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Genetic Transfer in Bacterial Mating

What mechanism insures the orderly transfer of DNA from donor to recipient cells?

Julian D. Gross and Lucien Caro

The genetic material controlling all the essential functions of *Escherichia coli* is organized into a single chromosome which consists, as far as is known, of a continuous double-stranded molecule of DNA, approximately 1100 microns long (*1–3*). Both genetic and microscopic evidence indicate that this chromosome has a circular structure (*3, 4*). Most of the DNA constituting the chromosome is packed into a loosely defined nuclear region less than 0.1 cubic micron in volume. In a fast-growing culture the cells of *E. coli* are 2 to 3 microns long and 0.8 micron in diameter. They contain two to four chromosomes, in various stages of replication. The cells grow by elongating, forming a constriction at the equator, and separating into two daughter cells each containing two chromosomes (*1, 5*). Each chromosome replicates once during each generation, and the products are segregated so that, at division, each daughter cell receives the appropriate number of chromosomes.

Cells of *E. coli* harboring the sex factor F or other similar elements, all of which are constituted entirely or primarily of DNA (*6*), can form a cellular connection with suitable recipient cells. DNA, corresponding to the sex factor, is then efficiently transferred from donor to recipient. In strains in which the sex factor has become associated with the chromosome (Hfr cells), conjugation results in the progressive linear transfer of the entire chromosome, at a rate such that transfer is complete in about 90 minutes (*7*). A striking aspect of this process is that, for any one Hfr strain, the chromosome is transferred in precisely the same sequence from all mating cells. The origin of the sequence is defined by the position at which the F factor had been inserted into the circular bacterial chromosome (*3*).

Various models have been proposed as to how the process of DNA transfer in conjugation may be related to the mechanisms which coordinate chromosome replication and cell growth. In this article we describe these models and discuss experiments which have a bearing on them.

Conjugation in E. coli

The most studied system of conjugation is the one, just mentioned, controlled by the transmissible sex factor F. There are three main mating types: F^-, F^+, and Hfr. F^- cells lack F entirely: they can act only as recipients in matings with donor cells, and they do so with much higher efficiency than either F^+ or Hfr cells. F^+ cells transfer their sex factor with high frequency to F^- cells, converting them to the F^+ type (*8*). The F factor itself is the only genetic material normally transferred in such matings. F^+ cells do occasionally, however, give rise to stable Hfr derivatives capable of transferring the entire bacterial chromosome (*3*).

Genetic experiments, which have been reviewed extensively (*3, 9*), indicate that transfer of the bacterial chromosome by an Hfr is rarely complete. Instead, the majority of the F^- cells receive only a segment of the Hfr chromosome. The frequency of transmission, for any chromosome determinant, decreases with the distance of the determinant from the origin of transfer. The sequence of transfer of genetic markers can be precisely determined by artificially interrupting the mating at various times and assaying for the inheritance, by the F^- cells, of a series of Hfr determinants. Transferred markers are expressed as a result of recombination between the Hfr chromosomal fragment and the F^- chromosome. In interrupted matings, the capacity to act as an Hfr donor is invariably the last character transferred. Hfr cells occasionally revert to the F^+ type or give rise to cells with variant sex factors (F′ factors) capable of transferring, in addition to F itself, a number of genetic markers previously located on one or both sides of the origin of transfer on the circular Hfr chromosome (*10*).

The properties of Hfr cells may be accounted for by postulating that they arise by integrating the F factor into the continuity of the circular bacterial chromosome at any one of a number of possible points. This would be accomplished by a pairing between the sex factor and the chromosome, followed by a reciprocal genetic exchange. The process could be reversed to pro-

