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Reprinted from AMERICAN SCIENTIST, Vol. 44, No. 3, July 1956

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THE recombination of genes stands on a par with mutation and selection as a cardinal element of biological variation. In the laboratory, recombination furnishes the experimental test of mutational change in genetic units. In nature, it leads to their fulfillment by generating a multitude of different combinations which are then sifted by natural selection. Until recently, genetic recombination has been closely identified with sexual reproduction: indeed geneticists consider it to be the principal biological function of sexuality, but other processes are now recognized as alternative means to the same end. In sexual reproduction, the fertilization of one intact cell or gamete by another precedes the formation of the new zygote and assures the union of a full complement of genes from each of two parents. In *genetic transduction*, by contrast, one cell receives only a fragment of the genetic content of another. As we shall see, the fragment can be defined not only in genetic but also in physical, chemical, or virological terms. That is, the fragment may be associated with subcellular constituents which are equally notable as virus particles or as macromolecules of DNA (desoxyribonucleic acid). So far genetic transduction is only known, with certainty, in bacteria but whether this limitation is one of fact or of technology must still be found out.

Historical Background

Scattered observations that might be attributed to transduction have been recorded since the infancy of modern microbiology in the last century but could not be understood or coordinated in the incomplete genetic theory of the time. During the past twenty years, however, an infusion of quantitative method has nourished bacterial genetics, and this discipline has enlarged that basic concordance of all living forms which comparative biochemistry had firmly substantiated.

In higher plants and animals, the concept of the gene originated from recombination studies, but in bacteria it arose and for some time depended exclusively on mutation studies. This concept simply states that the hereditary quality, the intrinsic differences of organisms, can be analyzed in terms of unit factors, markers or genes. Detailed factorial analysis of bacterial heredity has, of course, had to wait upon the recog-

* Paper No. 606 from the Department of Genetics, University of Wisconsin. The research in bacterial genetics in this department has been supported by grants (C-2157) from the National Cancer Institute, Public Health Service, and from the Research Committee, Graduate School, University of Wisconsin with funds allotted by the Wisconsin Alumni Research Foundation.

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nition of recombinational techniques, be they sexual or transductional. Unit factors that are subject to mutation, and can be used for genetic studies of bacteria, are related to such diverse traits as pigmentation, resistance to antibiotics and to bacterial viruses, biochemical aspects of nutrition and fermentation enzymes, cellular morphology, and antigenic specificity. The most distinctive and advantageous developments in microbiological technique are selective methods for the easy discovery and isolation of rare individuals of a specified genetic constitution. For an obvious example, a streptomycin-resistant cell, or at least a clone of its descendants, can be isolated from any number of sensitive cells among which it may be submerged, merely by plating the entire population into a culture medium containing the drug. Such efficient detective techniques were first applied to the study of mutation, but are readily adaptable to a search for recombinational processes.

The idea of transduction of hereditary fragments is still difficult to reconcile with our well established knowledge of highly organized chromosomal systems of heredity, but the problem can now be stated in terms clear enough that the synthesis, if not yet certain, can at least be experimentally sought. Earlier generations of microbiologists were, however, untroubled by subtleties of a genetic theory that had not yet been elaborated. For example, in 1893, San Felice [1] reported that nonpathogenic, "pseudo-tetanus" bacilli might acquire the ability to produce tetanus toxin by being grown in a culture medium that had been impregnated with metabolic products of the true tetanus bacillus. That bacteria might absorb toxins from their medium is at least plausible, but San Felice had taken pains to show that the treated bacteria would continue to produce the toxin after many generations of growth. A new principle of genetics would be implied by that experimental result, but at that time even the name "genetics" had not been coined for a science that was still dormant. Remarkably enough, although San Felice played an important part in the scientific classification of the tetanus bacilli, and these toxin-forming bacteria have been intensively studied for many years, there is no record of later attempts to confirm his "transduction" experiments. It would be easy to dismiss them as technical fallacies in the sterilization and control of a notoriously difficult group of bacteria, but these 60-year-old experiments warrant re-examination especially in the light of recent findings on toxin formation in diphtheria [2].

