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GENETIC AND FUNCTIONAL ASPECTS OF GALACTOSE METABOLISM IN *ESCHERICHIA COLI* K-12

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INTRODUCTION

The widespread application of micro-organisms to physiological genetics has led to renewed interest in the distribution within the genome of genes controlling related biochemical functions. The concept of a functional hierarchy in the organization of the chromosome had been formulated by Serebrovsky in 1930, for instance, as step allelism in connexion with bristle mutants of *Drosophila*. The simple correspondence between step alleles and elements of the phenotype was questioned by later investigators since extraneous factors were shown to intervene in the development of the phenotype (Goldschmidt, 1955; Wright, 1958).

On the other hand, many clusters of pseudoallelic mutants have been found (Green, 1954; Carlson, 1959). These are mutants believed to be genetically identical until later recombination tests on a still larger scale revealed rare (crossover) wild types among the intercross progeny. A central feature of many pseudoalleles in *Drosophila* is their failure to complement each other, that is to produce an effective wild-type phenotype when the two mutant genes are carried on opposite chromosomes (*trans* arrangement, or $+-/-+$). Whenever both mutant genes were coupled in tandem on the same chromosome and the corresponding wild-type alleles on the other one ($++/--$ or *cis* arrangement), the normal phenotype is seen. The discrepancy between *cis* and *trans* arrangements of two pairs of genes is called a 'position effect' (Lewis, 1951). The *cis-trans* test provides a more reliable index of functional identity of similar mutants than a comparison of their biochemical defects.

Benzer (1956) has redefined the conceptual basis of genetic fine structure analysis. In a study of a large collection of *rII* mutants of bacteriophage T4 he demonstrated that each recombinationally distinct unit or *recon* could be arranged on a genetic map in an unambiguous linear array. Moreover, groups of recons could be classified by *cis-trans* tests into *cistrons*. Mutants from different cistrons complemented one another, while members of the same cistron did not. The cistrons themselves may be bridged by some deletion mutants which overlap them.

The ideal completion of this analysis would be the isolation and characterization of the presumably defective proteins produced by the individual mutants.

Benzer has calculated that some recons can be resolved over intervals of a few nucleotide pairs. This supports one tenet of the molecular theory of gene action: 'Each mutation changes the nucleotide sequence at a defined position on the nucleic acid corresponding to a polypeptide chain, producing corresponding changes in the amino acid sequence and rendering the protein defective' (Brenner, 1959).

A parallel approach utilizing transduction by phage P22 (Zinder & Lederberg, 1952) in *Salmonella typhimurium* has been vigorously prosecuted by Demerec and his collaborators at Cold Spring Harbor (1956). Several systems of auxotrophic and fermentative mutants were examined. The frequency of wild-type recombinants was used as a measure of the map distance between two biochemically similar mutants. A unique feature reminiscent of step allelism was observed. For example, over forty transductionally distinctive histidine auxotrophic mutants were subdivided into seven groups representing a series of steps in the biosynthesis of histidine. The groups could be arranged on the map in the same order as the biochemical steps (Hartman, 1956). Functional complementation was assayed by means of an abortive transduction test (Stocker, Zinder & Lederberg, 1953) on cell lines which carry a non-reproducing extra fragment of genetic material. These cell lines give 'minute' colonies if the mutants are complementing. This method has proved useful in some instances but was unsuccessful in other applications (Demerec & Ozeki, 1959; see also Clowes, this Symposium, p. 107).

The two studies summarized above illustrate many such investigations now in progress with diverse organisms. Micro-organisms are favoured material because of the ease of gathering large numbers of a desired mutant type. Efficient screening procedures on large populations can reveal rare recombinations between similar mutants. The precision of tests of such high resolving power is the basis of 'fine structure analysis' (Pontecorvo, 1958). In addition accurate characterization of the genetic defect is possible in micro-organisms. The possibility of isolating altered proteins from a mutant series, as has been initiated by Yanofsky & Crawford (1959), is also more hopeful. Each system has certain advantages and disadvantages. The galactose mutants in *Escherichia coli* strain K-12 fulfil many of the desiderata, partly because of the utility of this strain for recombination analysis and tests of complementarity, and partly because the enzymes blocked by mutation have been identified.

GALACTOSE MUTANTS

A considerable number of galactose-nonfermenting mutants (Gal$^-$) have been collected in *Escherichia coli* K-12. Those that have been analysed are clustered in a small portion of the genetic map (E. M. Lederberg, 1958). The first set of mutants which were reported (Morse, Lederberg & Lederberg, 1956*a*, *b*) consisted of seven recons diagnosed by several recombinational techniques. As implied by the definition of recon, any pair of mutants always recombined to give some Gal$^+$ recombinants. They are all linked to the markers *Lp* (*Lambda* lysogenicity, Lederberg & Lederberg, 1953), and also to the linked markers *Hfr*-1 (Cavalli-Sforza & Jinks, 1956) and *Ind* (indole-requirement, Richter, 1959). Some exceptional mutants will be discussed later. Aside from a few which occurred spontaneously, they were induced by ultraviolet irradiation of a galactose-fermenting (Gal$^+$) wild-type culture in a single treatment. Each has a characteristic reversion rate to Gal$^+$ as observed by the papillation pattern of colonies plated on EMB-galactose agar. The reversions, where examined, appear to represent restorations of gene function at the original mutated site.

