THEOREM 4. *Let C be an analytic Jordan curve whose interior is denoted by D, and let Δ be a bounded region containing C + D. Let f(z) be analytic in D, and for each positive M let $F_M(z)$ be the (or a) function analytic and of modulus not greater than M in Δ for which the norm $\|F(z) - f(z)\|'$ in D is least; let μ_M denote the least value of this norm. Suppose $k + \alpha + 1/p > 0$, where k is an integer and $0 < \alpha \leqq 1$. Then, a necessary and sufficient condition that f(z) be on C of class $L_p(k,\alpha)$ if $0 < \alpha < 1$ and of class $Z(k,p)$ if $\alpha = 1$ is*

$$\limsup_{M\to\infty} [\log M \cdot {\mu_M}^{1/(k+\alpha+1/p)}] < +\infty. \tag{13}$$

It follows[8] that (13) is valid if (13) holds merely for the numbers $M = M_n$ of a monotonic sequence such that $M_n \to \infty$, $\log M_{n+1}/\log M_n < \infty$.

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[1] Walsh, J. L., and H. G. Russell, *Trans. Amer. Math. Soc.*, **92**, 355–370 (1959).
[2] Alper, S. J., *Doklady Akademii Nauk. S.S.S.R.*, **136**, 265–268 (1961).
[3] Walsh, J. L., *Proc. Amer. Math. Soc.*, **10**, 273–279 (1959).
[4] Quade, E. S., *Duke Math. Jour.*, **3**, 529–543 (1937).
[5] Walsh, J. L., these PROCEEDINGS, **45**, 1528–1533 (1959).
[6] Hardy, G. H., *Proc. London Math. Soc.*, **14**, 269–277 (1915).
[7] Walsh, J. L., *Interpolation and Approximation*, Colloquium Publications of the American Mathematical Society, vol. 20 (New York: 1935).
[8] Walsh, J. L., these PROCEEDINGS, **37**, 821–826 (1951).

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*CONTROL OF INDUCIBILITY OF ENZYMES OF THE GALACTOSE SEQUENCE IN ESCHERICHIA COLI**

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In *Escherichia* and in *Salmonella* there exists a sequence of gratuitously inducible enzymes of galactose metabolism largely determined by a sequence of closely linked genes collectively called the *gal* region. Genetic studies in *E. coli* have been facilitated by the discovery, due to Morse, Lederberg, and Lederberg,[1] that coliphage lambda transduces specifically genes of the *gal* region and that, apart from infrequent acts of recombination, the transductants tend to persist as partial hyperploids termed syngenotes. Where the hyperploid region is heterozygous they are termed heterogenotes. Transduction of genes of the *gal* region in *S. typhimurium* has been achieved with the aid of phage PLT-22 by Z. Hartman[2] and by Nikaido and Fukasawa.[3] This phage, however, is not specific for the *gal* region and does not form stable syngenotes. A number of galactose mutants have been characterized genetically, largely through transductional analysis by E. M. Lederberg working with *E. coli*[4] and by Z. Hartman working with *S. typhimurium*.[2] Of these, several have been analyzed biochemically[3, 5–7] with regard to the enzymes of galactose

metabolism, viz., galactokinase, galactose-1-phosphate uridyl transferase, and uridine diphosphogalactose 4-epimerase, hereafter referred to as kinase (K), transferase (T), and epimerase (E), respectively.

