacteria was similar in the mixtures containing irradiated phage and in control mixtures containing non-irradiated phage, being approximately 93% in both cases.

Tube contents	*% of parental ^{32}P in*		
	Low speed pellet	*Progeny phage*	*High speed supernatant*
Unlabelled T4r+ + labelled T4r (non-irradiated)	11	43	46
Unlabelled T4r+ + labelled T4r (e^{-3} survival)	31	24	45
Unlabelled T4r+ + labelled T4r (e^{-6} survival)	45	22	33
Unlabelled T4r+ + labelled T4r (e^{-10}survival)	42	30	28

To answer this question we first carried out experiments with labelled phage heavily irradiated with X-rays. Such particles retain the ability to adsorb to bacteria while the majority of them have lost not only their infectivity, but also the ability to kill the host cell and to transfer genetic specificity (WATSON[13]). The experiments presented in Table VII show, however, that heavy X-ray damage reduces the transfer values only from the normal 40 to 50% to about 25%. Thus, substantial amounts of phosphorus may be transferred from particles which do not participate in genetic exchange. It should be noted that nearly 50% of the phosphorus of the irradiated particles remain attached to the bacterial debris. This value is significantly greater than the values of 5 to 15% found when active phage reproduces. It is possible that as many as 50% of the irradiation damaged phages remains passively attached to the cell surfaces; if so, the transfer value per transferring particle is again about 50%.

Transfer of parental isotope without simultaneous genetic transfer can also be demonstrated in bacteria in which one of the infecting phages does not multiply because of the presence of an unrelated phage. This "mutual exclusion" (DELBRÜCK[14]) is well illustrated by the unrelated phages T3 and T4; if a bacterium is infected simultaneously by both phages, T3 multiplication is completely suppressed and only T4 progeny particles appear; even the infecting T3 particles are lost. In Table VIII are shown the results of an experiment in which bacteria were simultaneously infected with ^{32}P-labelled T3 particles and unlabelled T4 particles. After lysis, the progeny particles were isolated and tested for radioactivity, the specificity of which was shown by the extent of its precipitation with anti-T3 and anti-T4 serum. It can be seen that approximately 25% of the ^{32}P originally present in the T3 particles was transferred to the T4 progeny, another 25% was associated with bacterial debris, while the remaining 50% cannot be sedimented at high speed. The excluded phage thus does not sit passively on the bacterial surface but must penetrate to the interior of the cell where it is broken down into its simpler components. Our experiment, therefore, supports the conclusion of WEIGLE AND DELBRÜCK[15] that mutual exclusion must involve some mechanism other than the establishment of a barrier to penetration.

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TABLE VIII

TRANSFER OF ^{32}P FROM EXCLUDED PHAGE

Bacteria were mixedly infected with an average of 5 particles of T4r and 1 particle of labelled T3 per bacterium. Following lysis the progeny particles were isolated and tested for radioactivity.

Experiment No.	*Burst size*		*% of parental (T3) radioactivity in*				
	T3	*T4r*	*Low speed pellet*	*High speed pellet*	*% of high speed pellet radioactivity precipitated by*		*High speed supernatant*
					anti T3 serum	*anti T4 serum*	
1	2	145	20	21	—	—	59
2	4	162	24	27	4	92	49
3	5	148	19	16	—	—	65

DISCUSSION

We shall now discuss the results of the seven different types of experiments described in the preceding section in relation to the problem of virus reproduction. First it must be stressed, however, that in all our experiments only the nucleic acid portion of the phage has been labelled; entirely different results are obtained if a specific protein labelling isotope, like ^{35}S, is used, (HERSHEY AND CHASE[16]).

1. Infection of unlabelled bacteria with ^{32}P-labelled T4r phage results in the transfer of 40 to 50% of the label to the progeny particles. The transmitted phosphorus is truly incorporated into the new phage. When the infected bacteria lyse, 5 to 10% of the parental ^{32}P remain associated with the bacterial debris; this may mean that 5 to 10% of the adsorbing particles stay passively attached to the bacterial surfaces. In our experiments the transfer value is an extremely reproducible figure and must be close to the maximum value since (a) nearly all the adsorbed particles participate in the reproduction process, and (b) all the progeny particles are recovered. The transfer value is constant for different preparations of labelled phage.

2. For maximum ^{32}P transfer to occur, all the phage particles which adsorb on a bacterium must do so within about two minutes. Particles which adsorb more than two minutes after the primary infection of the cell do not transfer significant amounts of ^{32}P. Half the ^{32}P of such late adsorbing particles remains attached to the bacterial debris; the rest appears within a few minutes in the medium as material soluble in 5% trichloroacetic acid. In contrast, over 95% of the ^{32}P of the early adsorbing phage is retained in the infected cells until lysis. Under conditions of almost simultaneous infection, the transfer value per infecting particle is constant for multiplicities of infection up to ten.

3. ^{32}P-labelled T2 phage shows the same transfer as T4, and, according to PUTNAM AND KOZLOFF[1] and KOZLOFF[3], similar maximum values are obtained with the related phage T6. The unrelated phage T3 transfers about 40% of its phosphorus to the progeny. Thus an incomplete ^{32}P transfer of 40 to 50% may be a general characteristic of phage reproduction in *E. coli*.

4. If the purines of T2, T3 and T4 phages are labelled with ^{14}C, transfer values are obtained which do not differ from those obtained with ^{32}P. This suggests that the purine bases and the phosphorus are not transferred independently but as the constituents of nucleotides or larger units.