Similar notions of transduction by the passive absorption of "receptors" by different bacteria in mixed cultures or from filtrates doubtless underlay an extensive literature under the heading of "paragglutination" which dates from about 1910–1935 (reviewed, [3, 4]). The discovery that bacteria, when injected into rabbits, would evoke specific antibodies that would agglutinate the bacteria, led to the use of this

technique as a means of classifying bacterial strains by what we now call their "serotype." At first, the reactions were believed to be absolutely specific, but exceptions were soon found whereby antiserums against one strain might sometimes agglutinate quite different bacteria. Such cross-reactions are even found between certain types of human red blood cells and certain strains of pneumococcus, and are now ascribed to fortuitous similarities in chemical make-up; in the early 1900's, however, many workers sought to find a biological basis for serological cross-reactions. Perhaps the most striking cross-reaction is that between the bacterium, *Proteus* OX-19, and the *rickettsia* which are responsible for typhus fever. Most authorities today believe that this reaction, which is the basis of the famous Weil-Felix diagnostic test, is a coincidence of chemistry of no biological significance and that the *Proteus* is a harmless saprophyte; a few still speculate on the possibility of a closer relationship. One recurrent proposal has been that an indifferent *Proteus* acquired the rickettsial antigens by residing in infected animals where it might be exposed to the antigens or other products, i.e., by transduction, but the genetic implications of such proposals were rarely thought through. Similar proposals were backed by rather indecisive experiments to account for the cross-reactions of various enteric bacteria (*Salmonella; Shigella; Escherichia coli*) and the scores of papers that were published on paragglutination have had very little direct influence on the further development of bacterial genetics.

The "Pneumococcus Transformation"

A new turn of events was marked by Griffith's announcement [5] in 1928 (the same year as Muller's proof that X-rays caused mutations) of the transformation of serological types in the pneumococcus. Although he nowhere alluded to paragglutination or other conceptual precedents, it is hard to doubt that Griffith was influenced by the earlier reports. He had been working for some years on the serotypic classification of pneumococci, which was important for therapy as well as for diagnosis, since antiserum was almost the only specific treatment known at that time. The most prominent antigen of virulent pneumococci consists of a polysaccharide capsule that envelopes each cell, and is readily detected by an apparent "Quellung" (swelling) reaction in the presence of specific antiserum. Many serotypes can be differentiated by the specific chemical and serological reactions of characteristic polysaccharides. The presence of the capsules conditions not only the high virulence of these organisms, but also the appearance of their colonies on agar media, whence these are called "smooth" types. "Rough" variants are also known: these are relatively avirulent and lack the specific capsular substances. Some rough variants were more or less unstable and would occasionally revert to the parental smooth form;

others appeared to be absolutely stable. Griffith was impressed by the occasional occurrence of more than one serotype in a single sputum, and speculated on the possible interconvertibility of the types. His experimental design was fundamentally similar to that of earlier attempts at transduction: he prepared heat-killed suspensions of smooth bacteria, and inoculated mice with a mixture of this vaccine and some living, rough cells. Neither the killed vaccine nor the rough bacteria, separately, would be expected to generate an active infection in the mice, and none did in control experiments, but in a few inoculations, the mixture of the two gave a virulent infection from which living smooth pneumococci could be isolated. In further experiments, he also showed that the serotype of the recovered bacteria depended on the type of bacteria used to prepare the vaccine, rather than on the parentage of the rough cells. He had therefore justified the claim that rough bacteria, originally of one type, had been transformed to a different smooth type. The mouse played the part of a selective agent in obtaining a specific genotype, namely a new virulent form. To explain this transformation, Griffith did not use genetic language, but adopted what amounts to a transduction hypothesis, namely that the substances responsible for the formation of the specific capsules had been transferred via the vaccines to the deficient, rough cells. Some later workers have erroneously attributed the transformation to the polysaccharides themselves, but Griffith had already noted theoretical and experimental arguments against such views.

Griffith also studied another quite different "transformation," namely the production of rough variants by the action of antiserum on smooth types. The serum effect has not been reinvestigated in detail, but can hardly be a transduction, and is most likely a selective action of the serum that permits spontaneous rough variants to manifest themselves.

Fortunately, Griffith's observations were saved from limbo by their prompt trial in several other laboratories where they could be confirmed and extended. The problem was taken up in the laboratories of the Rockefeller Institute by a group of workers under the leadership of the late O. T. Avery, who had already contributed much of our knowledge of the immunochemistry of the pneumococci. Their extensive researches culminated in the report [6] in 1943, that the active material in the "pneumococcus transformation" was a macromolecular form of desoxyribonucleic acid, DNA, a substance that had already been found to occur characteristically in the nuclei and chromosomes of the cells of higher organisms, and was therefore believed to be intimately connected with the stuff of heredity. These workers had also developed more precise quantitative techniques for assaying the frequency of transformations, and methods by which this could be accomplished *in vitro*.