With regard to inducibility or constitutivity, all three enzymes respond as a unit without exception.[8] This appears particularly striking following lambda induction, which, after a lag period, causes a concerted increase in the three enzymes—kinase,[9] transferase, and epimerase.[7] A single regulatory system must therefore control the enzymes of the *gal* region. That the control is through repression and its release (depression) is suggested by the dominance of inducibility over constitutivity (see *Results and Discussion*) and by analogy with more thoroughly studied inducible enzymes.[10, 11] The extents of induction of the different enzymes under various conditions appear (within the limits of accuracy of the assays) to be subject to a coordinate repression such as has been described for the sequence of histidine enzymes.[12]

At least four separate cistrons have been shown to affect the system of repression of galactose enzymes:

1. Mutation within the cistron of the *gal* region designated "C" by E. M. Lederberg[4] leads to a loss, complete or nearly so, of all three enzymes.[6] Mutants of cistron "C" fail to complement with those of cistron "A," deficient in kinase, or of cistron "B," deficient in transferase, but they are neither multiple mutants nor deletions, since they readily revert to galactose-fermenting capacity.[4] This loss of the galactose enzymes is not simply the result of a mutant allele epistatic to alleles of the "A" or "B" cistrons, since introduction of transducing lambda from a galactose-fermenting strain into the galactose-negative "C" mutant W3099 (which is essentially lacking in kinase, transferase, and epimerase[6]) results in a heterogenote capable of fermenting galactose.[13] This is to say that the "C" mutation is dominant with respect to alleles of the "A" and "B" cistrons only when these are *cis* to the "C" mutation, but not in the *trans* configuration. This kind of position effect, coupled with the pleiotropism of "C" mutations, characterizes mutations in operator genes.[14] Operator genes are those genes whose characteristics make them suitable for the role of receptor sites or determiners of receptor sites for repressors.[14] The originators of the operator concept have suggested that the "C" cistron mutations described above could affect an operator.[14] Constitutive mutants which also satisfy the criteria for operator mutants have been recently isolated by Buttin,[15] but it is not yet established whether they arose by mutation in the "C" cistron or elsewhere.

The operator concept therefore appears to be applicable in the interpretation of the effects of certain mutations in the *gal* region. Further observations are desirable, especially since complementation between a "C" mutant, W3099, and an epimeraseless mutant, gal 22, has been found to occur.[16]

2. Mutations affecting inducibility of the galactose enzymes, but not behaving like operator mutations, have been obtained independently by Buttin[15] and by Horowitz.[17] In both cases, the loci involved are not transducible by lambda. The locus studied by Buttin, here designated "R" locus, was shown by him not to be linked to *gal* in mating experiments.[15]

3. Mutations affecting inducibility have also been found to occur in cistrons determining kinase, both in *E. coli* (cistron "A"[5, 6] and cistron(s) "E"[13]) and in *S.*

typhimurium.[3] It has been suggested that this is a case of pleiotropism.[6]

The work reported here deals with the enzyme levels of heterogenotes between strains differing in regard to the cistrons mentioned under (2) and (3). Cooperation is shown to occur, within these heterogenotes, between two genomes each separately defective in one of the different cistrons of regulation. Evidence is also furnished that the regulatory effect of the kinase cistrons is due to the removal, by kinase activity, of an inducer generated in the cell. Incidental observations made in the course of these studies are also reported.

Materials and Methods.—Bacteriological: Difco Bacto-Tryptone (1%) was used as growth medium unless otherwise indicated. Medium A,[18] a minimal medium (with NH_4^+ as nitrogen source) was used with the addition of 1% glycerol as carbon source. Eosine-Methylene Blue Agar plus 1% galactose (EMBgal) was used as indicator agar for galactose fermentation in isolated colonies. Tryptone-2,3,5,-triphenyltetrazolium chloride agar[19] plus 1% galactose (TTCgal) was used to identify galactose-fermenting colonies against a background of non-fermenters. On this medium, fermenting colonies form red papillae against a white background and are thus more easily identified than on EMBgal (where dark blue papillae form on a red background). Irradiation medium[20] is a UV-transparent buffer (7 gm/l K_2HPO_4, 2 gm/l KH_2PO_4, $10^{-3}M$ $MgSO_4$ added after autoclaving).

Escherichia coli K-12 strains, including several kindly supplied to us by various workers, were described previously.[6, 7, 21] Strain B78A was obtained by courtesy of G. Buttin and F. Jacob. All other strains were derived from these by procedures described below.

