a spectrum of cell destruction ranging from complete to absent CPE but also a paradoxical sparing effect on the cultures. Further studies are required to ascertain the genetic and nutritional conditions underlying this phenomenon. This sparing effect has some superficial resemblance to that of certain tumor viruses on cell cultures, but there is as yet no evidence that the cells spared have undergone genetic transformation.

Summary.—Primary human amnion cells cultured under defined conditions were inoculated with a variant of Type II poliovirus and observed for over four months. The initial cytopathic effect was overcome, and gradually confluent sheets of healthy-appearing cells of altered appearance developed. In contrast, uninoculated control cultures underwent gradual spontaneous degeneration.

The author is indebted to Drs. John F. Enders, William McD. Hammon and Horace M. Gezon for helpful criticisms.

* This investigation was aided by Grant E-2953 from the National Institutes of Health.

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*REGULATION BY COLIPHAGE LAMBDA OF THE EXPRESSION OF THE CAPACITY TO SYNTHESIZE A SEQUENCE OF HOST ENZYMES**

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Communicated by Herman M. Kalckar, October 17, 1960

A small group of closely linked genes specifying the enzymes of galactose metabolism is capable of being transduced by the coliphage lambda.[1] A region of the bacterial chromosome or a replica thereof encompassing the transduced genes is inserted into the genome of the transducing phage, replacing a portion of the viral genetic material.[2–4] This relatively stable situation makes possible investigation of the expression of bacterial genes situated in a foreign environment, i.e., within the genome of a prophage. Upon inducing the replication of transducing phage, cells may be obtained prior to lysis, each possessing several copies of a short region of bacterial genetic material. The present work was originally undertaken to investigate the position and gene dosage effects afforded by a study of *Escherichia coli* harboring transducing lambda. Its course has been altered somewhat by an unexpected result obtained in a control experiment using *E. coli* lysogenic for normal lambda alone. Following induction of phage replication, the activity of an enzyme of galactose metabolism was found to increase whether or not the capacity for synthesis of this enzyme, galactose-1-phosphate uridyl transferase, was carried by the phage. Increases in enzyme activity, apparently due to increased dosage of transduced genes, must be interpreted in the light of the increases in

enzyme activity due to induction of normal lambda phage alone. The viral and enzymatic specificity of the latter phenomenon are described here. This newly recognized mode of interaction between phage and host genome offers a new approach to the study of cellular control of metabolic activity. Work of a similar nature, but involving a different enzyme of galactose metabolism, galactokinase, is being pursued by Buttin, Jacob, and Monod.[5]

TERMINOLOGY

The terms of transduction used here are largely as defined by Morse, Lederberg, and Lederberg[6] and incorporate some minor modifications introduced by Arber.[3]

A bacterium susceptible to infection by a particular phage is *sensitive*. Upon infection by a temperate phage, such as lambda, some of the cells are lysed, others lysogenized. A *lysogenized bacterium* harbors the phage as *prophage*, a condition in which each viral genome replicates in association with a bacterial nucleus. In the lysogenized bacterium, the viral functions required for the lytic cycle are under a repression which various inducing agents, such as ultraviolet light or chemical agents, can suspend. Since the viral functions of homologous phages which may enter the cell are also suppressed, the lysogenized cell is said to be *immune*. Phage mutations affecting the specificity of immunity lie within the same locus of the viral genome as mutations affecting the site of prophage localization.[7] Prophage lambda locates adjacent to the complex of bacterial genes governing galactose metabolism, the *gal* region.[23]

The lysogenized bacterium is called an *active lysogen* if the prophage is capable of undergoing its lytic cycle of multiplication, spontaneously or upon induction. The designation *defective phage* (strictly speaking, a contradiction in terms[9]) is applied to a phage whose genome is so altered as to render it incapable of undergoing a complete lytic cycle in single infection, yet capable of conferring immunity upon the cells it lysogenizes. Attempts to induce phage replication in such cells—*defective lysogens*—result in cellular lysis[10] but fail to yield phage progeny or even detectable phage protein.[3] The replication of defective phage is made possible by the simultaneous presence of active phage, so-called *helper phage*. Helper phage serves to make good the deficiency in defective phage by a cooperative process and permits a mixed burst of active and defective phage particles.[2, 3, 11, 12]

Bacteriophage lambda transducing the galactose region of *Escherichia coli* is a defective phage. The functional defect results from deletion of a large region of the phage genome which is replaced by genetic material of bacterial origin. In double lysogens, carrying in addition to a defective transducing prophage an active lambda prophage, the replication of transducing phage, inclusive of transduced genetic material, occurs upon induction.[10] Transductants, being hyperploid for the transduced genetic region, are referred to as *syngenotes: defective syngenotes* if helper prophage is absent, or *lysogenic syngenotes* if helper prophage is present. A syngenote is either a *heterogenote* or a *homogenote* depending upon whether the transduced characters under discussion are genetically different from or identical to those of the recipient. Both defective and lysogenic syngenotes are somewhat unstable and segregate sensitives, predominantly of the original bacterial genotype, with frequencies of the order of $2 \cdot 10^{-3}$ to $2 \cdot 10^{-2}$ per cell division.[1, 3, 6]

An *HFT lysate* is a high-frequency transducing lysate in which the titers of

normal and transducing lambda phage are of the same order of magnitude. HFT lysates are prepared by induction of lysogenic syngenotes. In *LFT* (low-frequency transducing) *lysates*, prepared by induction of active lysogens, the proportion of transducing phages is of the order of 10^{-6} to 10^{-4}.[1, 3, 6] A lysate obtained directly upon infection of a strain sensitive to lambda fails to yield transducing phage.[1] Lysogenic heterogenotes may be selected on indicator agar following treatment of sensitive cells with LFT lysates, while lysogenic homogenotes (and defective syngenotes) are most easily obtained by use of HFT lysates.

The term induction is used here in two different senses: (*a*) in the sense of Lwoff[13] to denote the induction of the lytic cycle of phage development, e.g., by ultraviolet light, and (*b*) in the sense of Monod and Cohn[14] to denote enzyme induction, e.g., in response to a substrate. Since each of these phenomena may represent release from repression exerted by a cytoplasmic agent,[15, 16] the use of identical terms may have some merit.

MATERIALS AND METHODS

BIOLOGICAL

Parental Strains of Bacteria.—The strains of *E. coli* employed here are listed in Table 1. Two strains incapable of fermenting galactose, and therefore known

TABLE 1

CHARACTERISTICS AND DERIVATION OF *Escherichia coli* STRAINS EMPLOYED

Strain	Derivation: Parent	Derivation: Process	Galactose phenotype: *K*	*T*	*E*	Original source	Donor
Sensitives							
C600			+	i^+	+	Ad[8]	Kr
W3110′	W3104	Selection	+	i^+	+	A	
W3104	W3104(λ)	UV	+	−	+	A	
Lysogens							
C600(λ)	C600	Infection with the indicated phage	+	i^+	+		Kr
C600(λ*imm*[434])							Lu
C600(434)							P
C600(82)							P
C600(21)							P
W3110′(λ)	W3110		+	i^+	i^+	A	
W3092(λ)	W3100(λ)	UV	−	i^-	+	L	Ki
W3092(λ*imm*[434])	W3092(λ)	Infection				J	J
W3092A(λ)	W3092(λ)	Spontaneous	−	i^+	+	A	
W3104(λ)	W3110(λgal_2^-+λ)	Segregation	i^+	−	+	L	Kr
Syngenotes							
W3104(λW3092+λ)	See text		+	i^+	+	A	
W3092(λW3104+λ)			+	i^+	+	J	J
W3092(λW3092+λ+λ*imm*[434])			−	i^-	+	A	

All bacterial strains are derivatives of *Escherichia coli* K12. The phage harbored by lysogens are indicated within parentheses and are described under *Materials and Methods*. The bacterial strain from which the transducing phage of each syngenote originally derived is indicated following the letter λ.