Griffith had been rather lucky in his earlier experimental design. The living mouse proved to have *two* functions: a selective action, as already mentioned, and to furnish protein adjuvants from its serum that play an obscure part in conditioning the bacteria to enable them to take up the DNA particles.

Considerable progress has been made recently in the chemical analysis of DNA, and it is known to consist of extended chains of nucleotide units (probably arranged in a double spiral [7]). Each unit contains phosphoric acid; a five-carbon sugar (desoxyribose); and a purine or pyrimidine base (adenine, guanine, thymine, or uracil). The specificity of DNA is believed to depend on the sequence of these four alternatives; it is not surprising that very large molecules are required to store biological information of ultimate complexity in a language with such a simple alphabet. Actually, the true molecular size of pneumococcal DNA is not accurately known, for such long linear macromolecules are the most difficult to study by the physicochemical methods that have been developed for the proteins. It is generally accepted, however, that the material extracted from the pneumococci is a mixture of active and inactive material, and that the active constituents would consist of chains with hundreds or thousands of nucleotide units. The same difficulties have stood in the way of rigorous proof that *only* DNA is involved in the transforming substances, though this has been established to the limits of current analytical methods. One of the most challenging and exciting problems of research in molecular biology will be the resolution of these DNA preparations into homogeneous fractions with specific biological activity, for, as we shall see, this represents the most direct approach to the chemistry of the genes [8, 9].

These spectacular discoveries in biochemistry ran far ahead of the genetic study of the pneumococcus transformation, which relied on the capsule as a sole genetic marker. Until this study was broadened about 1951 with experiments on drug resistance and other markers [8, 9], a variety of opinions were forwarded (mostly on a purely speculative level) on the biological interpretation of Griffith's finding. They included the following versions of the transforming substance:

1. It was a specific mutagen with a special ability to direct a particular gene to mutate in a definite direction.
2. It was a polysaccharide autocatalyst (perhaps as a complex with DNA) that primed an enzymatic reaction for polysaccharide synthesis.
3. It was a bacterial virus, which on infecting the bacteria provoked capsular synthesis as a host reaction.
4. It was an autonomous cytoplasmic gene or a morphogenetic inducer.
5. It might be acting at a distance without penetrating the bacterium.

6. It was a fragment of the genetic make-up of the bacterium, the only one to have been tested to that time.

7. It was an element *sui generis* for which no general conception should be adduced.

In a more sophisticated sense, these hypotheses are not absolutely incompatible with one another, but they had to be sorted out from one another as the basis for genetic experimentation. The main distinction of the sixth hypothesis, later named transduction, is that it led to the study of additional markers. The subsequent success of this approach is enough justification for classifying these hypotheses. The transduction hypothesis had been ably stated by H. J. Muller in 1947 in the speculation that the DNA might correspond to "still viable parts of chromosomes (which might) undergo a kind of crossing over with the chromosomes of the host" [10]. But this conception was not unanimously adopted, and other workers have been less willing to correlate the pneumococcus transformations with other genetic systems.

During the past five years, transductional systems have been a topic of renewed interest, engaging many workers in the comparative study of several organisms. The first common link is perhaps the well-known chemical fact of the distribution of DNA as a major constituent of chromosomes in all organisms. However, so long as a single trait of a single organism was all that was being studied there could be no appreciation of the general biological significance of the pneumococcus transformation. This impasse unfortunately obtained for twenty years after Griffith's announcement. The much more detailed analysis that has been developed more recently owes its vigor in part to the tacit acceptance of transduction as a working hypothesis for similar phenomena in the pneumococcus and several other organisms.

The essential purpose of multi-factorial experiments is to measure the bounds of the genetic unit that is being transferred. In Griffith's own experiments, he was, for example, obliged to admit the (far-fetched) possibility that the apparently sterile suspensions of heat-killed bacteria still contained damaged but viable cells that were not detected in control experiments, and could begin to grow only in the presence of actively growing cells. However, if the donor and recipient differ in two or more markers, the isolation of recombinant clones which differ in part from both "parents" easily rules out this kind of experimental fallacy. (Unfortunately, this possibility is not so far-fetched in some recent claims of transduction which have not passed this genetic test.) Further studies with multiple marker differences, for example, of resistance to penicillin, resistance to streptomycin, and capsular antigens have shown that each marker is transmitted independently of the others, a finding which is the principal basis for classifying the "pneumococcus transformation" as an example of transduction. The idea of trans-

duction also had to be, and has been, supported by a demonstration that the potency of a DNA extract depends on the genetic quality of its source. That is to say, streptomycin-resistance can be transferred only from a resistant donor; one can only recover in any recombination system the elements that have been put into it. This criterion implies that a reported effect [11] of nucleic acids (among other substances) from penicillin-resistant staphylococci in transforming the *same* bacteria to penicillin-sensitivity must be explained by some other mechanism, still unknown, and not by transduction. This order of specificity of DNA goes even further than the reassuring observation that DNA from unrelated sources, e.g., beef spleen, has no transforming activity on bacteria.

