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[16] It has been found that replacement of Na by K in the buffer reduces the need for supernatant factor and improves enzyme formation.

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*THE DISTRIBUTION OF PARENTAL PHOSPHORUS ATOMS AMONG BACTERIOPHAGE PROGENY**

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Approximately half the deoxyribonucleic acid (DNA) contained in a population of T2, T4, or T6 bacteriophage particles reappears among the descendants ultimately issuing from phage-infected bacterial host cells.[1, 2] This transfer is not due to the reincarnation of entire, intact parental DNA units in progeny guise, since at least half the DNA of each of those descendant particles which harbor the transferred atoms must be of nonparental origin.[3] For an understanding of the mechanisms involved in the reproduction of the hereditary structures of the bacteriophage, it is desirable to know the distribution of the parental atoms among the progeny population, i.e., the extent to which the atomic identity of the parental DNA has been conserved or destroyed. It is the purpose of this communication to present the results of experiments which indicate that most of the transferred phosphorus atoms of the parental DNA are distributed over at least 8 but no more than 25 of the progeny. A more detailed description of these results will be presented elsewhere.

The basis of these experiments is that the bacteriophages lose their infectivity upon decay of radiophosphorus P^{32} incorporated in their DNA, the rate of inactivation being proportional to the number of P^{32} atoms per particle.[3] The fraction of

P^{32} disintegrations which inactivate the T2 or T4 particles in which they occur is 0.10 at 4° C., this "efficiency of killing" having been established for *"nonparental"* radiophosphorus atoms, i.e., for those assimilated into the phage DNA from the phosphorylated constituents of host cell or growth medium.[3, 4] Since neither the transferred phosphorus atoms nor those whose decay leads to inactivation appear to reside in any "special fraction" of the bacteriophage DNA,[4, 5] it would seem reasonable that the decay of *transferred* P^{32} atoms should similarly inactivate the progeny particles harboring them. We have adopted this at present unprovable assumption and have endeavored to detect the presence of parental P^{32} atoms in the descendant phages by observing the lethal effects of the decay of these atoms on the progeny population.

Survival of the Infectivity of Progeny of a Highly Radioactive Parent.—Most of the phosphorus atoms transferred by P^{32}-labeled T2 or T4 parents appear among the first fifty or so progeny particles to mature after the termination of the eclipse period; the last hundred or so progeny particles to mature just before lysis of the infected cell receive very little or none of the parental atoms.[2, 6, 7] An experiment was carried out to examine whether the parental DNA has been dispersed so effectively that its atoms are widely distributed among the individual early progeny particles.

A culture of strain B of *Escherichia coli* was infected at a multiplicity of 0.2 phage per cell with a 2-hour-old stock of T2 labeled with P^{32} at 2,100 mc/mg (or 2,800 atoms of P^{32} per particle). The infected bacteria were freed from unadsorbed phages by centrifugation and incubated in broth at 37° C. for 14, 16, 21, and 30 minutes, when potassium cyanide and chloroform were added to aliquots to induce premature lysis.[8, 9] The lysates, which corresponded to yields of 0.11, 2.2, 35, and 230 mature intracellular T2 particles per infected bacterium, were stored at 4° C. and assayed from day to day for their infective titer.

The result of this experiment is presented in Figure 1, where the logarithm of the titer of the surviving infective phages has been plotted against the fraction of P^{32} atoms decayed by the time of assay. It is seen, first of all, that the highly radioactive parental T2 stock loses its infectivity rapidly in the exponential manner characteristic of inactivation by P^{32} decay,[3, 4] so that less than 1 per cent of the initial titer remains after 8 hours. The progeny populations do not, however, appear to be subject to any significant loss of titer, none of them being inactivated at a rate superior to 0.2 per cent of that of the rate of inactivation of their parent. It appears, therefore, that *the majority of the individuals among even the earliest progeny do not contain more than 0.2 per cent of the parental phosphorus.* Since the *average* transfer to the first intracellular progeny has been estimated to be 2 per cent parental phosphorus per particle,[10] it would follow that most of the transferred phosphorus resides in a minority of the progeny population. The first progeny, therefore, are highly heterogeneous with respect to amount of parental phosphorus possessed by each.