TRANSDUCTIONAL ANALYSIS

A major impetus to the programme was provided by the discovery of transduction of Gal markers by the temperate bacteriophage, λ. This phage, normally carried as a prophage by lysogenic strains of *Escherichia coli* K-12, is able to transfer a fragment of genetic material (Morse, Lederberg & Lederberg, 1956*a*, *b*). The recipients may be either lysogenic or sensitive providing that they are able to absorb λ. The λ must be derived by induction of lysogenic (*Lp*$^+$) donors: λ prepared by infection of a sensitive host is inert. Other transductional systems allow the transduction of any marker but with relatively low efficiency. The unique quality of the λ-system is its limitation to the *Lp*-Gal segment.

The mutants could be separated either by sexual intercrossing of the strains or by the production of Gal$^+$ recombinants when the λ of one was applied to the cells of another. To show this, 0·1 ml. of a 10^{10} lysate of one Gal$^-$ strain was plated with 10^8 recipient bacteria (carrying another Gal$^-$ marker) on EMB-galactose agar. Where effective transduction had occurred, one to two hundred Gal$^+$ (dark coloured) papillae grew out of the Gal$^-$ lawn within 48 hours. No papillae appeared when the λ was plated on its strain of origin.

HETEROGENOTES

The galactose-positive papillae arising in transduction experiments are routinely subjected to careful single colony isolation for the establishment

of clones. However, after repeated purifications, these Gal$^+$ clones regularly throw off galactose-negative sectors, in contrast to the original wild-type strain which is stably galactose-positive. These unstable clones have been termed *heterogenotic* since the segregants appear to represent the persistence of galactose-negative genes from the two different parents. The segregants were identified as such by homology tests as follows:

(1) matings with the input parents, and with other testers;
(2) transductions by lysates from the segregants to known Gal$^-$ testers;
(3) transductions to the segregants by lysates from known Gal$^-$ testers.

These homology tests were all consistent: a Gal$^-$ segregant was either the same as the donor parent (*exogenotic*), the recipient parent (*endo-*

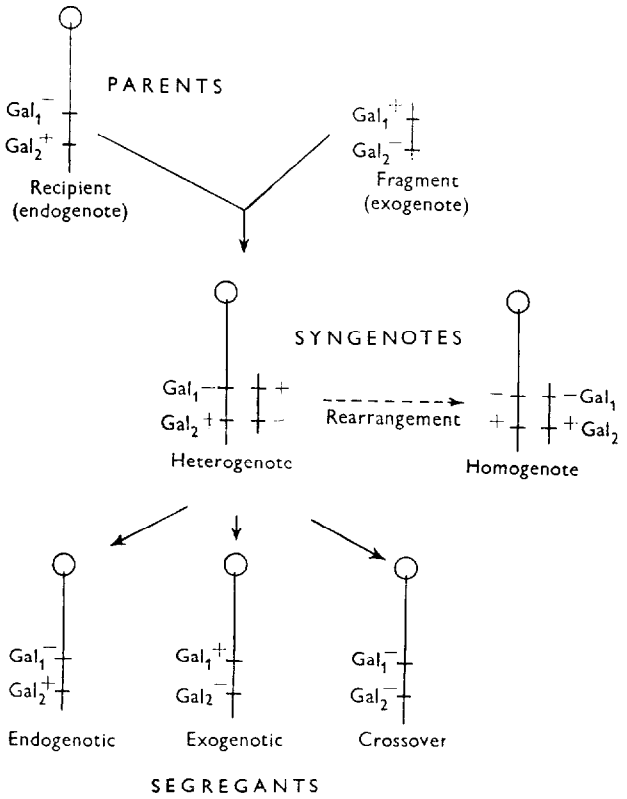

Fig. 1. Diagrammatic representation of transduction. This is exemplified by an experiment in which Gal$_1^-$ cells are treated with a Gal$_2^-$ lysate. The heterogenote which results can undergo two types of further changes: rearrangements to give derived syngenotes, and segregation to give the haploid types indicated. All of the genotypes diagrammed here can reproduce as such and persist indefinitely, but the syngenotes are unstable and repeatedly give off new types.

genotic) or was a double Gal⁻ recombinant, carrying one exogenotic, and one endogenotic Gal⁻ marker. Therefore, the conclusion was reached that the immediate product of transduction is a *syngenote*, that is a partial diploid clone in which the genome of the recipient is supplemented, but not yet supplanted, by a genetic fragment (the exogenote) from the donor (Fig. 1). In the λ system, only the Gal and *Lp* genes are so far known to be included in this fragment.