The success of Griffith and Avery has, of course, inspired many investigators to look for genetic effects of DNA with other bacteria. The greatest success to date has been met with in the influenza bacillus (*Hemophilus influenzae*) where again a capsular antigen and streptomycin resistance proved to be useful markers [12]. This system is closely parallel to the pneumococcus transformation, a result which is important, in itself, in confirming the generality of the potency of DNA in transducing activity. On the other hand, many trials with other bacteria have been definitely unsuccessful, though negative results do not, of course, receive equal attention. However, it is never possible to be sure that some technical detail (as the use of serum with pneumococci) will not turn the trick. This experience suggests that the critical problem may be to assure the penetration of undenatured DNA into the recipient cells, the mechanism of which is still quite unknown even in the pneumococcus. Unfortunately, the largest number of reports [4, 5] of possible transduction are still indecisive, the effects being either poorly reproducible or unsupported by the solid experimental evidence that has been accumulated for the pneumococcus and *Hemophilus*.

Transduction by Phage

A second mode of transduction, mediated by bacterial viruses rather than chemically extracted DNA, was discovered independently of the pneumococcus researches in the course of studies on *Salmonella* [13]. This group of bacteria is of medical interest because of their connection with diseases such as food-poisoning and enteric and typhoid fever. However, they are closely related to *Escherichia coli*, a species which has been shown to undergo recombination by a sexual mechanism, that is one that involves intact cells and the exchange of large blocks of genes [14, 15]. Originally, sexuality could be demonstrated in only one bacterial strain (K-12) but after suitable methods were developed, the same process could be found in many different strains of *E. coli*. It was therefore natural to extend these researches to other related organisms, of which *Salmonella* was a logical choice.

The principal criterion for sexual rather than transductive recombination is the concurrent exchange of several factors, a result that was also considered technically more reliable than the change of a single marker, for which simple mutation must always be considered. This caution led to many indecisive experiments: indications of exchange with single markers could not be confirmed in multi-factorial experiments. We eventually realized that genetic exchange was taking place in certain combinations of *Salmonella* strains, but in a different pattern from crossing in *E. coli*, as only single markers were being exchanged at each event. This realization was promptly followed by the corollary discovery that filtrates of certain mixed cultures could be freed of intact cells, and still transform individual traits of a recipient strain. The term "transduction" was introduced at this point for the hypothesis that genetic fragments were being transmitted from one strain to another, via cell-free filtrates, as seemed to occur in the pneumococcus too. However, *Salmonella* proved to be far more amenable to genetic study than the pneumococcus, and generalizations on this transduction could be based on studies with thirty or forty different markers in a relatively short time. The advantages of *Salmonella* in genetics were, however, compensated for in biochemistry. At first, the most important information about the transforming substance of *Salmonella* was that its activity was *not* destroyed by desoxyribonuclease, in distinction to the pneumococcus experiments. Filtration and sedimentation experiments then connected the activity with particles about 0.1 micron in diameter and hence just beneath microscopic visibility. (For comparison, an *E. coli* cell is about 1 micron wide and 3 to 5 microns long.) These particles were later identified as bacteriophage, or bacterial virus. The lack of effect of desoxyribonuclease was then easily explained, if the true agent of transduction were protected inside the skin of the virus particle. The inner agent might very well be DNA—but chemical studies so far cannot distinguish the bacterial genes from the DNA nucleus of the virus itself, and we must rely for this guess on the analogy with the pneumococcal transduction.

But where did the virus come from in these bacterial filtrates? One of the *Salmonella* strains proved to be "lysogenic," that is to say is infected by a latent virus. The latent virus, or "prophage" [16] is ordinarily transmitted as a hereditary quality, during the multiplication of the lysogenic cells. It would remain undetected indefinitely except for an occasional accident through which the prophage develops into active virus, kills its host, and is released into the medium. Once it is freed, the virus can infect other cells. Here it may behave alternatively as a typical lethal parasite, and grow rapidly at the expense of the host, or re-enter its latent form, and render the bacterium lysogenic. While empirical means have been discovered by which to influence these decisions, the actual steps that determine (*a*) whether a lysogenic bac-

terium will continue to reproduce as such, or break down and release active virus, and (*b*) whether a newly infecting virus will kill its host, or combine with it to form a lysogenic symbiosis are more speculated about than understood. We shall have to give further consideration to the genetics of the latent virus, but we may take this occasion to recapitulate the results of transduction genetics so far, by analysing the stages as follows (see Fig. 1).