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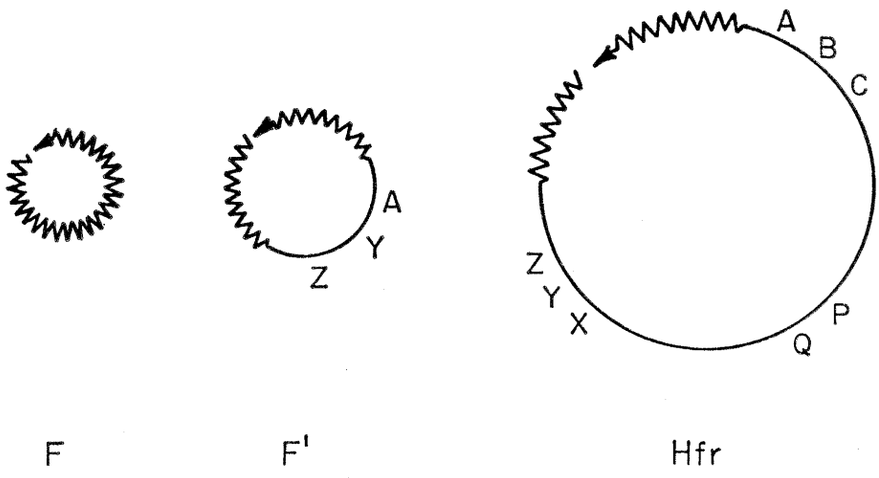

Fig. 1. Hypothetical structure of F and F′ factors and the Hfr chromosome. The genetic material of the F factor is represented by a zigzag line and that of the bacterial chromosome by a straight line. The arrows indicate the extremity of each structure which first penetrates the recipient cell, and the letters represent hypothetical chromosomal markers. F and the bacterial chromosome are not drawn to scale, F representing only 1 to 2 percent of the chromosome.

duce F+ or F′ strains. Figure 1 presents a hypothetical scheme for the structure of the material transferred by F+, F′, and Hfr cells (*9, 11*). This scheme is based on the assumption that F, like the bacterial chromosome, is circular and is itself transferred with a unique origin (indicated by an arrow in Fig. 1). The F′ factor and the Hfr chromosome are viewed, in this model, as hybrid elements which result from inserting into F a part of the whole of the bacterial chromosome. Transfer of F′ factors and of bacterial chromosomes would then simply be special cases of transfer of the sex factor.

Chromosome Replication

The F+ state is very stable: it is lost at a frequency of less than once per thousand cells per generation. If each F+ cell harbored many copies of F which were distributed randomly at cell division, this stability would not be surprising. There is, however, good reason to believe that there is only one or a very few copies of F per chromosome (*12*). F may thus be considered a small supernumerary chromosome in F+ cells. Both F and the bacterial chromosome must, therefore, replicate once per generation, and each daughter cell must receive a copy of each structure. It may be postulated that the mechanisms which insure replication and segregation are similar for both structures and that analogous mechanisms also perform these functions in F− and Hfr cells.

The autoradiographic studies of chromosome replication reported by Cairns (*2, 4*) indicate that replication proceeds from only one growing point, without opening of the circular chromosome, and results in the formation of two complete rings held together at one point. Separation produces two circular daughter chromosomes. Other lines of evidence have also demonstrated the existence of a single growing point in *Bacillus subtilis* (*13*) and in *E. coli* (*14*). Under usual growth conditions (37°C, medium containing glucose), replication occupies most or all of the generation time (*15*). Its initiation must be coordinated with cell growth and division so that it occurs once per generation. One important fact is available: the initiation of replication requires protein synthesis, but once replication has been initiated, it can be completed in the absence of further protein synthesis (*16, 17*).

Jacob and Brenner (*18*) proposed a scheme which would account, in a unitary fashion, for the control of replication and the segregation of the products. They suggest that each independently replicating structure, or "replicon," contains a unique site, the "replicator," at which DNA synthesis is initiated by the action of a diffusible gene product, an "initiator," specific for each replicator. They suggest also that each replicon is attached to the cell membrane at some point, probably the replicator, and that the reaction between initiator and replicator which triggers a new round of replication occurs at this point on the membrane, at a particular stage in the cell's life cycle. It is possible that the chromosome, or any other replicon, moves through this fixed site as it is replicated. Jacob and Brenner point out that this scheme could also account for the regular segregation of the two copies of each replicon at a subsequent cell division: upon completion of the replication of each structure the two products would still be held at the membrane; if the site of attachment itself split, growth of the membrane between the new sites would separate the daughter replicons and subsequent constriction of the membrane would distribute them to separate daughter cells.