Selection of revertants to galactose fermentation was carried out using TTCgal agar. The revertants were picked and purified by streaking on EMBgal agar.

Prophage substitution was used to free lysogenic strains of their prophage, either λ or λimm^{434}.[22] The latter phage was constructed by Kaiser and Jacob[23] to have the genetic complement of λ with the exception of the c_I region responsible for prophage localization and immunity. A strain lysogenic for one phage was grown in tryptone broth for 3 hr. in the presence of the other phage. In a high percentage of the progeny surviving this treatment, one prophage had replaced the other. The presence of prophage confers immunity to superinfection by homologous phage, and it was recognized by the absence of lysis when a loopful of an appropriate phage preparation was applied to a streak of the suspected lysogen.

Heterogenotes were prepared according to methods described by Morse *et al.*[1] and by Arber.[19] Transducing phage was prepared by induction of a lysogenic strain either by UV irradiation or by the addition of 5 μg/ml of mitomycin C.[24] Ultraviolet irradiation was carried out as described by Yarmolinsky and Wiesmeyer.[7] A senstitive host strain was than infected with the phage lysate, which is expected to contain transducing particles at a frequency of 10^{-4} to 10^{-6}. Whenever possible, the ability to ferment galactose was used to differentiate heterogenotes from other cells by means of TTCgal or EMBgal indicator agars. When this was not possible, an intermediate carrier heterogenote, harboring the desired transducing phage and identifiable by the above method, was prepared. A lysate of such a strain containing comparable titers of transducing and non-transducing particles was then used to infect the ultimate host. Lysates of all heterogenotes were tested for transducing phage carrying the suspected genes by means of apropriate indicator strains.

Enzymological: Enzyme assays were carried out on cells grown into logarithmic phase in tryptone broth unless otherwise stated. For enzyme induction, 10^{-2} *M* galactose was present for at least three generations. For transferase and epimerase assays, the cells were centrifuged in a desk-top centrifuge, washed with distilled water, resuspended in 0.5 to 1.0 ml irradiation medium, and sonicated for 45 sec with the 6 mm probe of the Mullard 20 Kc per sec sonicator. The sonicates, containing approximately 1 mg protein per ml, were centrifuged for one hr at 144,000 × *g* in a refrigerated Spinco preparative ultracentrifuge and the supernatant liquid stored at − 60°C.

Kinase was assayed according to a method communicated to us by K. Paigen. The assay was performed on lysozyme-treated cells, using galactose-1-^{14}C as substrate. The gal-1-P formed was precipitated with lead and collected on glass fiber filter paper, and the disintegrations were counted. The sensitivity of the assay was increased for use with kinaseless strains by separating the gal-1-P

from the galactose on Dowex-1 columns in the presence of carrier gal-1-P. The two methods gave essentially identical results for the specific activity of kinase in wild type.

Transferase activity was measured as TPN reduction by coupling the reaction with phosphoglucomutase and glucose-6-phosphate dehydrogenase. Epimerase was coupled with UDPG dehydrogenase and measured as reduction of DPN. The conditions for the assay of transferase and epimerase were essentially those described by Kalckar, Kurahashi, and Jordan[6] with the following modifications. In the transferase assay system, a partially purified extract of W3104 (transferase-less) was substituted for glucose-6-phosphate dehydrogenase, phosphoglucomutase, glucose 1,6-diphosphate, and cysteine. A sonicate of W3104 (*ca.* 5 mg protein per ml) was treated, at 4°C, with streptomycin (0.2% w/v final concentration) and the supernatant fluid brought to 55% saturation with respect to ammonium sulfate. Aliquots of the final supernatant fluid were used (20 μl per 1 ml reaction mixture). The activities obtained with this preparation were identical to those found with the purified components. Epimerase was assayed by the one-step method with the addition of 5×10^{-4} *M* KCN. This concentration almost completely inhibits the endogenous DPNH oxidase in the supernatant liquid after centrifugation while not affecting epimerase activity. In this way, essentially identical results were obtained by the one- and two-step methods.[6]

β-galactosidase was assayed on toluenized cells, using *o*-nitrophenyl-β-D-galactoside as chromogenic substrate.[25]

Protein was determined by a micro-biuret method.[26] Where the specific activities of kinase and β-galactosidase are given, protein was estimated from the optical density at 650 mμ of the culture sample. It was assumed that an optical density of 1.0 (representing 10^9 cells/ml) was equivalent to a bacterial wet weight of 1 mg/ml and that 20% of wet weight was protein.