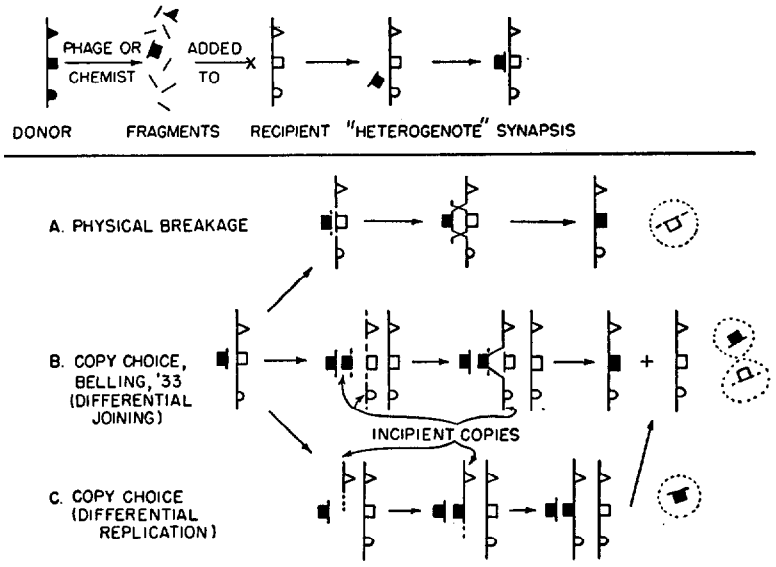

Fig. 1. Hypothetical schemes for the incorporation of transduced fragments. The top line shows the preliminary stages of fragmentation and reintroduction of fragments into recipient cells, common to all three proposals. The fate of the residual fragments, enclosed in dotted circles, is unspecified. From [15].

The first step is the disruption of the donor bacterium, and the formation of genetic fragments. This can be accomplished either by the chemist, as when he dissolves the pneumococcal cells in bile salts, or by a bacteriophage as it feeds on its host. In experimental practice, the next step is to purify these suspensions, either by filtration or by chemical precipitation; the main purpose of this step is to remove possible inhibitory substances and any remaining viable cells of the donor strain. Since the transducing agent, be it DNA itself, or encased in phage particles, is far smaller than the bacterium, this is not difficult.

The second major step is the introduction of the agent inside the bacterial cells. With DNA, it is not at all clear how this comes about but occurs when serum-treated cells are exposed to DNA solutions. On the other hand, viruses could not infect if there were not some special mechanism for their penetration, and recent studies suggest the follow-

ing stages: the virus attaches to the bacterial surface, usually by a protein appendage; there is then some enzymatic softening of the cell surface; then the DNA nucleus of the phage (and any passengers) enters the bacterium, leaving the phage skin outside [17]. These tricks of infection simulate the fertilization of an egg by a sperm, but whether the viruses or the cells discovered them first is an unanswered, if not a meaningless question of viral evolution.

After the agent has penetrated the bacterium, it may in due course exchange with the homologous genes of the recipient. How this is accomplished touches intimately on the very mechanism of genetic reduplication. Some possible formulations are presented in Figure 1, but it must be admitted that feasible experiments to decide among these alternatives have not yet been carried out. It is, however, virtually certain that this exchange step takes place promptly after penetration. In *Salmonella*, for example, this can be shown by means of transductions which involve flagellar antigens: when a new antigen has been effectively transduced, the previous homologue never reappears in the same clone [18]. If homologous replacement of genes thus takes place, we have to acknowledge some mechanism by which the homologues associate to precede the decision of which one will be kept. Cytologists had long since deduced a high order of genic specificity from the way in which whole chromosomes synapse with one another during the reduction-divisions, but it is none the less remarkable that in the large reaches of the cell a bit of DNA can find and recognize its opposite number as it must do to displace it. The datum of homologous replacement is also a strong indication that the genetic system of the recipient bacterium is organized (viz. into one or more chromosomes). If each one of the very many kinds of transducible fragment corresponded to an independent chromosomelet, it would be hard to see how an orderly process of homologous replacement could be managed, not to mention the problem of keeping each p and q in order during ordinary cell division. This intuition has, however, been substantiated by more explicit evidence, as we shall see. The dilemma of whether to postulate a disintegrated genotype in the whole life cycle of the bacterium, or an unknown mechanism by which a fragment could exchange with an integrated chromosome, was possibly the chief obstacle to bridging transductional genetics to the general theory of the science (for example, cf. [19]). The generality of homologous replacement in transduction experiments has given a definite direction to further research in this field in terms of integrated genic systems, i.e., chromosomes.

