THE CONTRIBUTION OF PROTEIN FROM PARENT TO PROGENY IN T2 COLIPHAGE[1]

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By infecting *Escherichia coli*, strain B, with P^{32} labeled phage, several workers have demonstrated that 30 to 50 per cent of the parental nucleic acid label is incorporated in the resulting progeny. An equally high parent to progeny transfer has been reported when the parental nucleic acid contained C^{14} labeled purines (Watson and Maaløe, 1953). It thus appears that about half of the parental desoxyribonucleic acid is contributed to the progeny. The first transfer experiments utilizing phage labeled in the protein with S^{35} (Hershey *et al.*, 1951) or uniformly labeled with N^{15} (Kozloff, 1952) suggested that some part of the parental protein also might be required by the progeny. However, further work by Hershey and Chase (1952) showed that after infection with S^{35} labeled T2 most of the sulfur containing phage protein could be stripped from the bacterial surface by means of a Waring blendor without interfering with phage production. The resulting progeny contained little or no S^{35}, indicating that growth of the new virus does not involve the incorporation of sulfur containing parental protein. It was considered desirable to extend the work of Hershey and Chase to phage labeled in the protein with a different isotope. For this study T2 phage has been used in which C^{14} is either incorporated chiefly in the protein or equally distributed between nucleic acid and protein. The results obtained are in agreement with those of Hershey and Chase (1952).

MATERIALS AND METHODS

Materials and methods in general were as previously described (French *et al.*, 1952). In addition two synthetic growth media were employed, namely a lactate medium containing per liter: sodium lactate, 10 g; NH_4Cl, 1 g; K_2HPO_4, 0.7 g; KH_2PO_4, 0.3 g; Na_2SO_4, 0.1 g; and $MgSO_4$, 0.01 g, at pH 7; and a glucose medium containing per liter: glucose, 4 g; NH_4Cl, 1 g; Na_2HPO_4, 6 g; KH_2PO_4, 3 g; NaCl, 5 g; $MgSO_4$, 0.1 g, at pH 7. A T2H phage, kindly supplied by Dr. A. D. Hershey, was used in the glucose medium where it was adsorbed usually at least 90 per cent in 5 minutes at 37 C.

The C^{14} compounds used were carboxyl labeled sodium lactate[2] and DL-lysine-2-C^{14} monohydrochloride (Tracerlab, 0.43 mc per mM). C^{14} was counted in a methane flow counter as dried and weighed barium carbonate on filter paper, the counts being corrected to zero thickness of barium carbonate (Yankwich *et al.*, 1947). Combustions were performed by the persulfate method (Calvin *et al.*, 1949). In certain experiments, where only relative assays were necessary, appropriate samples were plated on aluminum dishes, dried under infrared lamps, and counted directly.

EXPERIMENTAL RESULTS

Labeling of T2 using carboxyl labeled lactate. Unlabeled cells which had been grown freshly in lactate medium were sedimented and resuspended at 2×10^8 per ml in fresh medium at 37 C. Carboxyl labeled sodium lactate was added giving final concentrations of 188,000 to 366,000 cpm per ml. These cultures were infected immediately with 10 T2 particles per cell and aerated until lysis ensued. Four such lysates were produced varying in titer from 1 to 4×10^{10} phage per ml. The lysates were purified as previously described (Lesley *et al.*, 1950) and found to contain 1 to 3×10^{-8} cpm per phage. From 0.06 to 0.33 per cent of the added label was incorporated in the phage yield, the percentage uptake being roughly proportional to the lysate titer.

Labeling of T2H with 2-C^{14} lysine. Cells freshly

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TABLE 1

Lysine uptake and control experiments

EXPERIMENT	STAGE	PHAGE PER ML $\times 10^{-11}$	CPM PER ML $\times 10^{-8}$	CPM PER PHAGE	PER CENT RECOVERY OF PHAGE	PER CENT UPTAKE*
1	Lysate	2.4	336.0	1.4×10^{-6}	—	—
	purified	7.4	37.5	5.1×10^{-8}	52	3.6
2	Mixture	2.4	238.0	1.0×10^{-6}	—	—
(control)	purified	5.0	0.08	1.6×10^{-10}	42	0.02
3	Lysate	3.2	524.0	1.6×10^{-6}	—	—
	purified	7.2	29.2	4.1×10^{-8}	28	2.5
4	Mixture	3.4	597.0	1.7×10^{-6}	—	—
(control)	purified	9.4	0.03	3.0×10^{-11}	34	0.002

* Calculated by multiplying cpm per phage (purified) by the lysate titer and expressed as a percentage of the total counts added per ml.

grown in unlabeled glucose medium at 37 C were multiply infected with T2H when the culture had reached 5 to 6 $\times 10^8$ cells per ml. One minute later 26 to 48 mg per 100 ml of 2-C^{14} lysine were added and the culture incubated overnight in a Warburg bath at 37 C with continuous shaking. More reproducible and higher burst sizes were obtained by this method than by aeration. Lysates of 2.4 and 3.2 $\times 10^{11}$ phage per ml were obtained. Purification of the phage by the routine method yielded material of 4.1 and 5.1 $\times 10^{-8}$ cpm per phage, an uptake of 2.5 to 3.6 per cent of the C^{14} added (table 1).