Survival of the Transferability of the Transferred Phosphorus.—A second experiment was designed to probe further into the nature of the heterogeneous distribution of transferred parental atoms among progeny bacteriophages. This experiment is based on the notion that in the case of a population of P^{32}-labeled bacteriophages of which *a minority of the particles contain most of the* P^{32}, it should

be possible to determine the rate of inactivation of the radioactive minority through P^{32} decay by measuring at various times the fraction of P^{32} which the entire population can transfer *in single infection* to its progeny. Initially, when all the radioactive phages are still infective, the global P^{32} transfer to the progeny should be somewhere near 50 per cent; at later stages, when most of the radioactive phages have been inactivated by decay of some of the P^{32} atoms they contain, but when all the nonradioactive phages are still alive, the transfer of P^{32} to the progeny should be nearly zero. The *rate* at which the transferability of the P^{32} atoms contained by the heterogeneous population decreases with the fraction of P^{32} atoms decayed should, then, in principle, be a reflection of the specific P^{32} content of the radioactive minority.

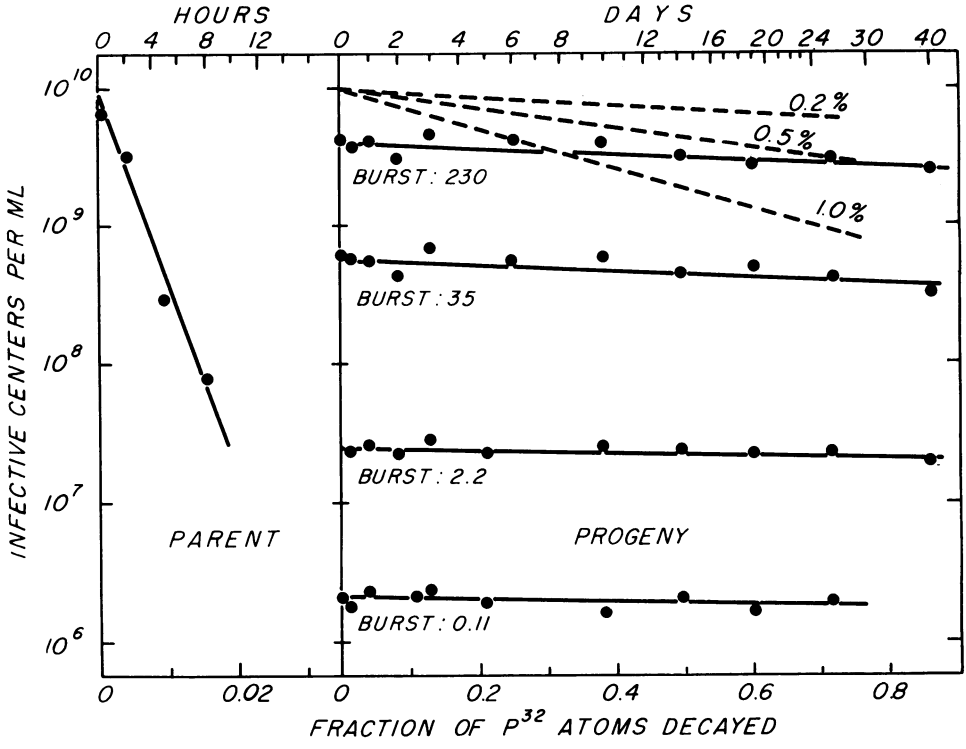

FIG. 1.—The infective titer as a function of the fraction of P^{32} atoms decayed of a parent stock of T2 labeled with P^{32} at 2,100 mc/mg (*left-hand panel*) and of its progeny collected at stages of intracellular growth corresponding to various burst sizes (*right-hand panel*). The stippled lines indicate survival curves corresponding to rates of inactivation constituting various percentages of the rate of inactivation of the radioactive parent.