In the *Salmonella* system, the genetic fragment has accompanied a bacteriophage, but in the transformed cell they appear to act independently [20]. A fraction of the bacteria are killed—an obvious condition of a workable system is the survival of a reasonable proportion of the in-

fected cells, either by the formation of lysogenic complexes, or by the suppression of the phage. One means of separating transduction from active virus infection is to treat the phage suspensions with ultraviolet light. This inactivates viral infectivity much more rapidly than the genetic action, so that many particles are formed that will transduce their contained fragments without transmitting viable virus. The same result is achieved by the use of bacterial strains to which the virus is poorly adapted. The ultimate fate of the viral residues is unknown, as is that of the leftovers shown in Figure 1.

The conclusion may be simply stated that the pneumococcus and *Salmonella* both manifest types of genetic transduction. In the latter, bacteriophage acts as passive carrier of the genetic (DNA?) fragments, but the viral nucleus has no other demonstrable relationship with its host companions. In both cases, any genetic marker that could be tested for was amenable to transduction.

Genetics of Prophage

The role of prior work on *E. coli*, for the development of information on *Salmonella* has already been outlined. While a sexual system (viz. recombination between intact cells of whole blocks of genes) has not yet been found in *Salmonella*, it was an obvious and early question whether transduction could be demonstrated in *E. coli*. This would be of particular use and interest just because of the genetic analysis that had been and could be done by sexual crosses, which had, for example, given some insight into the genetic nature of latent viruses. The latency of the virus in a lysogenic bacterium is very well exemplified by strain K-12 of *E. coli*, which had been used as a classroom type strain for twenty years, and an object of intensive genetic research for another five before this was appreciated. The virus, which was named *lambda*, is latent both in the sense of concealment, and potentiality for development. It was actually detected only when a mutant strain was discovered that was sensitive to lambda, and therefore could serve as an indicator for the free virus. The free virus, as already intimated, is released by about one cell per million in an ordinary culture; as Lwoff has shown, this release can be enormously accelerated by treating the bacteria with ultraviolet light. To account for the genetic quality of lysogenicity, many authors have long since tacitly assumed that the phage had somehow combined with the genetic make-up of the cell. This assumption was made even more necessary when it was learned that lysogenic bacteria, even when artifically opened, did not contain typical, infective virus particles. The term "prophage" has thus been given to the latent developmental phase of the bacterial virus, to the element of genetic continuity which distinguishes a lysogenic bacterium [16].

Before the development of crossing methods, the genetic localization

of the prophage could only be speculated about. On the analogy of the hereditary "kappa" particles in *Paramecium*, it was thought possible that the symbiotic virus might also live in the cytoplasm (whence the analogous symbol "lambda"). However, when lysogenic (lambda-carrying) bacteria were crossed with nonlysogenic strains, a regular segregation for this trait was found, which ruled out the cytoplasmic hypothesis. It was then found that the lambda-prophage was closely linked to one of the genetic markers of the bacterium, concerned with galactose-fermentation. Finally, hybrids were produced which segregated lysogenicity and galactose-fermentation as a pair of linked markers. One can even refer to the prophage as a dominant gene, since the hybrid is lysogenic, and like typical lysogenic bacteria is immune to the attack of the free virus, although it segregates both lysogenic and virus-sensitive, nonlysogenic offspring. It was concluded that the genetics of the prophage could not be distinguished from that of other elements of the bacterial genotype; the main distinction is that a bacterium which carries the prophage is liable to a sporadic transformation which results in the death of the bacterium and the appearance of infective virus. This result could be most readily understood on a dualistic version of the prophage: in the lysogenic bacterium, it is a part of the bacterial chromosomes; in the infective phage particle, it is the nucleus of an autonomous parasite, the virus [21].