That the cell membrane is involved in DNA replication has been supported by the electron microscopic demonstration, in *B. subtilis*, of an attachment of the nuclear regions to the mesosomes, membranous cytoplasmic structures continuous with the cell membrane (*19*). Furthermore, studies of cell fractions have indicated that newly replicated DNA is preferentially associated with the membrane fraction (*20*). Another aspect of the Jacob and Brenner model, the assumption of a single replicator on each replicon, appears to be valid in *B. subtilis*, in which Sueoka and his collaborators (*13, 21*) have demonstrated a unique sequence of replication of the genetic markers for two different strains. The situation is less clear for *E. coli*. Jacob and Brenner (*18*) assumed that in all strains of *E. coli*, F−, F+, and Hfr, the bacterial chromosome is replicated by the same unique replicator system. The F factor would have its own replicator, operative in F+ and F′ cells, but inactive in Hfr cells, where F would be replicated as part of the chromosome. This view is consistent with their observation that temperature-sensitive mutants of an F factor cannot multiply at high temperatures in the autonomous state but do so when incorporated in the bacterial chromosome (*18, 22*).

Lark *et al.* (*17*) found that in *E. coli* 15 T− the site at which replication is initiated in any given cell appears to remain constant for at least four generations. They cannot decide, however, whether this site is the same for all cells of the culture. The possibility that it is not is raised by the studies of Nagata (*23*), in synchronized cul-

tures, on the sequence of replication of two prophages located at widely spaced points on the chromosome. Nagata found that this sequence is not unique in F^- cells. On the other hand, there is a unique sequence in Hfr strains which depends on the strain: replication starts at or near the F factor and proceeds in the direction opposite to that of chromosome transfer. These results suggest that when the F factor becomes integrated into the bacterial chromosome it is not repressed, as proposed by Jacob and Brenner, but instead assumes control of the replication of the entire chromosome.

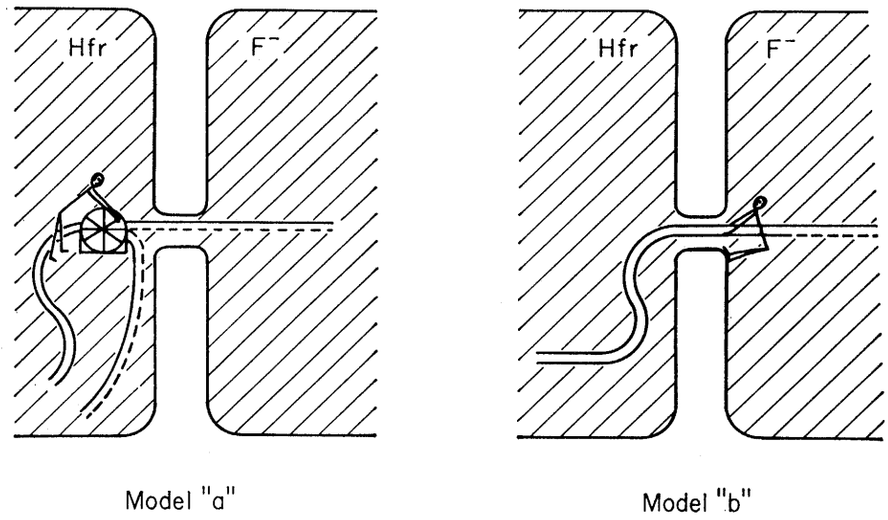

Fig. 2. Diagrammatic representation of two models of DNA transfer. (*a*) The model of Jacob and Brenner (*18, 22*); the DNA is replicated at the point of contact; one copy passes, as it is formed, into the female cell; replication provides the force for transfer. (*b*) The model of Bouck and Adelberg (*24*); DNA transfer starts when replication has been completed; the last portion of the molecule replicated is the first to be transferred and may therefore include a segment of DNA synthesized after Hfr and F^- cells are mixed; the nature of the force insuring transfer is not specified. ——, DNA synthesized before the cells are mixed. – – – –, DNA synthesized after the cells are mixed.