For enzyme induction, Sigma grade galactose, essentially free of glucose, was used (Sigma Chemical Company). Gal-1-P, UDPG, and UDPG-dehydrogenase (Type III) were obtained from the Sigma Chemical Company. UDPGal was prepared according to Wiesmeyer and Jordan.[27]

Results and Discussion.—Pleiotropic control of both galactokinase and inducibility of galactose enzymes: The data of Table 1 show that several kinaseless mutants derived from an inducible galactose-positive *E. coli* are constitutive with respect to the remaining functional enzymes of the galactose sequence; on the other hand, mutations studied which lead to loss of transferase or epimerase activity have no effect on the inducibility of the galactose enzymes. Revertants of kinaseless mutants to the galactose-positive state have regained inducibility along with kinase. An entirely similar situation has been shown by Nikaido and Fukasawa to occur among four separate kinaseless *Salmonella* mutants and a revertant.[3] A kinaseless strain may acquire the gene for synthesis of functional kinase not only by reversion but also by transduction, e.g. as in lines 3 and 6 of Table 3.[28] In either process, inducibility is regained. The data demonstrate, furthermore, that inducibility here is dominant with respect to constitutivity, suggesting that inducibility corresponds to the active state of the gene.

The previously entertained hypothesis that cistron "A" has pleiotropic functions[6] is supported by these observations accumulated here and in Japan.

A facultatively inducible kinaseless variant and influence of growth phase on inducibility: The above findings raise the question whether the loss of functional kinase necessarily entails the loss of inducibility. A fortuitously discovered variant of W3092(λ), W3092A(λ), answers this question in the negative.

W3092A(λ) is inducible for transferase and epimerase (Table 1), but, like the parent strain, does not ferment galactose. Kinase activity in this strain is less than 1% of uninduced wild type. Experiments with mixed extracts showed that wild type kinase activity at this low level was not inhibited by an extract of W3092A(λ).

The relative reversion rates to galactose fermentation of W3092(λ) and

TABLE 1

INDUCIBILITY OF GALACTOSE ENZYMES IN MUTANTS LACKING ONE OF THESE ENZYMES

Class	Cistron affected	Strain	Enzyme Specific Activities* (μmoles product/hr/mg protein) Kinase −gal	Kinase +gal	Transferase −gal	Transferase +gal	Epimerase −gal	Epimerase +gal
Wild type	—	W3100(λ)	0.5	2.2†	0.6	5.5	1.8	10.5
Kinaseless	A	W3092(λ)	<0.005	<0.005	4.4	4.4	6.5	7.2
	"	W3092A(λ)	<0.005	<0.005	0.94	5.4	1.7	6.3
	"	gal. 10	<0.005	<0.005	—	—	9.7	7.6
	"	gal 12	<0.005	<0.005	—	—	5.3	8.1
	"	gal 14	<0.005	<0.005	—	—	8.3	7.0
	E	gal 19	<0.005	<0.005	—	—	9.2	7.6
	"	gal 21	<0.005	<0.005	—	—	7.8	7.4
Revertants	—	W3092R(λ)	0.29	1.6	0.30	3.8	1.0	7.5
from kinase-	—	W3092AR(λ)	0.13	0.98	0.46	5.8	1.0	8.7
less to galac-	—	gal 19 R	—	—	—	—	1.3	9.0
tose fermentation								
Transferaseless	B	W3096(λ)	—	—	<0.1	<0.1	2.0	23
	"	W3104	—	—	<0.1	<0.1	1.5	32
Epimeraseless	D	gal 16	—	—	0.9	6.7	<0.1	<0.1
	"	gal 22	—	—	1.2	8.8	<0.1	<0.1

* Enzyme assays were performed as described in the *Methods* section. + and −gal refer to growth in the presence or absence of 10^{-2} *M* galactose for three generations.
† The lower value as compared with previous ones[6] warrants special study.