In the absence of similar crossing studies there is no certainty that other phages in *E. coli* or other organisms have a similar duality—whether they have a predetermined site in the bacterial chromosome or any at all—it would be premature to generalize from a single instance, but this kind of question is part of the catechism of any virus. As soon as the genetic functions of *Salmonella* bacteriophage were appreciated, the possible transducing ability of lambda in *E. coli* was examined, but at first with purely negative results on a number of tested markers. Later, M. L. Morse looked more closely at the matter, and it was discovered that lambda could indeed transduce, but only a single group of genetic markers, namely the galactose-factors to which the prophage itself was linked [22]. This specific relationship is quite different from the transductions previously analyzed, in which *any* marker was transducible and it speaks again for the proximity of the prophage to the galactose-genes. Again, only about one phage particle per million is effective, that is, will transform a galactose-negative recipient bacterium to the galactose-positive form, when the phage has been generated from galactose-positive bacterial hosts.

It was mentioned earlier that in the other examples of transduction, the newly transduced fragment promptly underwent exchange with the homologous elements of the recipient. The lambda system is different in a useful and interesting way, for it can be shown that here the fragment

does *not* quickly exchange, but may maintain its integrity for a long time and reproduce in parallel with the reproduction of the cell (see the "heterogenote" stage, Fig. 1).

Linkage in transduction

The further study of the fragment depends on linkage in transduction, which has not been mentioned so far. While the chief distinction of transduction is the transfer of a single marker, our discussion has represented this as a "fragment" rather than a "single gene." This caution follows from the fact that a bacterium must have thousands of genetic factors, only a fraction of which can have been detected by their mutant forms. It is therefore premature to label the unit of transduction of known mutants as an absolute "single gene," particularly in view of theoretical controversy as to the operational meaning of this concept [19, 23]. It would be more correct to state that transduction involves a unit small enough that in most cases it has been experimentally marked by only one factor. This reservation has been borne out by a few instances, both in *Salmonella* and in the pneumococcus, of linked transduction where the investigators have evidently been lucky enough to find markers so closely associated that they can be transmitted on the same fragment. More recently, still another transduction has been described in *E. coli*, by another phage, in which it has been possible to correlate linkage in transduction with linkage in sexual crossing [24]. Evidently, problems such as are raised by the exchange of a fragment with the chromosome are better studied with groups of linked markers. For this purpose, we have unfortunately been unable to find any genes other than the galactose-markers which are closely linked to lambda-prophage. However, these have proved to be genetically distinguishable but linked to one another and therefore serve the purpose. The diversity of the galactose genes was first revealed by crossing experiments. When galactose-negative mutants that had been obtained in different strains were crossed with one another, about one galactose-positive recombinant per thousand was found. It was therefore necessary to distinguish the mutants by different numbers: Gal_1; Gal_2; Gal_3; and so forth, to show how the same character may be affected by different genes. The result of the test cross would then be symbolized as: $Gal_1^- Gal_2^+$ X $Gal_1^+ Gal_2^-$, which can give some galactose-positive, $Gal_1^+ Gal_2^+$, recombinants as an application of a standard procedure for determining the identity or difference of mutant genes.

The same distinctions were revealed by transduction experiments. That is, phage grown on the Gal_1^- mutant would produce galactose-positives from Gal_2^- cells. This may be symbolized $Gal_1^- Gal_2^+ \rightarrow X$ $Gal_1^+ Gal_2^-$ to give $Gal_1^+ Gal_2^+$, and amounts to the transduction of the Gal_2^+ gene. However, the galactose-positive clones so obtained were

unstable for this character and would throw off occasional galactose-negative cells in the course of further growth. When these were typed, either by transduction or by crossing tests, they fell into three classes: the parentals, Gal_1^+ Gal_2^- and Gal_1^- Gal_2^+, and a new type Gal_1^- Gal_2^-. The formation of galactose-negatives is therefore a segregation process, and by adding up the markers we infer that the transduced fragment, Gal_1^- Gal_2^+ has remained intact in the same cell as the recipient chromosome [25]. When the fragment is lost or exchanges with the chromosome, the various negative types are generated. The Gal_1^- Gal_2^- combination is of particular importance in relating the exchange of fragment and chromosome to crossing-over as illustrated in Figure 1. The same sequence may be postulated for other transduction systems in which the pace of events is too fast for their separation.

Virus as gene; Lysogenic conversion

As in the *Salmonella* system, only a small proportion (about one per million) of the lambda particles carry Gal genes with them. However, when the lambda is obtained from bacteria which are already carrying supernumerary fragments, from previous transduction, these fragments are incorporated into virtually all of the phage particles. Since the fragments all trace their ancestry directly to the contents of an earlier infecting phage particle, this is tantamount to equating the fragment itself with the prophage. The analogy between the two elements is at least striking enough to justify classifying the virus infection as a special case of transduction, i.e., of that genetic element of the donor bacterium which we have called the prophage. In fact, experiments have recently been reported on transduction, *by other viruses*, of lambda prophage as a marker linked to Gal [24, 26].