The phenotypes with respect to three enzymes under the genetic control of the galactose region, namely, galactokinase, galactose-1-phosphate uridyl transferase, and uridine diphosphogalactose 4-epimerase, are given below the letters K, T, and E, respectively. The presence (absence) of an enzyme in galactose-adapted cells, as determined by direct measurement of enzyme activity in extracts or by inference from enzymatic capacities of the parental strain, is indicated by a plus (minus) sign. Enzyme inducibility upon growth of the strain in the presence of 10^{-2} *M* D-galactose is indicated only where it has been directly determined, either by ourselves or by Kalckar, Kurahashi, and Jordan;[17] i^+ and i^- designate the inducible and constitutive state respectively, and the reader is referred to Table 2 for the quantitative significance of these terms.

Investigators' names have been abbreviated as follows: A, present authors; Ad, R. K. Appleyard; C, A. M. Campbell; J, E. Jordan; Ki, K. Kurahashi; Kr, A. D. Kaiser; Lu, S. E. Luria; L, E. M. Lederberg; P, K. J. Paigen.

as gal-negatives, are included: W3092, which lacks the capacity to synthesize galactokinase[18] owing to the gal_2^- mutation[6] within the cistron designated "A"

by E. M. Lederberg,[19] and W3104, which lacks the capacity to synthesize galactose-1-phosphate uridyl transferase[17] owing to the gal_4^- mutation[6] within the "B" cistron.[19] The strains are not otherwise isogenic. Strain W3092 was derived by a single UV irradiation of the gal-positive strain W3100.[17] It differs from the wild type parent, not only in being incapable of galactokinase synthesis,[18] but also in that the transferase, inducible in W3100, is constitutive in W3092.[17] Spontaneous revertants of W3092(λ) to galactose-fermenting capacity are again inducible for transferase.[20] The above observations suggest an obligatory connection between the absence of galactokinase and constitutivity of transferase. Yet the connection is not obligatory, since a variant of W3092(λ), designated W3092A(λ), while still gal-negative, was found to be inducible for transferase. The nature of the differences among strains W3100(λ), W3092(λ), and W3092A(λ) is currently being investigated in this laboratory by E. Jordan. Strain W3104 was derived from a lysogen by a dose of UV lethal to the phage. The parent lysogen was a gal-negative segregant of a heterogenote consisting of W3110, a gal-positive strain, carrying lambda transducing the gal_4^- marker.[3] The gal-negative strains received were lysogenic for lambda and were freed of this phage either by UV treatment[21] or by displacement with the related phage λimm^{434}.[22] Sensitive clones were scored on basal EMB agar by the method of Lederberg and Lederberg.[23] A spontaneous gal-positive revertant of W3104 is designated here as W3110′ by reference to its parental wild type, W3110. The reversion is presumed to be due to back-mutation at the gal_4 locus rather than a suppressor mutation outside the galactose region because lambda-mediated transduction of genetic material from the supposed W3110′ into W3104 yields a gal-positive heterogenote.

The lambda prophages present in the lysogens may be characterized by the immunity they confer: strains C600(λ), W3092(λ), W3092A(λ), and W3104(λ) are each immune to phage obtained by induction from any of the other three. They are also immune to λ_{C71}.[24] They are all sensitive to λimm^{434}, described below, and to λ_{V2}, an inducing virulent mutant of lambda.[24] Phages λ_{C71} and λ_{V2} were supplied by Dr. Armin D. Kaiser. The lambda phage used for infection of strain W3110′ was obtained from a lysate of W3092(λ).

Phages *82* and *434*, described by Jacob and Wollman,[25] are related to lambda. Their prophage, like lambda prophage,[23, 26] locate close to the galactose region of the lysogenized bacterium.[54] A hybrid between lambda and *434*, obtained by Kaiser and Jacob,[7] is constructed so as to be isogenic with lambda except for the c_I region controlling prophage attachment site and immunity. This hybrid, designated λimm^{434}, is also capable of transducing the galactose region.[9] Phage *21*, while immunologically and genetically similar to lambda, is localized as prophage at a considerable distance from the galactose region.[25]

Preparation of Syngenotes.—Treatment of one suitable gal-negative bacterial strain with an LFT lambda lysate derived from another gal-negative strain with a complementary deficiency yields unstable clones of gal-positive heterogenotes recognizable on indicator agar. From these may be produced HFT lysates. An HFT lysate applied at high multiplicity to a lambda-sensitive derivative of the strain which served as source of the LFT lysate yields among the infected cells a high proportion of lysogenic homogenotes, recognized as such by their capacity to yield HFT lysates upon induction. Spotting of transducing lambda upon the

appropriate indicator bacteria insures that the intended marker is carried by the phage.

The syngenotes listed in Table 1 were prepared according to the steps indicated above as presented in detail by Morse, Lederberg, and Lederberg[6] and by Arber.[3]

For the rapid detection of gal-positive colonies against a crowded background of gal-negative colonies, tryptone-TTC-galactose agar of Arber[3] was used. The use of TTC-agar depends upon the ability of bacteria fermenting the added sugar to reduce the colorless 2,3,5-triphenyltetrazolium chloride (TTC) to the insoluble red dye formazan.[27] This agar sometimes gives misleading results, e.g., in the case of the various C600 lysogens, for reasons which remain to be explored. These strains, while galactose-negative on the TTC-agar, are galactose-positive on EMB-galactose agar.[1] TTC was obtained from the Dajac Laboratories Division of the Borden Co.

Syngenotes W3092(λW3104 + λ) and W3092(λW3092 + λ + λ*imm*^{434}) were prepared by transduction of W3092(λ*imm*^{434}) with the incidental elimination of the hybrid phage in the first case. Transducing phage from an HFT lysate was required for production of W3092(λW3092 + λ + λ*imm*^{434}). The source of the HFT lysate was the heterogenote W3104(λW3092 + λ).

Transducing phage was assayed on plates of tryptone-TTC-galactose agar, as described by Arber.[3] Helper phage derived from W3104(λ) and from W3092(λ) was used for assay of transducing phage with the recipient strains W3104 and W3092(λ*imm*^{434}) respectively. Lysates of each syngenote were demonstrated to be HFT with respect to the transduced markers they are reported to harbor. Lysates of each heterogenote were shown to be LFT with respect to the particular galactose markers characteristic of the bacterial host alone.

UV Irradiation en Masse for Phage Induction.[28]—Cells in the logarithmic phase of growth on tryptone-saline broth containing 0.01*M* $MgSO_4$[29] were harvested by centrifugation at 2000 × *g* for 10 minutes and resuspended in the UV-transparent irradiation medium of Campbell.[4] From the absorbance and volume of the resuspended culture, the irradiation period was calculated as indicated below. Cells were irradiated in a darkened room in a cylindrical vessel with vigorous mechanical stirring. They were then transferred to an equal volume of double-strength tryptone-saline-$MgSO_4$ broth in an Erlenmeyer flask of "low-actinic" glass (to prevent photoreactivation) and returned to the rotary shaker for incubation until cellular lysis occurred. An attempt was made to perform all operations, including centrifugation, at the same temperature, routinely 30°. At this temperature, lysis occurs at about 140 minutes after irradiation rather than at the 60 to 70 minutes characteristic of cells grown at the customary 37°.