W3092A(λ) are approximately equal, suggesting that if W3092A(λ) is a double mutant the second mutation by itself does not result in the absence of functional kinase. Galactose-fermenting revertants from strain W3092A(λ), designated W3092AR(λ), like those of W3092(λ), are inducible (Table 1).

There is no complementation between the genomes of the two kinaseless strains as shown by the absence of wild-type colonies when either strain is treated with a phage lysate prepared either directly from the other strain or from a heterogenote carrying transducing phage derived from that strain.

The phenotypic difference in inducibility between strains W3092(λ) and W3092A(λ) vanishes if the cells are permitted to go into stationary phase (Table 2). In stationary phase, the specific activity of both transferase and epimerase in W3092A(λ) is independent of whether the cells had been grown in the presence of galactose, whereas in logarithmic phase there is on the average a fivefold difference between induced and non-induced cells. The absolute values of the specific activities of both enzymes are also much higher in stationary phase than in logarithmic phase.

TABLE 2

INFLUENCE OF GROWTH PHASE AND GLUCOSE ON EPIMERASE LEVELS IN THE ABSENCE OF GALACTOSE

Strain	Logarithmic Phase −Glu	Logarithmic Phase +Glu*	Stationary Phase (Overnight cultures) −Glu	Stationary Phase (Overnight cultures) +Glu
W3092(λ)	10	3.2	30	2.6
	(3.7)	(1.1)	(11)	(1.0)
W3092A(λ)	1.7	1.5	20	0.7
	(0.8)	(0.8)	(10)	(1.9)
W3092R(λ)	1.1	0.7	2.3	3.0
	(0.6)	(0.7)	(2.7)	(2.3)
B78A	22	11	56	9

* Glucose was present at a final concentration of 1% for at least three generations.
Values in parentheses refer to enzyme activities of cells grown in medium A + 1% glycerol. Changes in transferase levels were found to correspond to those in epimerase levels.

The effect of growth phase on enzyme levels in W3092(λ), W3092R(λ), and B78A (constitutive "R" locus mutant) may be compared with that on W3092A(λ). In the three former strains, there is a two- to three-fold increase in specific activity in going from logarithmic to stationary phase. Still greater increases in specific activity, up to six-fold, have been obtained in the case of W3092(λ) and B78A when the cells were allowed to remain in stationary phase for longer times. The effect of post-logarithmic growth is readily reversed by dilution of the culture in fresh medium followed by two to three generations of logarithmic growth.

The presence of glucose represses enzyme synthesis in the two constitutive strains both in logarithmic and in stationary phase. In the absence of external inducer, strain W3092A(λ) is repressed by glucose only in stationary phase, whereas W3092R(λ) is not affected at all.

Evidence that a pleiotropic kinase gene is not an operator: It has been suggested as one possibility that the kinase gene constitutes a part of an operator controlling the galactose enzymes.[3] However, previously published enzyme assays of the heterogenote W3092(λW3104 + λ) (Yarmolinsky and Wiesmeyer,[7] Table 2 and *vide supra*, Table 3, line 3) rule out this possibility. If the kinase locus were an operator, then in this heterogenote the synthesis of transferase, determined only by the host chromosome, would be controlled by the mutated kinase gene on the same chromosome. It would therefore remain constitutive as in the host. The finding, however, is that transferase synthesis has become inducible in the heterogenote. This indicates that regulation can occur across chromosomes and hence must be mediated by a cytoplasmic agent.