LYSATES WITH HIGH TRANSDUCING ABILITY (HFT)

The accuracy of this recombinational system was greatly increased by the discovery of highly efficient or HFT lysates (Morse *et al.*, 1956*a*, *b*). The number of transductions approaches or equals that of the phage plaque titre of such lysates, whereas one transduction clone per million plaque particles is produced by standard lysates. Lysogenic heterogenotes always gave HFT lysates, as did occasional Gal⁻ segregants. The latter proved to be homogenotes: partial diploids homozygous for a Gal⁻ marker. The homogenotes arise by crossing-over and segregation within the heterogenote as diagrammed in Figs. 1 and 2. Homogenotes may later segregate haploid Gal⁻ clones.

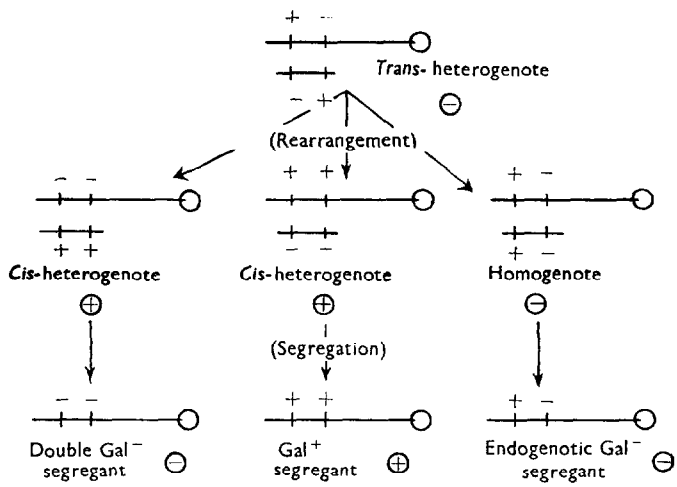

Fig. 2. Rearrangements and segregation in a heterogenotic clone. The figure shows a sample of the events which take place in a *trans*-heterogenote, in particular one involving a pair of non-complementing Gal⁻ mutants. This heterogenote then has a galactose-negative phenotype. The symbols + and — refer to the galactose-phenotype. Note that a — *trans*-heterogenote gives some + progeny, which accounts for the heavily papillated colonies formed by such heterogenotes.

What are the criteria which distinguish HFT from standard lysates? First of all, the lysate must originate from a syngenotic clone. Syngenotic

clones are defined as those containing a genetic fragment. The fragment and the chromosome may be either identical (homogenote) or different (heterogenote) with respect to their Gal alleles. Secondly, HFT λ is defective (*λdg*) for a definite portion of the phage genome. This attribute was observed by following the inheritance of genetically-labelled λ in transduction clones (Arber, Kellenberger & Weigle, 1957; Arber, 1958; Campbell, 1957). If sensitive bacteria were infected at low multiplicity with HFT lysates consisting of marked λ, none of the resulting heterogenotes were lysogenic. The segment of phage genome containing the determinants for this function and markers linked to it were missing, but the heterogenotic cells were still immune to superinfection with normal phage particles. The segregating immune heterogenotes previously described as $Lp^{r/s}$ (Morse, 1957) are now known to carry *λdg* in the exogenote. In our experience, all haploid segregants from $Lp^{r/s}$ heterogenotes have been λ-sensitive, i.e., the *λdg* is rarely if ever incorporated in the chromosome.

In principle, an HFT lysate should then contain two components: *λdg* which cannot mature to give plaques, but does carry Gal markers from the bacterial chromosome, and intact 'helper' phage which somehow cooperates in the functioning of the transducing particle, but does not appear to contain any Gal genes. The two types of phage in HFT lysates postulated from genetic experiments have now been separated in density gradients of caesium chloride solutions in the ultracentrifuge (Weigle, Meselson & Paigen, 1959). The reciprocal relationships suggest the hypothesis that the Gal region of the bacterial chromosome has replaced (through some as yet unknown mechanism of genetic exchange) the missing region of the DNA moiety of transducing λ (Campbell & Balbinder, 1959). Independent events leading to the production of transducing particles in LFT lysates evidently generate a series of λ types, clones of which can be separated by their varying densities (Weigle, personal communication).

The significance of the numerical relationship between transductions counted as papillae and the plaque titre of a lysate (T/P) as a measure of the transduction efficiency (Morse *et al.*, 1956*a*) is therefore obscure. A more meaningful measure would be transductions per total particles ($\lambda^+ + \lambda dg$). However, the *λdg* cannot be counted by routine methods of plating.