Irradiation was from a "Mineralight" Senior Model ultraviolet lamp, claimed by the manufacturer to emit 110 mWatts/ft^2 at 2537 Å at a distance of 2 ft. from the light source. The lamp was allowed to warm up 15 minutes before use. Solutions to be irradiated were placed at a distance of 28 cm for the lamp where they may be expected to have received 56 ergs/mm^2/sec at 253.7 mμ. Irradiation time was calculated from the formula

$$t = 38\, A \cdot V \cdot r^{-2}\, (1 - e^{-4.8\, A \cdot V \cdot r^{-2}})^{-1}$$

where t = irradiation time in seconds at the above dose rate, A = absorbance of

the cell suspension at 650 mμ (1 cm light path) as measured in a model DU Beckman spectrophotometer, V = volume in ml of the cell suspension, and r = radius in cm of the cylindrical vessel. The factor in parentheses reduces to unity if the solution is essentially opaque to the incident UV. The formula represents an empirical result at infinite dilution corrected for finite absorbance by the Beer-Lambert Law. As the formula was generally applied under a limited range of conditions, namely, $A = 0.6$ (i.e., $5 \cdot 10^8$ viable cells/ml), V = 50–100 ml, and r = 6 cm, no corrections were made for variations in light-scattering.

Conventional Techniques of Virology.—Viable counts, infective centers, and active phage were determined on pour-plates as described by Adams.[30] By infective centers are meant those cells which give rise to one or more active phage particles as measured by plaques on the sensitive indicator strain W3104.

With the K-12 strains of *E. coli*, grown in tryptone broth at either 30° or 37°, 10^8 viable cells per ml correspond to an absorbance at 650 mμ of about 0.09 in the 1 cm light path of the model DU Beckman spectrophotometer. This absorbance value is three times the figure reported by Roberts *et al.*[31] The discrepancy may be due, at least partially, to differences in the growth medium used.

The efficiency of mass-induction as measured by the ratio of infective centers, following irradiation, to viable cells, prior to irradiation, was found to be generally less than 50 per cent, although irradiation caused immediate reduction in the viable count by more than 95 per cent. Post-irradiation lysogenic cells do not divide, but do continue to grow linearly, as seen from the linear increase in absorbance of the irradiated cultures (see figures). Cellular lysis, as measured by decrease in absorbance at 650 mμ, appeared eventually to destroy the majority of the irradiated cells.

In general, the W3104 indicator cells used for the assay of phage and infective centers were starved for about 1 hour in 0.01 M $MgSO_4$ before use.[29] To insure that phage samples taken during the period of cell lysis are not rendered inhomogeneous by the presence of strands of deoxyribonucleic acid, crystalline deoxyribonuclease (Worthington Co.) was routinely added to the cultures at about the 100th minute after irradiation at a final concentration of 1 μg protein per ml. Phage were diluted in a lambda-diluent described by Kaiser[32] prior to assay. Intracellular phage were assayed in premature lysates obtained by vigorously shaking culture samples with a few drops of chloroform.[33]

BIOCHEMICAL

Enzyme assays.— (*a*) *Galactose-1-phosphate uridyl transferase:* The assays of this enzyme, hereafter abbreviated transferase, and of uridine diphosphogalactose 4-epimerase, hereafter abbreviated epimerase, are as described by Kalckar, Kurahashi, and Jordan.[17] The methods ultimately depend on the spectrophotometric measurement of pyridine nucleotide reduction in coupled systems. Both enzymes catalyze the interconversion of uridine diphosphoglucose and uridine diphosphogalactose; epimerase does this directly, transferase by an exchange of phosphorylated sugars. The specific measurement of transferase activity is accomplished by assaying the formation of α-D-glucose-1-phosphate dependent upon the presence of uridine diphosphoglucose and α-D-galactose-1-phosphate. In the presence of these two substrates and excess phosphoglucomutase, glucose-1,6-diphosphate,

glucose-6-phosphate dehydrogenase, and TPN, the initial linear rate of TPN reduction is a measure of transferase activity. It is occasionally necessary to subtract a low rate of TPN reduction observed prior to the addition of the last substrate (preferably galactose-1-phosphate). Routinely, proportionality to bacterial extract concentration was demonstrated. Reactions were initially run in the compartment of a model DU Beckman spectrophotometer without temperature control. In later experiments, incubation was performed in the water-jacketed compartment of a model-14 Cary recording spectrophotometer, in which the cuvettes were maintained at approximately 30°.

The purified enzymes used in the assay system were shown to catalyze the reduction of TPN in mole for mole stoichiometry with the amount of glucose-1-phosphate added. Once formed, the TPNH is not reoxidized at an appreciable rate even in the presence of crude bacterial extracts. The presence of 6-phosphogluconic acid dehydrogenase in these extracts does permit reduction of a second mole of TPN for every mole originally reduced. In general, this contaminating enzyme does not affect the initial linear rate of TPNH formation, as was shown by the failure of an extract of the transferaseless strain W3104(λ) to increase the apparent activity of a sample of transferase purified free of 6-phosphogluconic acid dehydrogenase activity. The sample of purified transferase was the gift of Dr. Kiyoshi Kurahashi.

The assay enzyme, phosphoglucomutase, was either the crystalline enzyme obtained from rabbit skeletal muscle and generously furnished by Dr. Victor Najjar or an amorphous preparation purified according to the method of Najjar[34] through the penultimate step. Each cuvette received 1 μg of this protein in a final volume of 0.6 ml along with $6 \cdot 10^{-4}$ μmoles of glucose-1,6-diphosphate. This is sufficient to insure a rate of TPN reduction by glucose-1-phosphate in the transferase assay system corresponding to an absorbance change at 340 mμ of 0.200 per min.

Glucose-6-phosphate dehydrogenase was Type III (substantially free of hexokinase and 6-phosphogluconic acid dehydrogenase) purchased from the Sigma Chemical Company. Each cuvette received 0.05 Kornberg units.[35] Both of the purified enzymes employed in the assay were made up separately in 10 mg per ml crystalline bovine plasma albumin (Armour Company) and stored at −60°.

Crystalline α-D-galactose-1-phosphate, dipotassium salt, substantially free of glucose-1-phosphate; crystalline glucose-1-phosphate, dipotassium salt; uridine diphosphoglucose, sodium salt; and TPN, sodium salt were purchased from the Sigma Chemical Company. The barium salt of glucose-1,6-diphosphate was generously donated by Dr. Luis Leloir of the Instituto de Investigaciones Bioquímicas, Buenos Aires through Dr. Victor Ginsberg of the National Institutes of Health, Bethesda, Maryland. It was converted to the sodium salt before use and assayed with phosphoglucomutase by reference to its Michaelis constant of $5 \cdot 10^{-7}$ *M*.[34] A second sample of partially purified glucose-1,6-diphosphate was obtained through the kindness of Dr. Herbert C. Friedmann, who prepared it through the first barium fractionation step in the method of Leloir *et al.*[36]

(*b*) *Uridine diphosphogalactose 4-epimerase:* This enzyme was assayed by the two-step procedure described by Kalckar *et al.*[17] Incubation mixtures preincubated at 30° with added uridine diphosphogalactose were heated at 100° for 90 seconds and then assayed for uridine diphosphoglucose by spectrophotometric

measurement of the DPN reduced upon the addition of DPN and uridine diphosphoglucose dehydrogenase. The dehydrogenase was purchased from the Sigma Chemical Company as Type III. Synthetic uridine diphosphogalactose, lithium salt, was the gift of Dr. Herman M. Kalckar. A sample was also prepared enzymatically[37] by Dr. Elizabeth Maxwell in collaboration with one of us. DPN was purchased from the Sigma Chemical Company.