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5. Most of the transmitted ^{32}P is incorporated into the early finished phage. An increase by a factor 2 to 3 in the phage yield per infected cell can be obtained in lysis inhibited cultures; such an increase in yield does not significantly increase the transfer.

6. The "second generation experiments" previously published (MAALØE AND WATSON[4]) show that uniformly labelled parental particles transmit phosphorus to all parts of the progeny particles which in turn become uniformly labelled.

7. Phage heavily damaged by X-rays or excluded from growth by the simultaneous presence of an unrelated phage transfer part of their nucleic acid. Isotope transfer is, therefore, not necessarily connected with the transfer of genetic specificity.

It is well known that infecting particles cannot be recovered by artificial lysis at any time during the first half of the latent period (DOERMANN[17]), and it is now firmly established that the nucleic acid components, phosphorus, adenine and guanine, are incompletely transferred from the infecting particle to the progeny. The transfer experiments, and especially the observation that chemical transfer may occur in the absence of genetic transfer, might, therefore, be viewed as evidence for an obligate and extensive breakdown of the infecting particle soon after adsorption. There is no doubt that such a breakdown can occur as evidenced by the unspecific transfer from radiation damaged or excluded phage (7). In both these cases, however, transfer takes place under abnormal conditions where the transferring particle does not participate in the reproduction processes. It is therefore unwarranted to conclude from these experiments that phosphorus is always transferred via genetically unspecific structures.

As they have turned out, the existing data on nucleic acid transfer, including the second generation experiments, do not decide whether transfer occurs via extensively degraded parental material or via large, genetically specific units. The data even fit with the assumption that nucleic acid structure of the infecting phage remains intact during replication and with a probability of about 50% becomes infective again and reappears among the progeny.

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SUMMARY

a. When ^{32}P-labelled phage reproduce in unlabelled coli bacteria a maximum of 40–50% of their label is transmitted to the phage progeny. Only 5–10% of the label stay associated with bacterial debris after lysis: the remaining about 40% appear as non-sedimentable material in the lysate.

b. The maximum transfer-values are very reproducible provided that all phages adsorbing on a given cell do so within about 2 minutes, and that the entire progeny is accounted for.

c. Experiments with T2, T3 and T4 all show a maximum ^{32}P transfer of 40–50%. The same phages labelled with ^{14}C in the purines yield identical transfer-values.

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d. The transmitted ^{32}P is found predominantly in the early formed phages. The latest formed progeny particles receive no ^{32}P from the parental particles.

e. Damaged or excluded phage particles which do not participate in reproduction or in genetic exchange nevertheless transmit considerable amounts of ^{32}P to the phage progeny.

RÉSUMÉ

a. Lorsque des phages marqués par ^{32}P se reproduisent dans des colibactéries non marquées, le 40–50% au maximum de leur ^{32}P est transmis aux phages nouveaux. Le 10% du ^{32}P seulement reste dans les débris bactériens après la lyse: le reste, 40% environ, apparaît comme matière non sédimentable dans le lysat.

b. Les valeurs maxima de transfert sont bien reproductibles pourvu que l'adsorption de tous les phages d'une même cellule ait lieu en moins de 2 minutes environ et que l'on tienne compte de tous les phages nouveaux.

c. Des expériences faites avec T2, T3 et T4 donnent toutes, pour le transfert de ^{32}P, un maximum de 40–50%. Les mêmes phages, marqués par ^{14}C dans les purines, donnent des valeurs de transfert identiques.

d. Le ^{32}P transmis se trouve surtout dans les phages formés les premiers. Les phages de la nouvelle génération formés les derniers ne reçoivent pas de ^{32}P des particules mères.

e. Des particules de phages endommagées ou "exclues" (c.à d., dont la croissance est empêchée par un autre phage) qui ne prennent pas part à la reproduction ou à l'échange génétique, transmettent tout de même des quantités considérables de ^{32}P à la nouvelle génération.

ZUSAMMENFASSUNG

a. Bei der Vermehrung von mit ^{32}P markierten Phagen in nicht markierten Colibakterien wird ein Maximum von 40–50% des markierten Phosphors auf die Phagennachkommenschaft übertragen. Nur 5–10% des markierten Phosphors bleiben mit den Bakterienresten nach der Lysis verbunden, die übrigen 40% erscheinen als nicht sedimentierbares Material im Lysat.

b. Die Maximumübertragungswerte sind sehr gut reproduzierbar, vorausgesehen, dass die Adsorption bei allen Phagen einer gegebenen Zelle innerhalb von zwei Minuten stattfindet und dass die gesamte Nachkommenschaft berücksichtigt wird.

c. Versuche mit T2, T3 und T4 zeigen alle ein Maximum der ^{32}P-Übertragung von 40–50%. Die gleichen, mit ^{14}C in den Purinen markierten Phagen ergeben identische Übertragungswerte.

d. Der übertragene ^{32}P findet sich überwiegend in den zuerst gebildeten Phagen wieder. Die zuletzt gebildeten Nachkommenteilchen erhalten keinen ^{32}P von den Elternteilchen.

e. Beschädigte oder von der Vermehrung ausgeschlossene Phagenteilchen, die nicht an der Vermehrung oder dem genetischen Austausch teilnehmen, übertragen trotzdem beträchtliche Mengen ^{32}P an die Phagennachkommenschaft.

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