Transducing activity then resides in *λdg* particles unable to carry out typical phage functions. Successful attempts to extract the active DNA component from *λdg* by removing the protein from λ preparations have been reported by Kaiser & Hogness (unpublished MS.). Preparations of

DNA isolated from HFT lysates transduced Gal markers when helped by normal λ-phage: neither the DNA nor the helper λ was active alone. The efficiency of Gal gene transfer by the extract was, however, reduced to about 10^{-8} of the original lysate.

HOMOLOGY TESTS AND CISTRON IDENTIFICATION

Despite their biological and chemical complexity, HFT lysates remain useful for genetic analysis. Those produced from homogenotes serve as specific reagents for resolving closely linked loci. The main limitation to the scope of the tests is the rate of spontaneous reversion.

The procedures for homology tests and preparing the necessary reagents are simple and may be outlined as follows: A Gal$^-$ mutant is infected with HFT Gal$^+$ λ. The resulting Gal$^+$ heterogenotes are allowed to segregate. The segregants are of two kinds: haploid Gal$^-$ and less frequent, homogenotic Gal$^-$/Gal$^-$ which are potential sources of HFT lysates. The segregant colonies are screened for HFT quality by replicating them on to an EMB galactose plate seeded with 10^8 complementary Gal$^-$ indicator bacteria. The plates are irradiated with ultraviolet light to initiate the liberation of λ phage and are then incubated for two to three days. Those colonies which show heavy Gal$^+$ papillation with the indicator strain are purified, rechecked by a second plating, and, if satisfactory, reserved as sources of HFT λ. Lysates are prepared from them when desired by ultraviolet induction of a broth culture. Each lysate is spotted on streaks of the known Gal$^-$ mutants (Plate 1). This test includes two controls: the level of spontaneous reversion as seen in untreated bacteria and the failure of productive transduction with homologous Gal$^-$ recipient strains.

The strong reaction of Gal$^+$ lysates on each of the Gal$^-$ indicators is shown by the figure. Some of the combinations (e.g. Gal_1^-; Gal_2^-) are equally intense. This is characteristic of the heterogenotes of Gal mutants classified as mutually complementary. However, certain reciprocal combinations (e.g. Gal_1^-; Gal_4^-) show a reduced yield of Gal$^+$ papillae. What is it in these combinations that corresponds to the Gal$^+$ heterogenotes observed in the strong interactions? When the spots from the weaker interactions were streaked out, a new class of Gal$^-$ colonies was discovered. At first they resembled the haploid Gal$^-$ parents. After one to two days' incubation, however, Gal$^+$ papillae appear densely packed in the centre of the colony. These colonies resembled the position-effect heterozygotes of two 'pseudoallelic' lactose-negative mutants (Lederberg, 1952). This suggested that the papillating Gal$^-$ colonies were non-complementary heterogenotes. The surmise was confirmed by the pattern

of segregants, identified as the expected stable endogenotic and exogenotic (parental) Gal$^-$ types. The Gal$^+$ papillae in the Gal$^-$ heterogenotes represent rearrangements from the *trans* (+−/−+) to the *cis* (++/−−) form (Fig. 2). In further confirmation, double Gal$^-$ segregants were isolated from such *cis* heterogenotes. The number of the Gal$^+$ papillae should reflect the frequency of crossovers between the two Gal$^-$ recons.

These observations became the basis for a *cis-trans* test for functional complementation. Tests of the standard mutants in all pairwise combinations permitted grouping into cistrons. Members of the same cistron interact to produce Gal$^-$ heterogenotes, while members of separate cistrons form wild-type or Gal$^+$ heterogenotes. The first group of mutants tested comprises two cistrons: group A (Gal_2, Gal_8) and group B (Gal_1, Gal_4, Gal_6, Gal_7). The colonies of Gal$^-$ heterogenotes may sometimes be distinguished from haploids by a slight colour on EMB galactose agar prior to their overt papillation. This indication of a limited galactose fermentation may be due either to Gal$^+$ crossovers which had not yet grown out as papillae or to some partial complementation between mutants classified as in the same cistron. Morse (1959) has reported such partial complementation for Gal_4^- and Gal_7^-. Accurate measurements on the enzymatic level of populations of heterogenotic cells are required for determining the degree of complementation.

Group C includes the spontaneous mutants Gal_3^- and Gal_9^- which formed non-complementary (phenotypically Gal$^-$) heterogenotes with each member of groups A and B. However, they are not considered to be multiple mutants or deletion types straddling both the A and B cistrons. Gal_3^- and Gal_9^- are unambiguously distinctive by recombination test: Gal$^+$ progeny are observed in crosses with any of the other known mutants and with each other. Both are readily revertible to Gal$^+$. Transducing lysates from such reversions are fully active on all Gal$^-$. For example, if unlinked suppressors of Gal_9^- had been selected, one might find their lysates active on all Gal$^-$ except Gal_9^- recipients, because they would be still carrying the same Gal_9^- mutation and in addition an alteration elsewhere on the chromosome. Deletion mutants would fail to recombine with some one or more mutants according to the length of the deleted segment. These mutants would be stable because of the low frequency of simultaneous reversions which would have to occur in them to appear as Gal$^+$. So far no such deletion mutants have been identified. Double and triple mutants may be constructed by mating or transduction of two non-allelic Gal$^-$ strains and thus simulate deletions.