Proposed Models for DNA Transfer

It is clear from what we have said that genetic transfer and chromosome replication have an important property in common: each appears to be polarized, beginning at one point and continuing progressively along the DNA molecule. This similarity led Jacob and Brenner (*18*) to suggest that there is a direct connection between the two processes. They assume that F, both in its autonomous and its integrated state, is attached by its replicator to a point on the cell membrane, at the site at which contact is formed with the recipient cell by means of the specific F antigen (see *9*). The formation of contact activates the F replicator, represented by an arrow in the structure shown in Fig. 1, and one copy of the DNA passes, as it is formed, into the recipient cell (Fig. 2, model *a*). This model is based on their assumption that the F replication system is inactive in vegetative Hfr cells. If the contrary is true, and the direction of replication during vegetative growth is the opposite of that of transfer (*23*), then for the model to be valid, the direction of replication would have to be reversed for transfer. Bouck and Adelberg (*24*) have proposed another way, based directly on the results of Nagata, of relating chromosome replication and transfer. They suggest that transfer of the Hfr chromosome can commence only after replication has been completed, when the finished structures are in some sort of "open" form; in this state, one of the daughter chromosomes would be transferred without further replication into the recipient cell (Fig. 2, model *b*) by an unspecified mechanism.

Arguments can be made for and against each model. Model *a* (Fig. 2) accounts for the unique direction of transfer: it is determined by the direction of replication; the model also provides in the replication process itself a possible force for transfer. Model *b*, on the other hand, does not explain why the same end of the completed chromosome always enters the recipient cell first, nor does it suggest a mechanism for transfer. Model *a* does present difficulties when one considers that most chromosomes are in a partially replicated state at any one time, and in particular at the time when transfer begins.

It is not clear on this model what happens when the two growing points meet, as they must at least when they progress in opposite directions. This problem does not arise on model *b*, since only completely replicated chromosomes can be transferred. A further comment concerns the fact that transfer of the entire chromosome requires 90 to 100 minutes and that this time is independent of the growth rate of the donor cells, whereas the time required for replication of the bacterial chromosome during normal growth appears to be less than 50 or 60 minutes. In model *a* one may suppose either that the F system of replication acts more slowly than the bacterial system (*22*) or that the process of transfer somehow imposes limits on the rate of replication. In model *b* there is, of course, no relation between the rate of transfer and the rate of replication.

A third way of envisaging the transfer process is to assume that there is no connection between transfer and replication: contact with the recipient would somehow trigger the transfer at the origin, and it would proceed without regard to the state of replication. Although this model has not previously been stated explicitly, it must be taken into account in considering experiments on the mechanism of transfer. We shall refer to it as model *c*.

Experimental Studies

1) *The interval between contact and the initiation of transfer.* Since, under the growth conditions generally employed in conjugation experiments, DNA replication occupies most of the generation time, the Bouck-Adelberg model predicts that there should be a variable delay, up to one generation time, between contact formation and the initiation of chromosome transfer by randomly growing Hfr cells. The other models do not make this prediction, since according to them transfer is directly triggered by contact with the recipient cells. Experiments in which the period of contact formation was deliberately restricted (*25, 26*) indicate that there is only a small, variable lag in the initiation of transfer

and that this lag is independent of the generation time of the donor cells. This is true for both Hfr and F′ donors. We have studied the kinetics of contact formation and of DNA transfer in the mating system used in our experiments, as well as the transfer of genetic markers from Hfr cells in which DNA synthesis had been synchronized. These experiments indicated that, even if the period of contact is not artificially limited, most cells start to transfer within considerably less than one generation time after mixing of donors and recipients; so it is unlikely that the ability to form contact is itself limited to the time when replication has been completed. These results favor models *a* and *c*, in which transfer is directly triggered by contact, but in view of the complex nature of the transfer process (*25*) they cannot be considered decisive.