TABLE 3

SPECIFIC ACTIVITIES OF ENZYMES OF THE GALACTOSE SEQUENCE IN HETEROGENOTES

Strain	Presumed Genotype† Host R	Host K	Host T	Host E	Transducing phage K	Transducing phage T	Transducing phage E	Enzyme Specific Activity (μmoles product/hr/mg protein) § No.‡	Transferase −gal	Transferase +gal	Epimerase −gal	Epimerase +gal
W3104	+	+	−	+					—	—	1.5	32
W3104(λW3092 + λ)	+	+	−	+	−	+	(+)	3	0.35	5.3	1.3	12
*W3092(λW3104 + λ)	+	−	+	+	+	−	(+)	2	0.12	4.1	2.0	39
W3104(λW3092A + λ)	+	+	−	+	−	+	(+)	3	0.33	5.3	0.9	13
*W3092A(λW3104 + λ)	+	−	+	+	+	−	(+)	2	0.18	3.2	1.7	22
*W3092(λB78A + λ)	+	−	+	+	+	+	(+)	2	—	—	2.4	9.6
B78A(λW3092 + λ)	−	+	+	+	−	+	(+)	1	—	—	17	20

* The host strains for these heterogenotes prior to transduction were W3092(λimm^{434}) or W3092A(λimm^{434}).[28]
† R: non-transducible gene of regulation. K, T, and E: genes determining kinase, transferase, and epimerase, respectively. Parentheses indicate that presence of gene is merely inferred from the genotype of the donor.
‡ Number of independently derived strains. The specific activities of the enzymes are the corresponding averages.
§ Enzyme assays were performed as described in the *Methods* section. + and −gal refer to growth in the presence or absence of 10^{-2} *M* galactose for three generations.

Cooperation between the pleiotropic kinase genes and the "R" locus: Constitutive synthesis of galactose enzymes in strain B78A, isolated and characterized by Buttin, is due to mutation at a locus not transduced by lambda and not closely linked to the *gal* region in mating tests.[15] Constitutive synthesis of transferase and epimerase by W3092(λ), on the other hand, is due to mutation of the kinase gene lying within the *gal* region. It was of interest to find out whether there was any cooperation between those two distant loci regulating the synthesis of the *gal* enzymes.

Heterogenotes of the two strains were prepared to investigate this question. Lines 6 and 7 of Table 3 show that W3092(λB78A + λ) is inducible for epimerase,

while the reciprocal B78A(λW3092 + λ) is constitutive. Two galactose-negative segregants from W3092(λB78A + λ) were isolated and found to be constitutive, demonstrating that the hyperploid state had been maintained prior to segregation and that the host is again constitutive on loss of the transducing phage.

The results indicate that a transducible locus functioning in B78A but not in W3092(λ) and a non-transducible locus functioning in W3092(λ) but not in B78A cooperates in the production of inducibility. Two alternative explanations of the necessity for two functional genes are possible. Repressor formation may require two steps, controlled by the separate loci (cf. Szilard[29]), or one locus may control the synthesis of a repressor while the other controls the removal of an internal inducer.

Evidence that pleiotropism of kinase genes is due to removal of internal inducer by kinase: Strong support for the theory that the regulatory function of kinase involves the removal of an internal inducer is provided by the experiment shown in Table 4. The experiment exploits the recent observation made by Buttin[15] that methyl-β-D-thiogalactoside (MTG) inhibits induction of the enzymes of the galactose sequence. This inhibition may be reversed by high concentrations of galactose. If the kinaseless strains are constitutive because of the presence of internal inducer, there should be a repression of enzyme synthesis, reversible by sufficient galactose, when the strains are grown in the presence of MTG. Table 4 shows that this indeed occurs. Strain B78A, on the other hand, defective at the "R" locus and presumably unable to make the corresponding repressor, is unaffected by MTG.