These fulfil the criteria of stability to reversion and of recombination pattern which would parallel those for deletion types.

For careful metabolic comparisons of biochemical mutants, it is important to minimize differences in background genotype, i.e., to prepare distinctive *isogenic* strains which differ, as far as possible, only in one given gene. The high efficiency of HFT lysates makes this feasible, allowing any stated Gal$^-$ to be inserted into a standard Gal$^+$ recipient. Since we have no convenient methods for selection of galactose-*negative* types, this can only be accomplished if they can be produced in appreciable numbers, as by HFT transduction. Gal$^-$ segregants seen after the exposure of Gal$^+$ cells to HFT Gal$^-\lambda$ must be checked against the original lysate to prove its specificity and that it is not a new spontaneous mutant. For instance, Gal_9^- had arisen in just such an experiment designed to obtain an isogenic Gal_4^-. It should be stressed that Gal_9 is recombinationally quite distinct from Gal_4; i.e., Gal_9^- is not a complex mutant which includes Gal_4^-.

A series of isogenic strains, carrying the then known mutants of groups A and B, was prepared for metabolic studies and were made available to H. M. Kalckar and his collaborators. Their results are quoted in the following sections.

GALACTOSE METABOLISM IN *ESCHERICHIA COLI* AND ITS MUTANTS

The pathway from galactose to the Embden-Meyerhof cycle comprises the following reactions as summarized by Kalckar (1957):

(1) kinase. The reducing group of D-galactose is phosphorylated to galactose-1-phosphate by a galactokinase;
(2) transferase. Galactose-1-phosphate is incorporated into a nucleotide, uridino-diphospho-galactose by an exchange reaction with UDP-glucose catalysed by the UDP-glucose transferase;
(3) epimerase. The 4-hydroxyl group of the hexose moiety of the nucleotide is epimerized by UDP-galactose 4-epimerase to regenerate UDP-glucose.

The net result of these reactions is the phosphorylation of galactose and its epimerization to give glucose-1-phosphate, which enters the general glycolytic cycle. The scheme might also include a pyrophorylase which first generates some UDP-glucose. A galactose-permease system may also be involved in the initial penetration of galactose into the cells.

The distribution of enzymes metabolizing galactose in the mutants was demonstrated by Kurahashi (1957). The mutants in group A are all

defective only in kinase whereas group B are defective only in transferase. Mixtures of extracts from a group A with a group B mutant incorporated galactose-1-^{14}C into uridine nucleotides as effectively as comparable Gal$^+$ extracts. The simple loss of enzymes in the mutants accounts better for the defect than their inactivation by a hypothetical inhibitor system on the basis of these data. Whether incomplete or modified proteins are formed in place of the active enzymes is not yet known.

The enzymatic picture of Gal_3^- and Gal_9^-, group C mutants, which are non-complementary with groups A and B, is more complex. Gal_9^- is completely devoid of epimerase and has somewhat less transferase and kinase activity than Gal$^+$. In this mutant galactose cannot be demonstrated in preparations of cell walls. All three enzymes could be identified in Gal_3^- at reduced levels (Kalckar, Kurahashi & Jordan, 1959). It may be restated that both of these multiply defective strains resulted from a single genetic alteration. We cannot decide now how to fit these mutants into the functional organization of the Gal segment. The immediate question for biochemical analysis is whether or not a unitary defect can be found to account for the diminution of three enzymes.

A fourth group, D, has emerged from a systematic search for more Gal$^-$ mutants. This cistron contains two members, Gal_{22}^- and Gal_{16}^-. Which enzyme is defective has not yet been identified. These mutants are non-complementary with each other, but complementary with all other mutants including Gal_3^- and Gal_9^-.

INHIBITION BY GALACTOSE

Kurahashi & Wahbe (1958) observed a decrease in growth rate of two transferase-deficient strains (Gal_4^- and Gal_6^-) when they were grown in a synthetic medium to which galactose had been added. Neither the Gal$^+$ nor Gal_2^- (a kinase mutant) were inhibited under the same conditions. The interference occurred with as little as 10 μg./ml. of galactose. The inclusion of yeast extract in the medium overcomes the galactose effect in some unexplained manner. The inhibition coincides with the accumulation of high levels of galactose-1-phosphate in transferase but not in kinase mutants (Kalckar & Kurahashi, unpublished).