A control experiment was carried out to test the practicability of this idea. Strain B of *E. coli* was infected at a multiplicity of 0.1 phage per cell with a mixed population of phages consisting of 97 per cent nonradioactive T4 particles and 3 per cent purified T4 particles labeled with P^{32} at 57 mc/mg (or 75 atoms of P^{32} per particle). The infected bacteria were separated from any unadsorbed radioactivity or phages by repeated centrifuging and washing in salt-poor broth, and the radioactivity adsorbed to the bacterial cells was counted. The infected bacteria were incubated in salt-poor broth for 27 minutes at 37° C., at which time

potassium cyanide was added to induce lysis,[8] yielding an average burst of 100–180 phages per infected cell. Bacterial debris and unlysed bacteria were then removed by centrifugation, and a second culture of bacteria was infected with an aliquot of the supernatant fluid, containing the progeny phages. After allowing adsorption of the progeny, the infected bacteria were centrifuged and the radioactivity contained in this last bacterial pellet counted. The ratio of radioactive counts in this last pellet to those initially adsorbed to the first bacterial suspension represents the fraction of the P^{32} transferred from parent to progeny. The mixed population of parental phages was stored at 4° C., and this transfer experiment was repeated on several successive days, while an ever decreasing fraction of the radioactive minority population of T4 still remained alive.

The results of this experiment are presented in Figure 2, *a*, where the logarithms of the percentage of the surviving particles of the radioactive T4 population and of the percentage of P^{32} transferred to the progeny by the mixed population of radioactive and nonradioactive parental phages are plotted against the fraction of the P^{32} atoms which have decayed by the day each transfer experiment was carried out. It is apparent that the transferability of the P^{32} does, in fact, decrease continuously from its initial value near 40 per cent to a value below 10 per cent at a rate similar to that at which the infectivity of the radioactive T4 particles themselves is lost. The two inactivation curves do not remain parallel after the transfer value has dropped below 15 per cent, because, due to certain experimental complications, minimum transfer values between 2 and 5 per cent are usually encountered even when multiplication or maturation of the adsorbed radioactive phage has been prevented.[7] This minimum may increase further when the radioactive parents have been inactivated by agents which prejudice the injection of the parental DNA into the host cell.[11] The control demonstrates, however, that *the rate of loss of transferability of the P^{32} contained in a phage population is an adequate reflection of the rate of inactivation, and hence of the specific P^{32} content, of the particles that possess the radiophosphorus.*

It has been shown that the first-generation progeny possessing P^{32} transferred to them from a randomly labeled parental population again transfer about 30–50 per cent of these P^{32} atoms to their own, second-generation progeny.[5] Hence it would follow that the rate of loss of the transferability of the parental radiophosphorus possessed by the first-generation progeny should reveal the amount of the parental P^{32} carried by individual progeny particles.

A suspension of *E. coli* B was infected at a multiplicity of 0.1 phage per cell with a purified stock of T4 labeled with P^{32} at 200 mc/mg (or 260 atoms of P^{32} per particle). The infected bacteria were permitted to lyse to yield a burst of 100 first generation radioactive progeny per cell. Analysis of these first-generation progeny showed that they contained 43 per cent of the parental P^{32}. The lysate was stored at 4° C., and a transfer experiment similar to the control experiment just described was carried out on successive days in order to determine the amount of P^{32} transferable from the first-generation to the second-generation progeny.

The results of this experiment are presented in Figure 2, *b*, where the logarithms of the percentage of the surviving particles of the radioactive parental T4 population and of the percentage of the P^{32} transferred from the first-generation to the second-generation progeny are plotted against the fraction of P^{32} atoms decayed.

It is seen that the rate of loss of transferability amounts to no more than 6 and no less than 2 per cent of the rate of death of the parental population. It may be concluded, therefore, that those particles of the first-generation progeny which contain most of the transferred P^{32} atoms appear to possess between 2 and 6 per cent of the phosphorus of their progenitor.