2) *Dependence of transfer on DNA synthesis.* Both the Bouck-Adelberg and the Jacob-Brenner models predict that transfer should be affected if DNA synthesis is prevented after donor and recipient cells have formed contact. On the former model a small fraction of the donor cells might have just completed replication when DNA synthesis was interrupted and might thus be able to transfer without further synthesis, but the majority of cells would be unable to initiate transfer. Once transfer was initiated, however, it could proceed in the absence of DNA synthesis. This is not true of the Jacob and Brenner model, which requires continuing synthesis. Model *c* is the only one in which transfer is independent of DNA synthesis, since this is the one and only postulate of the model.

Suit, Matney, Doudney, and Billen (*27*) have reported that transfer was not affected by amino acid starvation or ultraviolet irradiation, both of which inhibited DNA synthesis in the Hfr cells. This observation could rule out models *a* and *b*. In collaboration with Eggertson, we have irradiated Hfr cells with doses of ultraviolet light sufficient to kill 10 to 90 percent. In contrast to the results of Suit *et al.*, this treatment greatly reduced transfer in the survivors; moreover, those cells that could still transfer did so with a pronounced lag. In the experiments of Suit *et al.* the Hfr and the F^- cells were immobilized on a filter during mating and were thus held in close proximity. Since our crosses were done in liquid medium, the differences in mating conditions could account for some of the differences in results. It would be important, in connection with the observations on the effect of amino acid starvation, to determine whether DNA synthesis was in fact inhibited under the conditions of mating.

Pritchard (*26*) has studied the effect of thymine starvation on chromosome transfer by thymine-requiring Hfr cells. He found that thymine deprivation was virtually without effect on transfer by cells of one Hfr strain. In another strain the fraction of cells which were able to transfer in the absence of thymine declined, during starvation, from about 50 percent immediately after removal of thymine to about 5 percent after 1 hour. Even in this case thymine deprivation appeared to affect only the ability to initiate transfer, not the ability to continue transfer at the normal rate once started. These results appear to rule out both models *a* and *b*, because of the high proportion of cells able to initiate and continue transfer in the absence of thymine. They imply that DNA synthesis is not normally necessary for transfer (model *c*). The critical question in interpreting these observations is: how much DNA synthesis occurs in these Hfr strains under conditions of thymine deprivation? Measurements of incorporation of C^{14}-uracil into alkali-resistant, acid-insoluble material indicated that there was little DNA synthesis in the absence of thymine. Thymine-requiring cells, however, have many poorly understood properties, such as their loss of viability in the absence of thymine (*28*) and their frequent requirement for extremely high concentrations of exogenous thymine (*29*). In addition, many mutants show greater "leakiness" at room temperature than at 37°C (*30*). It is therefore difficult to be sure that residual incorporation is a true reflection of the amount of thymine available, and that none of the cells or the chromosomes, perhaps specifically those which are taking part in transfer, are able to replicate in the absence of exogenous thymine.

Two recent sets of experiments give results that are most easily explained by the model of Jacob and Brenner. Roeser and Konetska studied the effect of phenethyl-alcohol, which has been reported (*31*) to act like amino acid deprivation in that it allows the completion of DNA replication but prevents its reinitiation; they observed that if the inhibitor was added to a mixture of Hfr and F^- cells after transfer had started it did not prevent further transfer, whereas if it was added at the time the cells were mixed no transfer took place, although contact formation was not inhibited (*32*). These results suggest that the ability to complete replication is not sufficient to allow transfer, as it should be according to the Bouck-Adelberg model, but that a new initiation is required.

The other experiments involve the use of nalidixic acid, which is reported to inhibit DNA synthesis specifically and rapidly (*33*). Bouck, Adelberg, and Pritchard (*34*) have observed that this inhibitor completely blocks transfer whether it is added prior to mating or once transfer is in progress.

It is clearly impossible to derive a unified conclusion from these various experiments. The difficulty stems from the many uncertainties which exist with regard to the action of inhibitors of DNA synthesis: how complete is their action? what is their mode of action? do they affect various kinds of DNA replication similarly? Until these questions are answered the interpretation of experiments such as those just described will remain precarious.