A genetic defect in transferase is characteristic of the disease *hereditary galactosaemia* in man (Kalckar, 1957). Heterozygous carriers show levels of transferase intermediate between those of affected and normal individuals (Bretthauer, Hansen, Donell & Bergren, 1959). Galactose-1-phosphate is accumulated by galactosemic individuals after intake of galactose (Schwarz, Golberg, Komrower & Holzel, 1956). This may be

a clue to galactose toxicity in human subjects as well as in cultures of mutant bacteria.

Further investigations of the 'galactose-induced stasis' showed that after 15–24 hours a revival of growth may occur (Kalckar & Kurahashi, unpublished). At this stage mutants which had lost their galactose-sensitivity could be isolated. They proved to be still Gal$^-$ rather than reversions to Gal$^+$. In at least one such secondary mutant, reduced galactokinase activity was found in addition to the transferase defect. This would block the phosphorylation of galactose, and the excessive accumulation of galactose-1-phosphate had in fact disappeared. A genetic diagnosis of the galactose-resistant secondary mutants is in progress.

A second system of galactose sensitivity was discovered by Fukasawa & Nikaido (1959) in strains of *Escherichia coli*, *Salmonella enteriditis*, and *Salmonella typhimurium*. Certain galactose non-fermenting strains, termed *M* mutants, showed altered colonial form after growth in low concentrations of galactose. In hypertonic broth, conversion from rods to spherical protoplasts was observed, concomitant with their osmotic fragility. The production of this effect by galactose was compared to the similar production of protoplasts by penicillin (Lederberg, 1956*a*). The hypothesis that galactose as well as penicillin might inhibit an intermediate process of cell-wall synthesis is under test by these investigators. The epimerase of these strains appears to be blocked (Kalckar, personal communication), in contrast to those from K-12 blocked in transferase which are not lysed by galactose. Such mutants, unfortunately, have not been found so far in *Escherichia coli* K-12 where they would be amenable to genetic analysis.

OTHER GROUPS OF MUTANTS

Exceptional mutants: group E

Practically every Gal$^-$ mutant may be transformed to Gal$^+$ by λ lysates but some exceptions have been found. The following two classes were not studied further: glucose non-fermenters on the one hand, and strains which do not adsorb λ. The remaining mutants included Gal$_5$ which is present in a number of widely used marker stocks, W-677 and W-1177. This marker, however, is really a slow fermenter, and consisted of more than one genetic component as tested by outcrossing (Morse *et al.*, 1956*b*). Another exception, W-3142, not yet definitely mapped, carried a defect for kinase, similar to group A mutants (Kurahashi, 1957). The third exception not yet studied biochemically was shown to be clearly

linked to *Lp* and the other Gal loci (Calef, personal communication). Both of these last two mutants are susceptible to λ.

SUMMARY OF NEW MUTANTS

Over 250 Gal$^-$ mutants had been collected from time to time, mostly after ultraviolet light treatment, from a variety of Gal$^+$ sources. A very few were spontaneous. About 8% have already been assigned locus designations on the basis of mutual non-identity while the remainder are still pending. Almost all have been tentatively grouped by preliminary trials with at least one member of each cistron or group (Gal_1^-, Gal_2^-, Gal_{22}^-, Gal_9^-). As an illustration of the distribution of mutants from the ultraviolet irradiation of a standard Gal$^+$ culture, one lot has been tentatively classified into the following groups: A, 10; B, 27; D, 2; E, 10. In addition three mutants, amenable to transduction, were neither A nor B and may be D or another cistron. Mutants with high reversion rates were excluded from the series since Gal$^+$ reversions would be confused with recombinants in the tests. This experiment involved survivors of a single irradiated broth culture, a procedure which might allow mutant sibling clones to be scored more than once. To avert this, mutants have been screened from a series of single clones, 300-500 survivors of each being examined. On the average less than one mutant was seen per clone.

Deletion types, covering more than one recombination unit, have not yet been identified in the Gal system; on the other hand, they occur fairly frequently among lactose negative mutants (Cook, 1958). Very few recurrences of a previously isolated mutant have been ascertained, none with certainty: almost all of the mutants are easily identified as recombinationally distinct. Temperature-sensitive mutants such as have been found in the lactose and maltose series (Lederberg, Lederberg, Zinder & Lively, 1951; Lederberg, 1955) have not been recognized; nor have any galactose-*dependent* mutants that might be expected to occur if galactose is an essential metabolite for *Escherichia coli*. The loci now extant are listed in Table 1.

ATTEMPTS AT MAP CONSTRUCTION

Two lines of work were instigated to determine the order of mutational sites within the Gal region of the genetic map. Both utilized sexual recombination; neither is at this point yet conclusive.