TABLE 4

EFFECT OF AN INHIBITOR OF INDUCTION (MTG) ON EPIMERASE LEVELS IN CONSTITUTIVE AND INDUCIBLE STRAINS

	Epimerase Specific Activity* (μmoles product/hr/mg protein)			
Strain	−gal −MTG	−gal +MTG	+gal −MTG	+gal +MTG
B78A	26	24	(21)†	—
W3092R(λ)	1.2	0.6	13	6
gal 19	6.0	1.3	(7.6)	—
gal 12	5.5	1.6	(8.1)	—
W3092(λ)	13	2.1	(14)	4.3
W3092A(λ)	1.8	1.6	(6.3)	—

* Enzyme assays were performed as described in the *Methods* section. Where indicated, galactose at 10^{-2} *M* MTG (thiomethyl-β-D-galactoside) at 5×10^{-3} *M* were present for more than three generations.
† Numbers in parentheses are based on separate experiments.

An explanation for the exceptional behavior of W3092A(λ) is suggested by the above findings. The strain might have acquired a new mechanism for removal of internal inducer (presumably galactose, or a derivative of it, which might be formed in this strain from UDPGal directly or indirectly). An indication that this may be the case was obtained by an examination of β-galactosidase induction in strains W3092(λ) and W3092A(λ). It was found that the strains differ with regard to the lowest concentration of galactose which will induce the β-galactosidase (Table 5). In the presence of 10^{-5} *M* galactose, the enzyme is induced fourfold in W3092(λ) but not at all in W3092A(λ). This difference could be due to the presence in W3092A(λ) of a mechanism which can reduce the intracellular concentration of galactose to a level ineffectual for the induction of β-galactosidase. In strains W3092R(λ) and B78A, the threshold concentration of galactose required for β-

TABLE 5

INDUCTION OF β-GALACTOSIDASE IN STRAINS DIFFERING IN KINASE ACTIVITY

	β-galactosidase Specific Activity (μmoles product/hr/mg protein)			
Strain	—	10^{-5} M gal	10^{-4} M gal	10^{-3} M gal
W3092(λ)	3.8	16.3	58.0	59.0
W3092A(λ)	3.0	3.0	12.4	57.5
W3092R(λ)	1.5	1.9	1.8	—
B78A	0.7	—	—	0.9

The strains were grown for about eight generations in the presence of the indicated concentrations of galactose. The assays were performed as described in the *Methods* section on cultures which had reached an optical density >1.0 at 650 mμ in the Beckman DU spectrophotometer.

galactosidase induction correlates well with the level of kinase in each strain, a finding which supports the hypothesis.

If the constitutivity of the kinase defectives is due to a failure to remove an endogenous inducer, a question arises as to the origin of the inducer. Although the endogenous inducer may not be identical with galactose itself, it seems most likely that it stems from free galactose and that phosphorylation of galactose prevents the formation of the inducer. If this is correct, a kinaseless mutant with an additional mutation blocking the synthesis of functional epimerase should be inducible for transferase unless it is transduced with λgal defective in kinase only. The latter heterogenote should again be constitutive because of the functional epimerase gene of the lambda. In other words, in this case constitutivity should be dominant because the internal induction relies on a functional epimerase gene. This type of study should therefore be able to clarify further the nature of the pleiotropism of the kinase gene.

Summary.— Three types of genes interact in the regulation of galactose sequence enzymes. Two of these have the characteristics of repressor-forming and operator genes respectively; the third comprises genes determining galactokinase. Most strains lacking functional kinase are phenotypically constitutive. The following lines of evidence suggest that this condition is due to an internally produced inducer. Introduction of a functional kinase gene by transduction leads to loss of constitutivity for all the galactose enzymes whether determined by genes cis or trans to the mutated kinase genes. This indicates that there is no interference with the function of the (non-transducible) repressor-forming gene in kinaseless strains and that the mutations to kinaseless are not operator mutations. Constitutivity may be prevented by growth of the cells in the presence of a known inhibitor of induction of the galactose enzymes. Presumably, the basal level of kinase in wild type strains is sufficient for the removal of the internal inducer, making these strains inducible. There is also evidence that in an exceptional inducible kinaseless strain another reaction for the removal of the internal inducer may have appeared.