(*c*) *Uridine diphosphoglucose pyrophosphorylase:* This enzyme, like galactose-1-phosphate uridyl transferase, is also a uridyl transferase, the acceptor molecule here being pyrophosphate. The assays of the two enzymes, dependent upon measurement of glucose-1-phosphate formed, are similar.[38] It was found essential to use a high concentration of pyrophosphate ($6 \cdot 10^{-3}$ *M*) in order to obtain a linear time course of reaction. Pyrophosphate is apparently rapidly hydrolyzed in crude *E. coli* extracts.

(*d*) *Beta-galactosidase:* The method of Lederberg, employing *o*-nitrophenyl-β-D-galactoside, *o*NPG, as chromogenic substrate was used.[39] Incubations were carried out at 37°. Calculations of enzyme activity are based on an extinction coefficient at 420 mμ of $\epsilon = 4.6 \cdot 10^3$ cm^2 mmole^{-1} for *o*-nitrophenol in 0.67 *M* Na_2CO_3. *o*NPG was purchased from the California Corporation for Biochemical Research and *o*-nitrophenol was from Eastman Organic Chemicals.

(*e*) *6-Phosphogluconic acid dehydrogenase:* This enzyme was assayed by the spectrophotometric procedure of Horecker and Smyrniotis,[40] incubations being performed at approximately 30° in the compartment of a model-14 Cary recording spectrophotometer. The sodium salt of 6-phosphogluconic acid was purchased from the Sigma Chemical Company.

Conditions of Enzyme Extraction.—The following procedure has been used throughout the experimental work. Culture samples ($3\text{–}5 \cdot 10^8$ viable cells per ml) of approximately 20 ml are centrifuged for two minutes in 12 ml centrifuge tubes in a desk-top centrifuge to sediment the cells. At this point, the drained pellets may be frozen at −60°. The pellets are resuspended in $^1/_2$ to 1 ml of the potassium phosphate-$MgSO_4$ buffer identical to the medium in which cells are irradiated.[4] One-half ml aliquots of the suspension are transferred to lusteroid tubes in an ice bath and each is submitted to 45 seconds of sonication with the 6 mm diameter probe of the Mullard 20 kc per sec sonicator. The sonic extracts were centrifuged in stainless steel tubes for one hour at 144,000 × *g* in the refrigerated Spinco preparative ultracentrifuge. The supernatant fluid is then assayed or stored at −60° to be assayed at convenience.

Sonication is the method of cell disintegration used by Kurahashi and Sugimura for purification of the enzyme[41] and is superior or equivalent to alternative methods tried.[42] In exploratory studies of enzyme stability and extractability in small samples, attention was largely confined to transferase activity released by sonication. Below are briefly summarized the results of these studies. The bacterial strains used were either W3092(λ) or W3104(λW3092 + λ) grown in the absence of galactose.

Transferase appears to be a soluble enzyme. All of the transferase activity present in a crude bacterial extract, prepared through sonication as described above, remains in the supernatant fluid after centrifugation at 144,000 × *g* for one hour. Increasing the sonication time to 2 minutes does not appreciably alter the amount

of enzyme activity or protein extracted. Further sonication causes some enzyme inactivation. Sonication for 30 seconds appears adequate to cause maximal extraction of enzyme activity from cells which have not been irradiated. Cells which have been induced by irradiation are fragile just prior to lysis and require less sonication, but the transferase they release exhibits no increased lability to further sonication. The transferase in extracts prepared by harvesting induced lysogens towards the end of the latent period and allowing them to lyse spontaneously in a small volume of buffer is of no greater specific activity than is attained by sonication of the same cells. The presence of 10 mg per ml bovine plasma albumin or 10^{-2} *M* galactose during sonication is without effect.

An extract subjected to 5 cycles of freezing and thawing suffered no detectable loss of transferase activity. Packed cells and enzyme extracts have been successfully stored at $-60°$ for several days without losing enzymatic activity.

Reproducibility of enzyme extraction and assay was determined in an early experiment by assaying five separately sonicated aliquots of a bacterial culture. The mean specific transferase activity and standard deviation were 4.3 ± 1.2 μM TPNH/hr/mg protein.

Centrifugation of the sonicates serves to clarify them and is assumed to remove a constant proportion of the total protein which is then neglected in calculations of specific activity. This assumption is only approximately correct. Protein removed by centrifugation represents between 20 and 40 per cent of the total.

Protein Determination.—Sonicates containing 1 to 2 mg of bacterial protein per ml were freed of contaminating tryptone by precipitation at 0° with 0.35 *M* trichloroacetic acid. The protein of the tryptone broth is precipitated with less than three per cent the efficiency of precipitation of bacterial protein. The protein pellets were washed once with 2 ml 0.35 *M* trichloroacetic acid by centrifugation and then dissolved in dilute alkali and assayed for protein by a simple microbiuret procedure.[43]

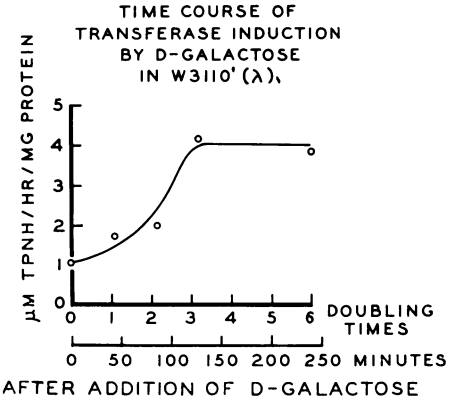

FIG. 1.—To a logarithmically growing culture of W3110'(λ) in tryptone broth $+10^{-2}$ *M* $MgSO_4$, at 30°, D-galactose was added at the start of the experiment to a final concentration of 10^{-2} *M*. The culture was maintained in logarithmic growth by dilution in a fresh medium at the time of each sampling.

Enzyme Induction by D-*Galactose and* D-*Fucose.*—It can be seen from Figure 1 that by the third generation of logarithmic growth in the presence of 10^{-2} *M* D-galactose the transferase activity of strain W3110' is maximally induced. The ratio of transferase specific activities, maximally induced to uninduced, is low, 4.0 in this case; in the strains tested, this induction ratio does not exceed a value of 10 whether D-galactose or D-fucose (6-deoxygalactose) is used as the inducing sugar (Table 2). A strain in which the induction ratio reaches or exceeds a value of 2 will be considered inducible and will be designated i^+. Where the ratio does not reach this value, and if transferase activity is appreciable, the strain will be considered constitutive and will

TABLE 2

TRANSFERASE INDUCIBILITY

Part	Strain	Inducing sugar: None	Inducing sugar: D-galactose	Inducing sugar: D-fucose	Maximum inducibility ratio
A	W3110′	0.66 1.00*	4.4	...	6.7
A,B	W3110′(λ)	1.0 0.92*	4.0	2.9	4.0
B	W3104	<0.02	<0.02	...	...
B	W3092(λ)	2.8 2.3	2.9 2.3	2.7	1.0
	W3092A(λ)	1.4	11.1	...	7.9
B	W3104(λW3092+λ)	1.3 2.6	5.0 12.1	7.2	5.5
B	W3092(λW3104+λ)	1.0	8.3	6.2	8.3
	W3092(λW3092+λ+λ*imm*^{434})	5.1 8.3	7.8 9.2	8.1	1.6
	C600	1.3	5.4	...	4.2
	C600(λ)	1.1	5.1	...	4.6

Cells were grown at 30° in tryptone-saline-$MgSO_4$ broth[29] and harvested in the logarithmic phase after more than four generations in the presence of 10^{-2} *M* inducer. The values of the first three columns are specific activities of transferase in μM TPNH/hr/mg protein. Values marked by an asterisk are the average of four separate determinations. The last column expresses the ratio of maximally induced activity to uninduced activity. These ratios are more significant than comparisons of specific activity values in different rows.

be designated i^-.