1. Mapping two non-allelic Gal$^-$ with reference to *Lp* as an unselected marker. Since the *Lp* locus is linked to the Gal segment, the relative order of two Gal$^-$ genes should influence the segregation ratio of an *Lp* marker among Gal$^+$ recombinants. Stocks

Table 1. *Preliminary classification of* Gal *loci in complementation groups*

Complementation Group					
A	B	C	D	E	Pending
Gal_2 *k*	Gal_1 *t*	Gal_3 *k*± *t*± *e*±	Gal_{16}	Gal_{21} *t*	Gal_{13}
Gal_8 *k*	Gal_4 *t*	Gal_9 *k*± *t*± *e*	Gal_{22}	Gal_5	Gal_{18}
Gal_{10}	Gal_6 *t*			Gal_{19}	Gal_{20}
Gal_{12}	Gal_7 *t*			Gal_{23}	Gal_{17}
Gal_{14}	Gal_{11}				Gal_{24}
Gal_{26}	Gal_{15}				Gal_{25}

Groups A, B, and D constitute simple cistrons. Group C is non-complementary with all mutants of groups A or B. Group E contains mutants not amenable to transduction by λ. Nine mutants have been studied biochemically by Kalckar and Kurahashi. The enzymatic defects are given by *k*, *t*, and *e*, for losses of kinase, transferase, and epimerase, respectively.

carrying wild-type λ (λ^+) or a host range mutant (λ^h) were therefore prepared so as to carry various Gal⁻ markers, and then intercrossed. In a cross Gal_a^+ Gal_b^- $\lambda^+ \times Gal_a^-$ Gal_b^+ λ^h the Gal_a^+ Gal_b^+ recombinants should be mainly λ^h if the order is Gal_a, Gal_b, *Lp* as indicated; mainly λ^+ if the order is Gal_b, Gal_a, *Lp*. In fact $\lambda^+ : \lambda^h$ ratios were found over a wide range and were influenced as much by sexual polarity and extraneous variables as by the Gal relationships.

2. Time-dependent transfer of Gal⁺ into zygotes. The linkage group of *Escherichia coli* K-12 has been successfully mapped by the interruption of conjugating pairs to time the progressive transfer of markers into the zygote (Wollman, Jacob & Hayes, 1956). Their technique has been applied in an attempt to map the Gal loci: the same Gal⁺ male strain was mated with different Gal⁻ female strains to determine if the wild-type alleles for the various Gal⁻ genes had characteristically different times for initial transfer. The strains were also marked by the $\lambda^h : \lambda^+$ pair. Preliminary experiments (Cavalli-Sforza & Lederberg, unpublished) gave a characteristic time, under our conditions, of 22 minutes for Gal_2^+ followed rapidly by the Gal⁺ alleles of other group B loci. Gal_3^+ appeared to be delayed several minutes longer. Apart from this distinction, the several loci are not easily resolved by this method, but efforts are being made to improve its acuity.

F-LINKED TRANSFER OF GAL GENES

The mating types of *Escherichia coli* are determined by the presence or absence of the *F* factor. At least one of the parents must be F⁺ or male

for a cross to occur: the combination of two F^- or female strains is sterile. The *F* agent is infectious, however, so that brief contact in mixtures converts females into F^+ males (Lederberg, Cavalli & Lederberg, 1952).

The *F* factor may occupy any one of a number of potential sites within the male cell. In those males termed F^+ the *F* particles are free in the cytoplasm and are transmissible with very high efficiency in mixed culture thereby converting the F^- into F^+. A second category of males, termed *Hfr*, differ from F^+ by displaying a higher rate of chromosomal transfer in matings but do not usually transfer *F* to their F^- mates since the *F* particle is chromosomally bound (Wollman *et al.*, 1956; Lederberg & Lederberg, 1956; Lederberg, J., 1958). In its extrachromosomal state, the *F* particle is accessible to disinfection by acridine orange whereas it is resistant when attached to the chromosome (Hirota & Iijima, 1957; Hirota, 1959).

Recently another state has been discovered (Adelberg & Burns, 1959). In so-called *F*-prime strains, as have been isolated from several old *Hfr* cultures in our collection (Hirota, 1959), an *F* particle has become associated with some chromosome fragment. By analogy with the λ system the contagious transfer of markers in a fragment coupled with *F* may be termed *F*-linked transduction (see Jacob *et al.*, this Symposium, p. 74). The fragment in one of the *F*-prime strains included the Gal^+ markers and its transfer to Gal^- testers resulted in unstable heterogenotes (Sneath, unpublished). Preliminary trials with complementing pairs of Gal^- mutants have established segregating Gal^+ heterogenotes (Lederberg, unpublished). Segregation and crossing-over occurred within the heterogenote, just as in the λ system, allowing the serial passage of *F*-prime particles in association with several non-allelic Gal^- mutants. The *Lp* locus was not involved in the transduction of Gal *via* the *F*-prime mutant. A promising avenue has thus been opened for investigations of portions of the genome not amenable to other transductional techniques.