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The following abbreviations are used: DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; UDPG, uridine diphosphoglucose; UDPGal, uridine diphosphogalactose; Gal-1-P, α-D-galactose-1-phosphate.

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[1] Morse, M. L., E. M. Lederberg, and J. Lederberg, *Genetics*, **41**, 142 and 758 (1956).

[2] Hartman, Z., M.S. thesis, Cornell University, 1956.

[3] Nikaido, H., and T. Fukasawa, *Biochem. Biophys. Res. Comm.*, **4**, 338 (1961).

[4] Lederberg, E. M., in *Microbial Genetics*, Tenth Symposium of the Society for General Microbiology, ed. W. Hayes and R. C. Clowes (Cambridge University Press: 1960), p. 115.

[5] Kurahashi, K., *Science*, **125**, 114 (1957).

[6] Kalckar, H. M., K. Kurahashi, and E. Jordan, these PROCEEDINGS, **45**, 1776 (1959).

[7] Yarmolinsky, M. B., and H. Wiesmeyer, *ibid.*, **46**, 1626 (1960).

[8] The apparent exception found previously in W3092(λ) has been eliminated.[6] On being assayed again, this strain proved to be constitutive for both epimerase and transferase (see Table 1).

[9] Buttin, G., F. Jacob, and J. Monod, *C. R. Acad. Sci. (Paris)*, **250**, 2471 (1960).

[10] Vogel, H. J., these PROCEEDINGS, **43**, 491 (1957).

[11] Pardee, A. B., F. Jacob, and J. Monod, *J. Mol. Biol.*, **1**, 165 (1959).

[12] Ames, B. N., and B. Garry, these PROCEEDINGS, **45**, 1453 (1959).

[13] Yarmolinsky, M. B., E. Jordan, and H. Wiesmeyer, *in* Cold Spring Harbor Symposia on Quantitative Biology, vol. **26** (1961), in press.

[14] Jacob, F., D. Perrin, C. Sanchez, and J. Monod, *C. R. Acad. Sci. (Paris)*, **250**, 1727 (1960).

[15] Buttin, G., *in* Cold Spring Harbor Symposia on Quantitative Biology, vol. **26** (1961), in press.

[16] Lederberg, E. M., personal communication.

[17] Horowitz, E. B., in *Abstracts, Fifth Annual Meeting of the Biophysical Society* (February, 1961), p. TA3.

[18] Hartman, P. E., in *Genetic Studies with Bacteria*, Carnegie Institution of Washington, Publication 612 (1956).

[19] Arber, W., *Arch. Sci. (Geneva)*, **11**, 259 (1958).

[20] Campbell, A., *Virology*, **4**, 366 (1957).

[21] Soffer, R. L., *J. Bact.*, **82**, 471 (1961).

[22] The method described is that of D. K. Fraser (personal communication). It was used because usual methods of curing lysogenic strains by UV irradiation were not always successful.

[23] Kaiser, A. D., and F. Jacob, *Virology*, **4**, 509 (1957).

[24] Otsuji, N., M. Sekiguchi, T. Iijima, and Y. Takagi, *Nature*, **184**, 1079 (1959).

[25] Lederberg, J., *J. Bact.*, **60**, 381 (1950).

[26] Zamenhof, S., in *Methods in Enzymology*, ed. S. P. Colowick and N. O. Kaplan (New York: Academic Press, 1957), vol. 3, p. 702.

[27] Wiesmeyer, H., and E. Jordan, *Anal. Biochem.*, **2**, 281 (1961).

[28] Strain W3092(λimm^{434}), which was used as host for the preparation of W3092A(λW3104 + λ), is a constitutive strain though derived from the inducible W3092A(λ). The nature of this change is currently being investigated.

[29] Szilard, L., these PROCEEDINGS, **46**, 277 (1960).