The conditions of transferase induction by D-galactose appear inadequate to induce β-galactosidase much above its basal level in those strains in which this enzyme was assayed. The enzyme may be induced to much higher levels by an inducer such as thiomethyl-β-D-galactoside.

D-galactose was purchased from Pfanstiehl Laboratories and D-fucose from the K and K Laboratories, Jamaica 33, New York.

RESULTS

Transduction and Dominance of Transferase Inducibility.—Small differences in the specific activity of transferase form the basis of the distinction between i^+ and i^- strains (Table 2), whether induction is effected by D-galactose or by D-fucose

TABLE 3

EFFECT ON TRANSFERASE ACTIVITY OF MIXING BACTERIAL EXTRACTS

No.	Source of extract	Transferase specific activity: Found	Transferase specific activity: Calculated
1	(*a*) Uninduced cells	0.53	...
	(*b*) D-galactose-induced cells	4.0	...
	(*c*) Mixture of (*a*) and (*b*) extracts (3:1 by protein)	0.92	0.80
2	(*a*) Unirradiated cells	4.2	...
	(*b*) Irradiated cells at start of lysis	11.6	...
	(*c*) Incubated mixture of (*a*) and (*b*) extracts (3:2 by protein)	7.0	7.1

In experiment No. 1, designed to determine whether extracts of uninduced cells inhibit the assay of an extract of induced cells, the bacteria were W3110′(λ) and were induced by growth in the presence of 10^{-2} *M* D-galactose. The mixture was not preincubated. Specific activities are expressed in μM TPNH/hr/mg protein.

In experiment No. 2, designed to determine whether the lytic enzyme of a UV-induced cell extract can increase the transferase activity of an unirradiated cell extract, the bacteria used were W3092(λW3092+λ+λ*imm*^{434}). Crude uncentrifuged extracts were preincubated, either separately or combined, for 30 min at 30°, then assayed without centrifugation. Negligible loss in enzyme activity occurs during preincubation. Specific activities are expressed in μM TPNH/hr/mg total protein.

(6-deoxygalactose). We can ascertain, by a mixing experiment such as shown in Table 3, experiment 1, that the specific activity differences between an i^+ and an i^- strain are *not* due to the presence of an inhibitor of enzyme assay in extracts of the i^+ strain which is absent or lower in concentration in extracts of the i^- strain. The experiment shows that the activity of mixed extracts is simply additive.

In two further respects, the inducibility of transferase behaves like other instances of enzyme inducibility which have been more intensively studied. Firstly, a gene determining inducibility of transferase, a "gene of regulation," appears to be distinct from a "gene of structure" specifying the enzyme.[44] Thus, from Part B of Table 2, it can be seen that the transferaseless strain W3104, derived from the i^+ strain W3110, appears to retain a functional inducibility gene since the heterogenote W3092(λW3104 + λ) is i^+. This observation suggests the existence of a transducible inducibility locus. The restrictive nature of lambda transductions, noted earlier, places this new i-gene within the *gal* region; just as in the case of the *lac* region the corresponding i-gene is found within that region.[44] A second point apparent from the enzyme levels of the two reciprocal heterogenotes is that irrespective of how the i^+ and i^- factors are distributed between host and transducing phage, the heterogenote is always inducible, i.e., the i^+ state is dominant over the i^- counterpart. We refrain from speaking of alleles where allelism remains to be established. Dominance of the i^+ state is consistent with the theory, first proposed by Vogel,[46] that inducibility results from the existence of a specific repressor of enzyme synthesis which is antagonized by inducers, while complete constitutivity results from the absence of repressor.

Absence of Lysogenic Conversion by Normal Lambda of Transferase Inducibility.— The proximity of the attachment site of lambda prophage to the *gal* region suggests the possibility of an influence of lambda prophage on the expression of genes within that region. Specifically, the possibility that the differential rate of transferase synthesis might be altered by lysogenization with lambda was examined in the progeny of four separately lysogenized cells of W3110′. The averages reported in Part A of Table 2 reveal no significant effect of lysogenization with lambda upon transferase levels in these inducible cells.

Increased Transferase Activity following Induction of Transducing Lambda.— Two reciprocal heterogenotes and a homogenote have been prepared in this laboratory, the heterogenotes being inducible, the homogenote constitutive or nearly so. The specific activity of transferase plotted against time after the initiation of phage development by UV irradiation is shown, for each of these strains, in Figure 2. These experiments, carried out at 30°, yield, upon compression of the time scale, kinetic curves identical in shape to those obtained when the experiments are carried out at 37°, but the extended latent period at 30° is somewhat more convenient for repeated samplings. For this reason, all the experiments presented here were run at the lower temperature.

Each graph is provided with a caption indicating the presumptive genotype of the strain; allelism of alternative inducibility states is assumed merely for simplicity of presentation.

Preliminary attempts at quantitative measurement of transducing lambda have not proved sufficiently accurate to be reported here.

The absorbance measurements reported were made immediately upon sampling

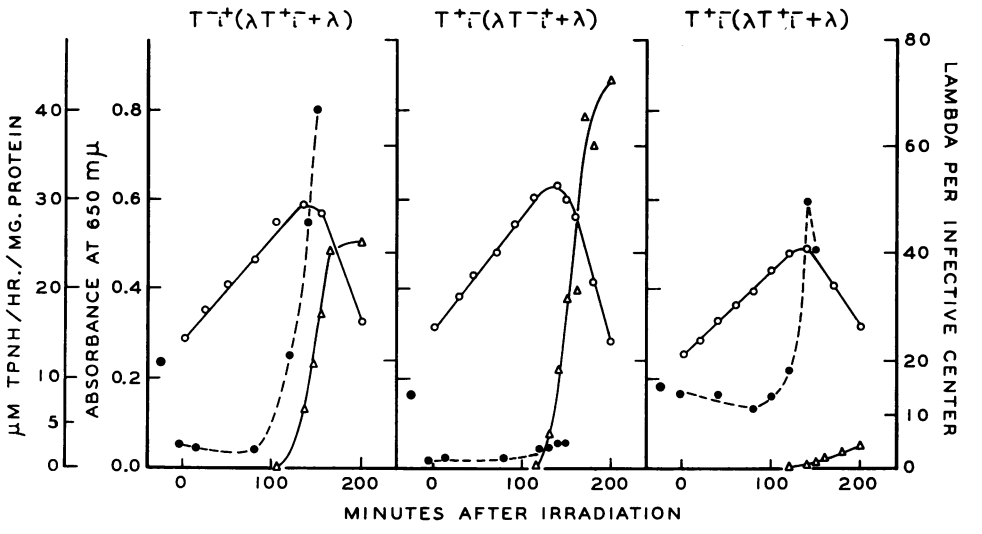

FIG. 2.—Transferase activity following induction of phage replication in syngenotes. The bacterial strains employed are, from left to right, W3104(λW3092 + λ), W3092(λW3104 + λ), and W3092(λW3092 + λ + λ*imm*^{434}). The key to the symbols used is provided in Figure 3. The ratios of infective centers measured promptly following irradiation to viable count prior to irradiation for the three experiments are, from left to right, 0.39, 0.39, and 0.45. Lambda titrations were performed with the indicator strain W3104 and therefore in the case of the homogenote include lambda and λ*imm*^{434}. The low phage yield with the homogenote is not atypical for this strain.