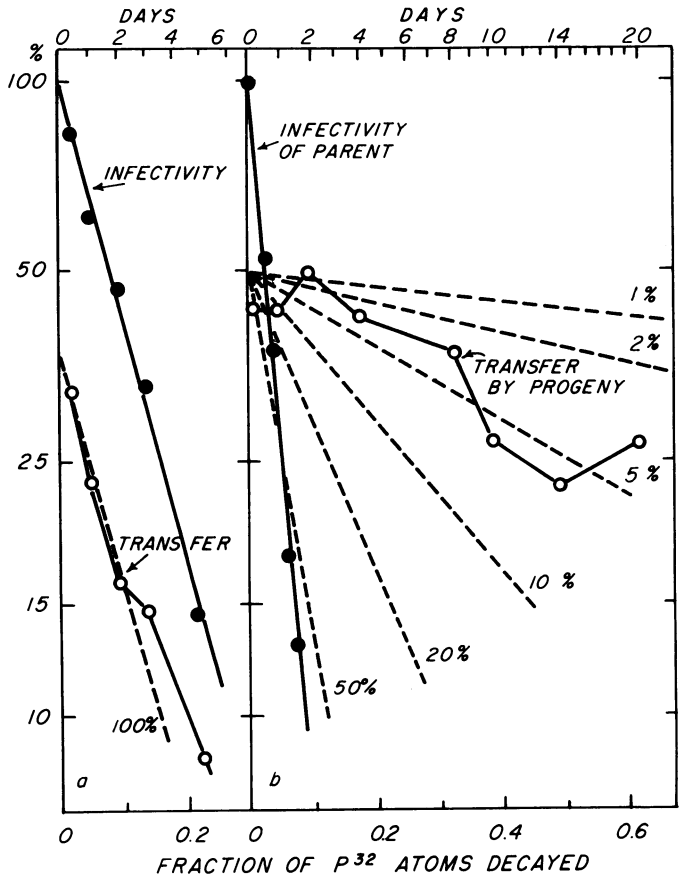

FIG. 2.—*a:* The percentage of the initial infectivity and of the transferable radiophosphorus of a stock of T4 labeled with P^{32} at 57 mc/mg as a function of the fraction of P^{32} atoms decayed. *b:* The percentage of the initial infectivity of a parent stock of T4 labeled with P^{32} at 200 mc/mg and the percentage of the parental phosphorus contained in the first-generation progeny transferable to the second-generation progeny as a function of the fraction of the P^{32} atoms decayed. The stippled lines indicate survial curves corresponding to rates of inactivation constituting various percentages of the rate of inactivation of the radioactive parent.

Discussion.—The survival of the infectivity of progeny of a highly radioactive parent, presented in Figure 1, indicates an *upper* limit to the dispersion of the parental phosphorus atoms, for the accuracy of titration of the infective centers in that experiment would appear to be such that the initial inactivation of no more than a third of any of the progeny populations should have escaped our notice. The bulk of the transferred phosphorus can, therefore, reside in no more than a

third of the first 50 or so progeny, that is to say, in no more than about 15–20 particles. These particles would then carry at least 3 times the average of 2 per cent, or 6 per cent parental phosphorus each. The survival of the transferability of the transferred phosphorus, presented in Figure 2, *b*, indicates a similar dispersion, for the 50 per cent of the parental atoms which are transferred to the descendants appear in progeny particles each of which seems to contain between 2 and 6 per cent of the parental phosphorus. From this it would follow that the number of phages which carry the bulk of the transferred phosphorus atoms must be between 50/2 and 50/6, i.e., between 25 and 8 particles. The fact that the parental atoms are distributed over at least 8 particles indicates that the parental bacteriophage DNA experiences a certain dissociation in the course of its reduplication within the host cell. It should be noted that this dissociation proceeds in *single infection*, i.e., under conditions in which it is certain that the progeny are, in fact, the true offspring of the individuals whose radioactive atoms they bear. Previous experiments, by which evidence for a dispersion of the parental DNA had been adduced, were invariably carried out in *multiple* infection, where it was possible, and sometimes certain, that some of the transferred atoms had not actually been derived from that parent which was responsible for the issue.[2, 11, 12] The quantitative inferences drawn from the present experiments, however, depend for their part on the as yet unverified assumption that the "efficiency of killing" by decay of transferred P^{32} atoms is also 0.10. The significance of the present findings for various proposals concerning the mechanism of DNA replication will be considered elsewhere.

Summary.—The descendants of highly P^{32}-labeled T2 or T4 bacteriophages are inactivated by radiophosphorus decay in a manner which indicates that the bulk of the atoms transferred from the deoxyribonucleic acid of the infecting parental virus particle to that of its progeny is distributed over no less than 8 and no more than 25 progeny particles.

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