The equivalence of virus and gene is here a formality of the means of hereditary transmission. The prophage-gene may be related more or less closely to the rest of the genetic system, but in the cases so far can be readily separated, e.g., by crossing-over or by a cycle of parasitic growth of the virus. In other situations, however, a prophage may act as a gene of the host bacterium not only in a formal but in a functional sense. For example, certain *Salmonella* strains may be classified into the serotypes E_1 and E_2. Phage grown on E_2 cells will convert E_1 into E_2. This conversion is, however, inseparable from infection by the phage, which is both a necessary and sufficient condition for the conversion [27]. The same phage may casually and sporadically transduce other markers, but the E_2 serotype is invariably associated with the phage and vice versa [28]. The prophage may therefore be considered as the gene for E_2. This lysogenic conversion is an example of transduction in the sense that it is transduction *of* the prophage, rather than of other genes *by* the phage. An equally striking example of lysogenic conversion

has been described in the diphtheria bacterium, where phage in filtrates of toxin-forming strains will convert nontoxin-forming cells so that they will also form the toxin [2]. If San Felice's observations [1] are vindicated and the same pattern holds for tetanus toxin, our excursion among viruses and genes will have turned full circle both in concept and in experiment.

Applications and Prospects

The study of transduction in bacteria has been the labor of many scientists of whom only a few are listed in the references here. But the story has only well begun. Of the thousands of bacteria species, only a few have been examined at all, and each study has revealed a new facet. So far, only a minority of attempts to demonstrate recombination mechanisms (sexual or transductive) in bacteria have been successful It is a fair caution that the first essential is a selective technique, a workable means of detecting new types even when they are extremely rare. Partly for this reason, and partly because the experimental organisms have usually been economically unimportant, recombinational techniques have had more analytic than practical utility. (Artificial serotypes in *Salmonella* have been of some use in the preparation of diagnostic serums.) The vast applications of genetics in practical agriculture are, however, a sufficient portent of what may be accomplished in due course with the microbes that are important in medicine and industry.

Some of the hopes that have been expressed, however, are too extravagant in the light of present knowledge, for example for the massive transformation of virulent or drug-resistant bacteria in an infected host to more innocuous forms. To be therapeutically effective, such transformations would have to involve virtually every bacterium, which is too much to ask of a recombination process (barring the exceptional examples of lysogenic conversions). A similar misconception has provoked the suggestion [29] that transduction could account for the spread of drug-resistance from one mutant cell to a large population (a result that needs no elaborate explanation other than selection). In every case so far, genetic transduction is achieved at the expense of the life of the donor cell: in the most favorable cases, the DNA or the lambda from one or a few bacteria has been enough to transform a single recipient, which speaks for the recovery of much of the original genetic material.

So far, no definite case of transduction has been reported for higher organisms. Claims that DNA from tumor cells would induce tumors in normal mouse tissues are controversial [30, 31] but they do illustrate the impact of transduction on experimental cancer research. The concept may also have some bearing on mysterious changes in tumor cells that are transplanted to new hosts. And speculations correlating the pneu-

mococcus transformation to embryonic inductions perhaps have to be inverted and reviewed with the genetic understanding of the former. However, before a convincing search for transduction in higher organisms can be executed, efficient selective methods will have to be developed as they have been for bacteria.

The question of whether transduction is unique to the bacteria, or occurs more generally, is important for its bearing on general genetic theory. It has been suggested that the postulated "chromosomes" of bacteria and viruses are chemically and structurally less elaborate than the cytogenetically verified chromosomes of higher plants and animals. However, the generalization of concepts and techniques learned from these organisms has been the most productive approach to the analysis of transduction in bacteria. Conversely, transduction has pointed up the weaknesses of some traditional formulations of chromosome behavior. In crossing-over, for example, can we believe that two chromosomes will regularly break at precisely corresponding points? The impending translation of genic differences as chemical (or grosser structural) differences in DNA has also provoked a reexamination of the concept of the "single gene" [23]. We are reminded again of the first principle of genetics, that we cannot recognize genes directly but only their differences. In turn, we should not insist on genes as self-reproducing units, but as units or markers of a more complex self-reproducing system. Nevertheless, the representation of genic differences in chemically purifiable DNA is the closest approach to the reduction of genetics to biochemistry, an enterprise which can challenge the skills and imaginations of specialists in a dozen sciences.

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