The unconnected symbols representing transferase specific activity drawn to the left of zero minutes give the values obtained for parallel cultures grown in the presence of 10^{-2} *M* D-galactose for at least 6 doubling times.

the culture; after the initiation of lysis, cells allowed to stand in the cuvette lyse more rapidly than in the incubating culture itself.

In each case, the specific activity of transferase increases during the final third of the latent period of phage development. This increase is most striking in the case of the heterogenote in which the T$^+$i$^-$ transducing phage replicates against the background of a T$^-$i$^+$ host. The reciprocal heterogenote shows only a small, but nevertheless significant, increase in enzyme activity. If transducing genetic material were functional during the latent period of the phage development, in the sense of initiating enzyme synthesis, we might expect the rate of transferase synthesis to increase in the first-mentioned heterogenote, but no such rise should occur in the reciprocal heterogenote. In the latter, we might expect an increased repression of enzyme synthesis owing to replication of the i$^+$ gene. It would appear that an explanation other than increased gene dosage must be invoked to explain the fact that transferase activity increases in both heterogenotes upon phage induction.

Experiments have been performed in which the transferase of the heterogenote W3104(λW3092 + λ) was induced prior to irradiation by growth in the presence of 10^{-2} *M* D-galactose. During the latent period of phage development, which was also allowed to proceed in the presence of the sugar, the specific activity of transferase increased above the normal galactose-induced level. The time course of this enzyme increase resembles that seen following induction of the homogenote of Figure 2.

The rise in transferase activity which occurs in the homogenote is again towards the end of the latent period of phage development. Again the specific activity of transferase attained subsequent to phage induction is greater than is attained with either D-galactose or D-fucose as enzyme inducer. The possibility that an inactive form of transferase might be activated by the lytic enzyme present in cells about to lyse was investigated by determining the specific activity of transferase in crude extracts preincubated together (Table 3, experiment No. 2). Additivity of enzyme activities fail to support this hypothesis.

Increased Transferase Activity following Induction of Normal Lambda.—Since the induction of transducing lambda in the previous experiments involves the

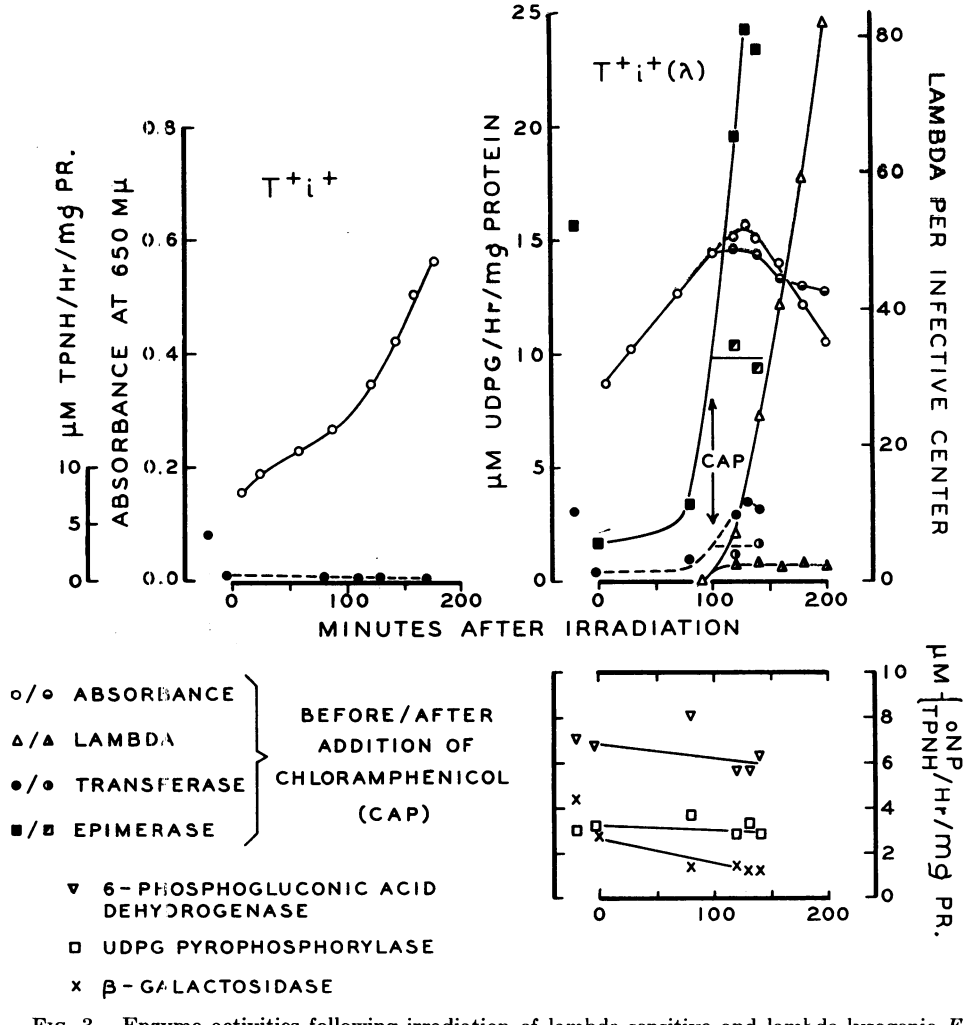

FIG. 3.—Enzyme activities following irradiation of lambda-sensitive and lambda-lysogenic *E. coli*. The bacterial strains employed are W3110′ (left) and W3110′ (λ) (right). The ratio of infective centers, measured promptly following irradiation of the lysogen, to viable count measured prior to irradiation is 0.42. The scales are the same as in Figure 2. For transferase, UDPG pyrophosphorylase, and 6-phosphogluconic acid dehydrogenase activity, refer to the scales labeled μM TPNH/hr/mg protein; for epimerase activity, μM UDPG/hr/mg protein; for beta-galactosidase activity, μM oNP/hr/mg protein. As in Figure 2, the unconnected symbols representing enzyme specific activities drawn to the left of zero minutes give the values obtained for parallel cultures grown in the presence of 10^{-2} M D-galactose for about 6 doubling times. At 100 minutes after irradiation of the lysogen, a portion of the culture was transferred to a separate containing flask chloramphenicol (Parke, Davis and Co.) at a final concentration of 40 μg/ml.

simultaneous induction of helper phage, it is necessary to determine the effect upon transferase activity of induction of normal lambda as well as the effect of the inducing irradiation alone upon a lambda-sensitive strain. Irradiation of a sensitive strain, either W3110′ (Fig. 3, left) or C600 leads to a lag period after which the culture resumes logarithmic growth. Irradiation does not serve to bring about an increased activity of transferase.

Irradiation of an active lysogen, W3110′(λ) or C600(λ), does bring about a rise in the specific activity of transferase. This is shown by the dashed curve on the right in Figure 3. The line is drawn partly on the basis of two replicate experiments. In these experiments, transferase specific activity increased, by the time of lysis, 8 fold and 12 fold, respectively, as compared with 9 fold in the experiment of Figure 3. Here, as in the case of the induced syngenotes of Figure 2, the rise in transferase activity occurs late. Also upon irradiation of the constitutive lysogen W3092(λ), increases of two to three fold in the specific activity of transferase have been found to occur.

Since induction of normal lambda alone in i^+ or i^- normal lysogens is capable of increasing transferase activity, this raises the question of how much of what occurs upon induction of a lysogenic syngenote is due to induction of the helper phage.