Two types of control were performed (table 1) for the lysine labeling experiments. In one control (experiment 2) purified unlabeled T2H was suspended in glucose medium and labeled lysine added to give phage and lysine mixtures at concentrations approximately the same as in experiment 1. After one hour at 25 C and overnight at 4 C the phage was purified in the routine manner. In another control (experiment 4) a fresh unlabeled lysate was prepared at the same time and from the same culture of cells used to make the second labeled T2H preparation (experiment 3). After lysis 2-C^{14} lysine was added to approximately the same concentration as used in experiment 3. The mixture was incubated for two hours at 37 C and then purified in the same manner and at the same time as the labeled phage.

Table 1 shows beyond doubt that a genuine incorporation of C^{14}, and not merely adsorption or exchange, has occurred in the uptake experiments. It was of particular importance to establish this fact since, as shown below, the C^{14} incorporated in T2H from lysine labeled medium appears almost entirely in the lysine of the phage protein.

Distribution of C^{14} in labeled phage preparations. Aliquots of the labeled phage preparations were mixed with small amounts of crystalline egg albumin to act as carrier and fractionated by the method of Schneider (1945). This involved extraction with cold trichloracetic acid, hot ethanol, and hot trichloracetic acid, the insoluble protein residue being dissolved in dilute alkali. The average distribution of C^{14} obtained for the four "lactate" preparations and the two "lysine" preparations is presented in table 2. It is apparent that whereas the "lactate" type of phage preparation is labeled approximately equally in both protein and nucleic acid, the "lysine" type is labeled nearly specifically in the protein.

In order to determine the distribution of C^{14} in the protein of the lysine labeled phage, further aliquots were refluxed overnight with twice distilled 6 N HCl. The HCl was removed *in vacuo* and the residues dissolved in distilled water. Such hydrolyzates were run as two dimensional paper chromatograms, the solvents being: phenol-water and butanol-acetic acid-water. Although the runs were only about 20 cm by 25 cm, 15 amino acids were readily identified when developed with ninhydrin and compared with known controls. The only amino acids which showed any radioactivity were lysine and possibly histidine and arginine which tended to overlap with lysine. However, electrophoretic separations on paper, utilizing the technique as developed by Markham and Smith (1952), showed that histidine contained no activity and

that arginine contained less than 5 per cent of the total C^{14}. The best conditions found for the run were 0.02 M phosphate buffer, pH 7.8, 900 to 1,000 volts being applied for 4 hours.

Transfer of C^{14} from parent to progeny. The transfer experiments (table 3) with "lactate" labeled phage were carried out in tryptose broth where adsorption was at least 98 per cent complete in 5 minutes at 37 C. Cells freshly grown in broth were sedimented, resuspended in fresh broth at 2×10^8 ml, and infected with 10 T2 particles per bacterium. Ten minutes later 5 unlabeled phage per cell were added to ensure uniform lysis inhibition, and the culture was aerated. After 60 minutes (experiment A) and 100 minutes (experiment B) the infected cultures were run into sterile ammonium sulfate solutions so that the final concentrations were 5 per cent ammonium sulfate (French *et al.*, 1952). The cultures were stored at 4 C overnight and had lysed by morning. A similar experiment (C) was performed omitting the use of ammonium sulfate, and in a control experiment (D) the cells were first infected with 5 unlabeled phage and ten minutes later with 10 labeled phage per cell. The phage from each lysate was purified and the contribution of parental C^{14} calculated. Approximately 13 per cent (experiments A–C) of the parental phage C^{14} was transferred to progeny as contrasted with a transfer of only one per cent under conditions of superinfection (experiment D).

Transfer experiments with lysine labeled T2H were performed in the glucose medium. Three such experiments were performed with two different preparations. Cells were grown freshly at 37 C in glucose medium to 4 to 5×10^8 per ml and infected with 7 to 10 labeled phage per cell. By 15 minutes 88 to 99 per cent of the phage had adsorbed, and titrations through anti-T2 serum gave full recovery of infected cells. The infected cultures were incubated at 37 C with continuous shaking until lysis. It had been observed that some phage in glucose medium lysates may be lost by readsorption. Preliminary experiments in which infected cultures were diluted one hundredfold in 0.01 M potassium cyanide (Doermann, 1952) and titrated after 16 hours at room temperature showed that intracellular phage production reached its maximum in about 5 hours at 37 C. Therefore, in progeny experiments, dilutions were made into cyanide at 4 and 6 hours after infection. When such samples gave higher titers than the final lysate, the higher value was used in calculating the transfer of parental C^{14}. The results obtained with lysine labeled phages are included in table 3. These results are all uncorrected for the presence of small amounts of unadsorbed parental phage in purified progeny, in any event amounting to less than 1 per cent. An average of less than 2 per cent of the parental phage protein label was found to be incorporated in the resulting phage progeny yield (experiments 1–3). In a control experiment, not included in the table, performed under identical conditions with P^{32} labeled phage, a transfer of 28 per cent was obtained.