3) *Characterization of the DNA transferred during conjugation.* The experimental approach that has given the most promising results is to label the donor DNA in order to distinguish between DNA synthesized prior to mating and DNA made during mating, and then to test the predictions which the different models make concerning the DNA transferred to the recipient cells.

Herman and Forro (*35*) grew F^+ cells in tritiated thymine and mated them with F^- cells in the absence of label. They studied, by autoradiography, the distribution of label among the cells in microcolonies grown from single F^- cells that had received an F factor from the donors. They found that in many instances only one cell in the microcolony was labeled. When more than one cell was labeled the total number of autoradiographic grains over the microcolony was, in general, no higher than when the grains were concentrated over a single cell. Since it is known that F replicates soon after entering a cell and continues to do so at least once per generation, these results can be interpret-

ed by assuming that the DNA entering the F^- has only one of its two strands labeled. Most cases in which the label is distributed over several cells can be explained by a fragmentation of that single labeled strand. Although there are intrinsic limitations to the analysis of these data, the results do suggest that the DNA of the F factor has replicated once before entering the F^- cell.

Ptashne (*36*) studied the transfer of an F′ factor carrying a segment of DNA corresponding to prophage λ. He had found previously that (*37*) when λ-lysogenic cells were infected with the related phage 434, the resulting lysates included, in addition to phage 434, a few λ particles, one-third of which contained exclusively prophage DNA. This indicated that in some cases both strands of the prophage DNA had been released from the bacterial chromosomes and incorporated into a phage particle without further replication. This observation provided a means of determining whether the λ prophage segment of the F′ factor was transferred to the F^- cells without replication. The DNA of the F′ cells was completely labeled by growth in heavy isotopes, and the mating took place in light medium with recipient cells previously infected with phage 434. The phages released when the recipient cells lysed contained many λ particles with one labeled strand but none with two labeled strands. Ptashne obtained evidence that the absence of fully labeled λ particles was not due to an obligatory replication of the prophage DNA subsequent to its transfer, and concluded that the F′ particle carrying the prophage invariably replicated prior to or during DNA transfer. These results are predicted by the model of Jacob and Brenner, which requires that replication take place simultaneously with transfer. To account for these results in terms of the Bouck-Adelberg model one would have to assume that the sex factor is never in the "completed form" at the time of contact formation but must replicate to be converted to this form. Model *c* appears to be definitely excluded.

We have examined the relationship between DNA replication and chromosome transfer by Hfr cells. In this case models *a* and *b* both predict that the region of the chromosome transferred early during conjugation will have been replicated before transfer, but they differ in one important respect. In model *a* replication and transfer are simultaneous, whereas in model *b* replication precedes the initiation of transfer. Our results favor model *a*.

In one experiment thymine-requiring Hfr cells were mated for 50 minutes with morphologically distinguishable adenine-requiring recipients that were starved for adenine so they would not synthesize DNA during mating. The Hfr cells had grown in H^3-thymine for several generations, and the labeling was either continued throughout the period of mating or stopped by the addition of an excess of unlabeled thymine at the time of mating or 20 minutes later. The amount of radioactive DNA appearing in the recipient cells was then measured by autoradiography after mechanical agitation to separate the couples. The number of labeled F^- cells did not increase significantly between 20 and 50 minutes, an indication that in most cases transfer had already started at 20 minutes. The effect observed was the same whether labeling was stopped at the time of mating or 20 minutes later: in each case the amount of label subsequently appearing in the F^- was half as much as in the mixture in which labeling was continued. In a second experiment, unlabeled Hfr and F^- cells were mixed and H^3-thymine was added to the mixture 20 minutes later: the recipient cells became labeled immediately, and the amount of labeling increased progressively with time; if the H^3-thymine was diluted with unlabeled thymine 10 minutes after its addition, further labeling of the F^- was halted immediately. The same results were obtained in experiments in which the recipient cells were prevented from synthesizing DNA during mating by prior irradiation with a heavy dose of ultraviolet light rather than by adenine starvation. These results are what would be expected if replication and transfer occurred simultaneously; on the other hand, if replication were completed prior to transfer, as in the model of Bouck and Adelberg, the removal or addition of label to the medium after the initiation of transfer should not affect the labeling of the DNA which is subsequently transferred. There is, however, another conceivable explanation for our results. This is that the Hfr chromosome is transferred without any replication and is immediately replicated in the F^-. This explanation is not very plausible, since one would have to assume that, when the F^- cells are starved of adenine, they either receive just sufficient adenine from the male cells to allow replication of an amount of DNA equivalent to the incoming Hfr DNA, or they receive more adenine but only synthesize that amount of DNA. In order to eliminate this explanation we have performed an experiment, suggested by Mark Ptashne, which demonstrates that not more than one strand of preformed Hfr DNA is transferred during conjugation.