Limitation of Specific Activity Increases, following Induction of Normal Lambda, to Enzymes of the Gal Region; Inhibition by Chloramphenicol.—A possible limitation of the enzymes whose activities are increased following lambda induction to those genetically determined by the *gal* region is suggested, in part, by the observation of Buttin *et al.*[5] that galactokinase activity is increased by lambda induction just as is transferase activity. The hypothesis is supported by a study of the activities of enzymes under separate genetic control and one additional enzyme determined by the *gal* region, uridine diphosphogalactose 4-epimerase. The enzymes assayed in the experiment of Figure 3 include, apart from transferase and epimerase, two enzymes involved in galactose or galactoside metabolism (uridine diphosphoglucose pyrophosphorylase and β-galactosidase). The last mentioned, like transferase and epimerase in this strain, is inducible, although D-galactose under the conditions of the experiment is an exceedingly poor inducer. A third enzyme, 6-phosphogluconic acid dehydrogenase, is of particular interest since its activity may affect the measurement of transferase activity, of which more will be said later. Most striking about the curves of Figure 3 is the contrast between enzymes determined by genes lying within the *gal* region and those determined by genes outside the *gal* region. Following lambda induction, the specific activities of the former enzymes increase together (upper graph), whereas those of the latter group decrease slightly (lower graph).

Epimerase activity is considerably greater than transferase activity and may even be underestimated in the experiment of Figure 3. Linearity of the assay with time of incubation of substrate with sonicate was not attained, although the two-step assay of Kalckar *et al.*[17] was used. The shape of the epimerase specific activity curve is probably not seriously affected by the inaccuracy thus introduced.

The reliability of transferase assays may be criticized on the basis of two considerations. The first of these is that the number of molecules of TPN reduced by each molecule of glucose-1-phosphate depends upon the contribution of 6-phosphogluconic acid dehydrogenase to the oxidation of the sugar phosphate. This con-

tribution is not necessarily constant and unless taken into account may lead to an error by a factor of as much as two. A slight decline in 6-phosphogluconic acid dehydrogenase specific activity following irradiation has been found to occur not only in the lysogen of Figure 3, but also in the sensitive strains and in the heterogenotes. Therefore, the error introduced by the changing proportion of 6-phosphogluconic acid dehydrogenase activity can only lead to an underestimate of the transferase specific activity increases reported here.

A second and more serious concern is the specificity of the assay designed to measure transferase. It is possible to write two reactions, catalyzed by one or perhaps two pyrophosphorylases, which sum to yield an over-all reaction identical to the one catalyzed by the galactose-1-phosphate uridyl transferase:

(1) UDPG + PP = UTP + G-1-P
(2) Gal-1-P + UTP = PP + UDPGal
Sum: UDPG + Gal-1-P = UDPGal + G-1-P

An enzymatic catalysis of reaction (1) has already been demonstrated by Kurahashi[18] to occur in extracts of *E. coli* K-12. Reaction (2) is known to occur in extracts of galactose-adapted yeast.[47] On the assumption that reaction (2) also occurs in *E. coli* extracts, an apparent change in the activity of galactose-1-phosphate uridyl transferase could be due to an alteration of pyrophosphorylase activity. The assays of uridine diphosphoglucose pyrophosphorylase of Figure 3, showing no specific activity increase subsequent to phage induction, diminish the possibility of such an artifact.

The increase in transferase or epimerase activity can be halted abruptly by addition of chloramphenicol to the culture (Fig. 3). Since protein synthesis is thereby specifically inhibited,[48] the observed increases in enzyme activity must represent, or at least be shortly preceded by, the synthesis of protein.

In the experiment of Figure 3, the time of addition of chloramphenicol is such as to largely dissociate the rise in enzyme activities from the formation of mature phage. A recent observation by Dr. Maurice Bessman that under a nitrogen atmosphere the formation of phage-induced deoxyguanylate kinase may occur in the absence of the synthesis of the majority of phage proteins[49] suggested to us the possibility of dissociating bacterial from viral protein synthesis in this way. However, in preliminary experiments, appreciable increases in the specific activity of transferase were not observed when the irradiated lysogen W3110′(λ) was maintained anaerobically.

Relation of Transferase Activity Increase to Prophage-Attachment Site following Induction of Lambda-Related Phages.—The transferase activity increase upon phage induction has been examined to determine the phage specificity of the phenomenon. The phages investigated are related to lambda as shown by certain similarities of phenotype and by their ability to undergo genetic recombination with lambda.[7, 25] They are all lysogenizing and become associated as prophage each with a specific locus along the bacterial chromosome. They are inducible by UV-irradiation. The locations of the prophages, determined by Jacob and Wollman,[54] are as follows: prophage *82* lies closest to *gal* region, next is lambda, then *434* (and λ*imm*^{434}) closely following; prophage *21* is located somewhat further along the chromosome.

Of the phages listed in Table 4, those which become situated in closest proximity to the *gal* region cause increased production of transferase following irradiation. On the other hand, induction of phage *21*, which lies at some distance from the *gal* region, fails to alter the level of transferase. The prophages prior to induction are without effect. While the results with phage *82* are ambiguous (owing perhaps

TABLE 4

TRANSFERASE ACTIVITY FOLLOWING INDUCTION OF LAMBDA AND RELATED PHAGES

Phage	Per cent viable cells induced	Transferase Specific Activity	
		Cells not irradiated	At time of lysis
None	..	1.3	...
82	21	1.3	1.7
λ	50	1.1	5.0
λ*imm*^{434}	41	1.4	4.1
434	38	1.2	3.0
21	69	1.1	1.1

The phages were initially present as prophage in strain C600. They are listed in order of increasing distance between the site of prophage localization and the gal region of the host. Infective centers, used in calculating per cent viable cells induced, were determined with the indicator bacteria C600 for *82*, λ*imm*^{434}, and *21*, C600(λ) for *434* and W3104 for λ. Transferase specific activity is in μM TPNH/hr/mg protein.

to the fact that the efficiency of induction is low), it would appear that alterations in the phage genome do not necessarily abolish the phenomenon of transferase increase, provided the prophage is not displaced too far from the *gal* region.

Construction of reciprocal hybrids between phages *21* and lambda (*21imm*$^{\lambda}$ and λ*imm*^{21}) should permit a more decisive evaluation of the role of prophage localization site in determining the capacity of a lysogenizing phage to cause increases in enzymes of the *gal* region after its induction.

DISCUSSION

The ontogeny of lambda during the lytic cycle is accomplished through the orderly functioning of phage genes whose capacities are latent in the prophage. It is of interest to know how a segment of bacterial genome behaves at various stages in the life of lambda when present as an insertion in the genome of the phage. Following the introduction of a transducing *gal*$^{+}$ determinant into a recipient cell, phenotypic expression has been found by several workers to occur almost immediately[5, 50, 51] and it persists following reduction to prophage. Even when the vegetative replication of phage has been initiated, the transduced genes apparently continue to function since they are capable of causing a further increase in enzyme synthesis, e.g., in the induced heterogenote W3104(λW3092 + λ) of Figure 2. Thus, throughout these stages in phage development, the transduced bacterial genes lying within the phage genome maintain their capacity to initiate enzyme synthesis.

The functioning of transduced genes is not, however, independent of the state of the phage. On the contrary, a series of experiments on galactokinase transduction into kinaseless recipient bacteria (lysogenic or sensitive to lambda) has led Buttin *et al.*[5] to conclude that when these bacteria are not or are no longer immune to lambda, transduced kinase synthesis, normally inducible, becomes constitutive.

In the course of our own studies and those of Buttin *et al.*, it was discovered that the phage genome can also affect the functioning of bacterial genes not inserted

within the phage genome but merely associated with it. The bacterial genes affected, those of the *gal* region, lie near the site of prophage localization and are influenced only upon phage induction (Fig. 3) and not by the mere presence of prophage (Table 2, part A, and Table 4). Because of the relative simplicity of the phenomenon, our primary concern has been a description of the influence which the induction of normal lambda and related phages exerts upon the *gal* region.