DISCUSSION AND SUMMARY

Genetic transduction has been defined as the introduction of a fragment of the genetic content from one cell into another by means of any of a number of vectors (Lederberg, J., 1956*b*). 'Phage-mediated' transduction involving λ appears now to depend on a defective phage particle which has lost some genetic elements needed for the independent function of the λ as a phage. The DNA isolated from the transducing λ is also effective in transduction though at a very much reduced efficiency. A third mechanism of Gal transfer is mediated by a variant of the mating-type determinant *F* whose point of attachment to the chromo-

some, like that of λ prophage, is linked to the Gal markers. Particles such as these which alternate between extrachromosomal (infectious) and chromosomal (segregating) states have been categorized as episomes by Jacob & Wollman (1958) and will be discussed more thoroughly at this Symposium by Jacob *et al.* (p. 67).

Recombinational analysis, by means of transduction and sexual conjugation, has separated a group of mutants defective in galactose metabolism into a series of distinctive recombinational units or recons. Most of them could be classified without difficulty into three functional mutually complementary groups or cistrons. Two of the three cistrons have been tested biochemically. In these all but one of the enzymatic steps involved in galactose metabolism were normal. In two mutants belonging to one cistron, group A, the missing enzyme was galactokinase, and in four mutants belonging to the other cistron, group B, uridino-diphospho-galactose transferase was missing. A simple correspondence between genetic alterations as grouped in cistrons and enzymatic defects has thus been supported by investigations of these mutants. Mutants of the third cistron, group D, have not yet been tested biochemically. A fourth group, group C, consists of two mutants which behave genetically as if distinctive point mutations had occurred just as do all the other recons. These mutants complement neither the transferase nor the kinase cistrons and showed a complex enzymatic disturbance. Gal_9^- was defective in the 4-epimerase acting on UDP galactose and showed reduced activity for the kinase and the transferase. Gal_3^- showed a reduced activity for all three enzymes. The unitary alteration underlying these multiple defects is not yet known. The results do point to some inherent inconsistencies and difficulties in complementation testing of functional relationships. The cistrons have not yet been mapped in a linear sequence as has been done with a number of systems reviewed at this Symposium.

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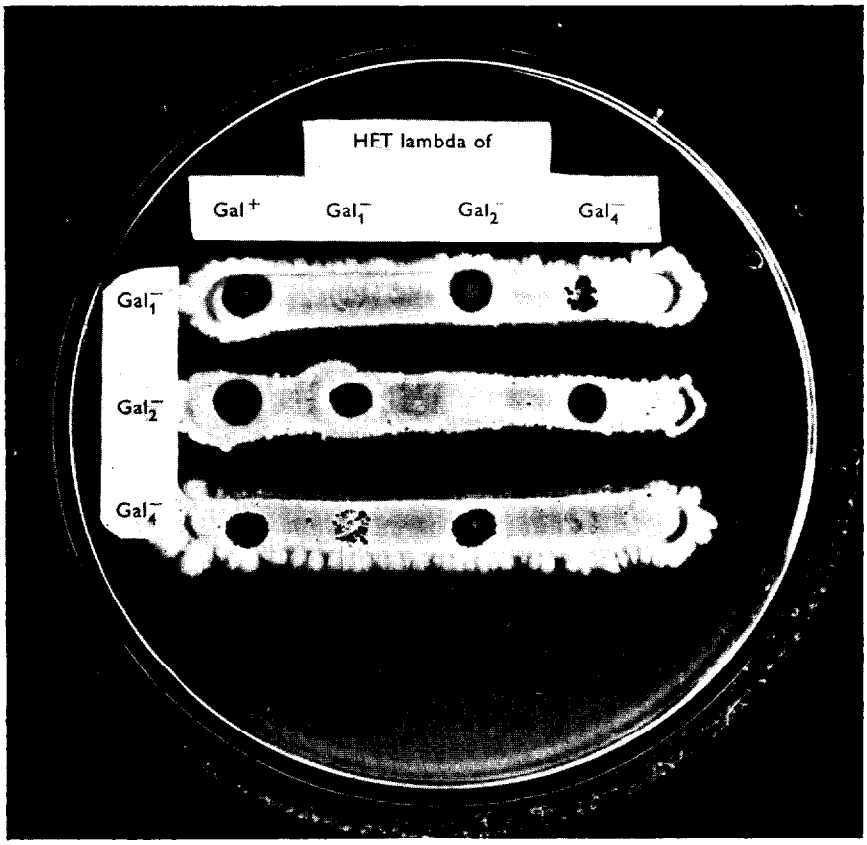
PLATE 1



(*Facing p.* 131)

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EXPLANATION OF PLATE

Homology or allelism tests. The recipient Gal$^-$ strains are labelled at the left, and the donor lysate at the top of the photograph. The transductions are observed as dense clusters of Gal$^+$ papillae at the junctions. (From Morse *et al.*, 1956*b*.)