We compared the label transferred during conjugation, in unlabeled medium, by two parallel cultures, one labeled until the time of mixing with the F^-, the other placed in unlabeled medium 1.3 generations before mixing to insure that all the DNA molecules would be either hybrids, with one radioactive and one nonradioactive strand, or completely nonradioactive. In the first mating twice as many F^- cells received label as in the second. However, the amount received by individual cells was the same in both cases: since in one case the maximum amount of label that could be transferred was that contained in one radioactive strand, it follows that this remains true even when labeling is continued until the time of mating. This experiment shows that, at most, one strand of preformed DNA can be transferred in mating.

Earlier experiments, in which the donor DNA was labeled with P^{32} prior to mating and the effect of P^{32} decay on the viability of the transferred material was measured, had suggested that this material was uniformly labeled (*38*). The interpretation of these experiments is difficult, however, since the pool of P^{32}-containing material in bacterial cells is large, so that DNA synthesized for several minutes after removal of P^{32} from the medium will continue to be heavily labeled (*39*).

Conclusions

It is evident that at present no definite conclusion can be drawn from the experiments that we have summarized. The rapidity with which transfer is initiated implies that it is directly triggered by contact with the recipient cells and does not require that the donor cells reach a special stage in their division cycle. Although certain

of the results obtained with inhibitors of DNA synthesis suggest that transfer is not dependent on DNA replication, the DNA transferred under conditions of inhibition has in no case been characterized. Consequently it is not yet certain whether or not that DNA is replicated. On the other hand, the various approaches used to characterize the DNA transferred under normal conditions have in general indicated that it does replicate before entering the recipient cell. In the case of transfer by Hfr cells, there is good evidence that the replication takes place simultaneously with transfer. Since this approach appears to us to be the most direct and reliable one, we believe that the basic characteristics of the transfer process are in accord with the model proposed by Jacob and Brenner. It does, however, remain to be proved that the observed replication of donor DNA is a *necessary* condition for transfer.

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Health, Education, and Welfare

Remarks on the Department by the new Secretary.

John W. Gardner

Three months ago President Johnson asked me to head up the Department of Health, Education, and Welfare. Probably very few people have an accurate knowledge of what that department is and does.

The Department is big, but not colossal. It employs about 90,000 people, as against 600,000 for the Post Office and more than a million civilian employees for the Defense Department. As a basis for comparison, AT&T and its affiliated companies employ 750,000, General Motors 660,000.

This year we will spend roughly $10 billion from general revenues and $20 billion from the Social Security trust funds. As in any insurance plan, Social Security benefits are paid out of funds that the beneficiaries originally paid in. The total annual expenditure of about $30 billion puts us far behind the Department of Defense, which will spend around $50 billion. (Although we rank a poor second in that comparison, we like to say, "We try harder.")

The Department is made up of eight major agencies—the Public Health Service, the Office of Education, the Food and Drug Administration, Social Security, the Welfare Administration, Vocational Rehabilitation, the Administration on Aging, and a new agency for Water Pollution Control.

Let me describe the work of some of these agencies.

In 1798 the young American nation undertook to provide medical services to merchant seamen in its busy ports—and that was the start of what is now the Public Health Service. Today the Service still provides direct medical care not only to American merchant seamen, but to American Indians and Alaska natives, Peace Corps volunteers and federal prison inmates.

It also maintains surveillance against contagious disease. The Communicable Disease Center in Atlanta is a command post of modern medical science,

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