The increases in enzyme activities which follow induction of lambda are interpreted as reflecting the differential rate of synthesis of the proteins involved. The ability of chloramphenicol to halt the enzyme increases once they have started supports this interpretation. Results of mixing experiments (Table 3, Exp. No. 2) and the narrowness of phage specificity offer strong evidence against zymogen activation by a lytic enzyme or other agent formed in the course of phage development.

Prior to lysis of phage-infected *E. coli.*, leakage of various substances from the cells is known to occur.[53] It might be argued that, during the latent period of phage development, loss of a repressor (or a substance required for repressor formation) could be responsible for the apparent derepression of enzymes of the *gal* region. This argument does not seem tenable in view of the ineffectiveness of phage *21*, closely related to lambda, in provoking the enzyme increases, although the phage matures and brings about lysis of the host.

The enzymes whose specific activities are increased following induction of lambda, viz., kinase, transferase, and epimerase, belong to one biochemical pathway and are controlled by a cluster of genes collectively called the *gal* region. The levels of all three enzymes are diminished[17] by single point mutations within a cistron of the *gal* region designated "C."[19] Group "C" mutations are noncomplementary with all mutations in cistrons "A," "B," or "C."[19] The proposal by Jacob *et al.*[45] that the genes of the *gal* region constitute an "operon," i.e., a genetic region under the coordinated pleiotropic control of a single "operator gene," would appear to be based on these properties of mutations in the "C" cistron. According to the concept of Jacob *et al.*[45] that the operator (or a cytoplasmic replica of it) is the seat at which repression is exerted, the enzymes of a sequence governed by the same operator should not be capable of separate induction. The kinaseless strain W3092 in which a previous report[17] and Table 2 show transferase to be constitutive and in which Kalckar *et al.*[17] have reported epimerase to be inducible may present a paradox and is therefore the subject of reinvestigation. The inducibility of at least transferase is affected by mutation within a presumed "gene of structure" determining kinase and has suggested an auxiliary role of kinase in controlling the formation of or sensitivity to a repressor substance.[52] Whether or not transferase inducibility is under separate control, the fact remains that all three enzymes of the *gal* region undergo a concerted increase following induction of lambda.

A survey of a few lambda-related lysogenizing phages reveals that the effect we are studying is sensitive to changes in prophage localization. This observation, but more particularly, some ingenious experiments reported by the group in Paris,[5] leads us to conclude that the host-phage interaction responsible for initiating increased enzyme activity is not mediated by a cytoplasmic agent. The experiments just mentioned compare kinase levels, which follow lambda induction, in partially diploid lysogens heterozygous at the *gal* region and in which the prophage is as-

sociated exclusively with either the *gal*$^+$ or the *gal*$_2^-$ bacterial chromosome. Synthesis of kinase becomes constitutive only where lambda prophage was originally associated with the *gal*$^+$ chromosomal segment.

The ability of lambda induction to cause coordinate increases in the same enzymes whose coordinate expression is presumed to be controlled by a cytoplasmic repressor and its receptor, the operator cistron "C," suggests that lambda induction affects either the repressor substance or the operator gene. As already indicated, available evidence appears to preclude the mediation of any cytoplasmic agent. We are therefore inclined to ascribe the enzyme increases, which occur upon lambda induction, to a direct interaction between a portion of the lambda genome and the "C" cistron of the bacterial *gal* region. Since lambda induction in i$^+$ lysogens has on occasion resulted in enzyme levels in excess of those achieved by induction with D-galactose or D-fucose, we must assume that the derepression normally afforded by these sugars is incomplete. The increases in transferase levels observed upon lambda induction in the constitutive strain W3092 require us to assume that transferase synthesis is to some extent repressed in this strain but not subject to derepression by either of the above sugars. Further experiments are required to determine the validity of the hypothesis that an operon of the host and a portion of the phage interact upon induction.

A number of factors may be expected to influence the specific activity of transferase following the induction of transducing lambda in lysogenic syngenotes. Not only may there be position effects due to surrounding genetic material of the transducing phage and perhaps the associated genetic material of helper phage, but there may also be dosage effects due to the increasing numbers of presumably functional genes determining enzyme structure and inducibility.

The large differences in the two reciprocal heterogenotes of Figure 2 between transferase levels following phage induction might be most simply attributed to the effects of gene dosage. Assessment of the role of gene dosage must, however, await less ambiguous experiments. The problem might be somewhat simplified if galactose markers could be inserted into the genome of a prophage which locates some distance from the *gal* region, e.g., λgal^+imm^{21}. Such a phage would be recognized by its capacity to transduce the *gal*$^+$ marker and confer immunity to phage *21* and not to lambda.

Lysogenizing phages which locate close to the genetic determinants regulating additional groups of enzymes and perhaps viruses inhabiting the nuclei of animal cells deserve attention to ascertain how general the phenomenon described here may be.

Summary.—(1) Evidence is presented for genetic regulation in *Escherichia coli* of the inducibility of galactose-1-phosphate uridyl transferase, inducibility being dominant over constitutivity. The determinant is transduced by lambda phage and is therefore presumed to be located within the *gal* region. (2) Where a gene for functional transferase synthesis is present in the transducing phage, induction of phage multiplication in cells doubly lysogenic for normal and transducing lambda is shown to cause a rise in transferase specific activity to levels higher than are attained by enzyme induction with D-galactose or D-fucose. Smaller enzyme increases occur where the transducing phage does not contribute a gene for the synthesis of enzymatically active transferase. (3) Evidence is offered, in part based

on the work of Buttin, Jacob, and Monod,[5] that induction of normal lambda specifically increases the differential rate of synthesis of the sequence of enzymes determined by the *gal* region of the host, viz. galactokinase, galactose-1-phosphate uridyl transferase, and uridine diphosphogalactose 4-epimerase. (4) Studies of lambda-related phages suggest that prophage localization site (near the *gal* region in the case of lambda[23]) is important in determining the effect of phage induction upon the subsequent activity of *gal* region enzymes. (5) The hypothesis is offered that an interaction between the "C" cistron of the bacterial *gal* region,[11] considered as the operator gene of the *gal* operon,[45] and a portion of the phage genome is responsible for the effects of phage induction upon *gal* region enzyme levels. Unresolved related problems are briefly discussed.

We are much indebted to those investigators whose names are listed in Table 1 (as donors) for providing us with bacteria and phage and for advice on matters of technique. Dr. Philip E. Hartman has also materially aided our understanding of virological aspects of this work.

We are again pleased to acknowledge our considerable debt to Dr. Herman M. Kalckar for advice and encouragement.

Our thanks are also due to Miss Elke Jordan, Mr. David M. Shapiro, and Dr. Herbert C. Friedmann for critically reading the manuscript.

* This work was supported by a grant from the National Institutes of Health, U.S. Public Health Service. A preliminary report has appeared: H. Wiesmeyer and M. B. Yarmolinsky, *Bacteriol. Proc.*, P120 (1960).

The following abbreviations are used: TPN, triphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide; DPN, diphosphopyridine nucleotide; UDPG, uridine diphosphoglucose; UDPGal, uridine diphosphogalactose; G-1-P, α-D-glucose-1-phosphate; Gal-1-P, α-D-galactose-1-phosphate; UTP, uridine triphosphate; PP, pyrophosphate; *o*NPG, *o*-nitrophenyl-β-D-galactoside; *o*NP, *o*-nitrophenol.

† Post-doctoral fellow of the National Science Foundation.

‡ Contribution No. 305 of the McCollum-Pratt Institute.

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