TABLE 2

Average per cent distribution of C^{14} in labeled T2

PHAGE LABELED FROM	PER CENT SOLUBLE IN			PROTEIN RESIDUE
	Cold 4 per cent TCA*	Hot 95 per cent ethanol	Hot 5 per cent TCA*	
Lactate.....	3	3	45	49
Lysine.....	4	2	2	92

* Trichloracetic acid.

TABLE 3

Transfer of C^{14} from parent to progeny

EXPERIMENT	PHAGE LABELED FROM	MEDIUM USED IN TRANSFER EXPERIMENT	BURST SIZE*	PER CENT TRANSFER TO PROGENY
A	lactate	broth	149	12.3
B	lactate	broth	166	13.8
C	lactate	broth	525	11.2
D	lactate	broth	575	1.1†
1	lysine	glucose	332	2.5
2	lysine	glucose	496	1.9
3	lysine	glucose	600	1.2

* Phage titer per ml divided by infected cells per ml.

† Infected first with 5 unlabeled and 10 minutes later with 10 labeled phage per cell.

Distribution of C^{14} in a lysate from lysine labeled phage. During the purification of one of the lysates (table 3, experiment 3) the distribution of C^{14} in some of the fractions obtained by differential centrifugation was followed. The fresh lysate was centrifuged in a Sorvall angle centrifuge for 15 minutes at 5,000 G and the resulting supernatant for 60 minutes at 22,000 G. Table 4 shows the distribution of C^{14} in the four

TABLE 4

*Distribution of C^{14} in a lysate from lysine labeled T2H**

FRACTION	PER CENT
Low′ speed sediment	83
Low speed supernatant	17
High″ speed sediment	6
High speed supernatant	11
Purified phage	1.2

* Experiment 3, table 3. ′ 15 minutes at 5,000 G; ″ 1 hour at 22,000 G.

fractions so obtained. The bulk of the parental C^{14} was attached to cellular debris containing very little phage. Although 6 per cent of the isotope was found in the high speed sediment containing nearly all the phage, further differential centrifugation by the routine procedure eliminated most of this C^{14}. This is shown by the fact that the final purified progeny contained only 1.2 per cent of the parental isotope.

DISCUSSION

The above results with C^{14} labeled phage protein are in agreement with those obtained by Hershey and Chase (1952) using S^{35} labeled phage. Less than 2 per cent of the parent C^{14} protein label was incorporated in progeny, the 83 per cent remaining attached to cellular debris presumably corresponding to the dispensable 80 per cent of S^{35} label which Hershey and Chase were able to remove from the infected bacteria by means of the Waring blendor. This finding with a nonsulfur label increases the probability that there is little or no transfer of any parental protein or amino acid.

The results obtained with T2 equally labeled with C^{14} in protein and nucleic acid components are also best explained by the assumption that only parental nucleic acid labels are incorporated in progeny phage. The 13 per cent transfer obtained is in agreement with an average of 16[3] per cent predicted on this basis. Moreover, the transfer is almost completely suppressed when T2 infected cells are superinfected with uniformly labeled T2, a procedure which had been shown previously (French *et al.*, 1952) to suppress the transfer of nucleic acid P^{32}. Kozloff's findings for parent to progeny transfer using N^{15} labeled phage are inconclusive and have been criticized already on technical grounds (Hershey, 1953).

Although parental T2 protein is largely, if not completely, excluded from progeny and probably takes no part in the intracellular multiplication of phage, it is difficult to believe that its sole function is to act as a vehicle for transporting nucleic acid. Certainly adsorption of the protein phage "ghosts" effects a profound modification in the metabolism of the host since it has been observed here that respiration is inhibited quickly and completely (unpublished experiments). It has been observed also by Herriott (1951) that "ghosts" cause lysis of the host cells.

SUMMARY

T2 bacteriophage was labeled by infecting unlabeled *Escherichia coli*, strain B, in media containing carboxyl labeled sodium lactate or 2-C^{14} lysine. The incorporated C^{14} was distributed equally in the protein and nucleic acid of the phage produced in the first medium but was incorporated nearly completely as lysine in the protein of the phage produced in the second medium. About 13 per cent of the parental C^{14} was incorporated in progeny from the "lactate" labeled phage but less than 2 per cent in progeny from the protein labeled phage. In a lysate obtained from the protein labeled phage, 83 per cent of the label was found to be attached to cellular debris.

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[3] Calculated from an average parental P^{32} transfer of 35 per cent (French *et al.*, 1952), under identical conditions, from the above observed value of 45 per cent of the C^{14} label in nucleic acid and from the fact that P^{32} and C^{14} nucleic acid labels are transferred to progeny with nearly equal efficiencies (Watson and Maaløe, 1953), thus $35 \times 45/100 = 15.8$ per cent.

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