studied, the high molecular weight form in higher plants behaves typically for genetic material. This does not appear to be true for the low molecular weight form. A number of reports have been published concerning the existence of DNA in chloroplasts[8] and of "nonfibrous" DNA in nuclei of calf thymus.[9] No satisfactory evidence exists for presuming a homology between the form studied here and those reported elsewhere. The fact that label can be almost entirely "chased" out of the molecule argues against its being a genetic determinant in the conventional sense. Its rapid formation in embryos induced to germinate suggests rather a physiological role. If so, the gross composition here reported may not be a constant characteristic, even though in roots of both wheat and corn the guanine-cytosine content appears to be greater than that of the high molecular weight form. At this stage of study the most important conclusion to be drawn is that a form of metabolically labile DNA exists.

Summary.—Growing tissues of higher plants have two forms of DNA. One of these has an average molecular weight of the order of 10^5 and is characterized by a relatively rapid rate of turnover. The composition of this DNA appears to differ from its high molecular weight counterpart.

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*INTERFERENCE WITH GLYCEROKINASE INDUCTION IN MUTANTS OF E. COLI ACCUMULATING GAL-1-P**

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The inhibitory effect of galactose on the growth of mutants of *E. coli* lacking gal-1-P-uridyl transferase has been established by the work of Kurahashi and Wahba[1] and Yarmolinsky *et al.*[2] The inhibition is very pronounced in a synthetic medium containing glycerol as sole carbon source, and the addition of glucose to the medium promptly relieves inhibition.[2] The growth inhibition is accompanied by the accumulation of gal-1-P in the cell.[3] Similar findings have been reported from this laboratory with another mutant of *E. coli* which lacks the enzyme UDPG synthetase.[4]

It is not clear how galactose causes growth inhibition in these mutants. The

favored hypothesis is that gal-1-6-P, formed presumably by phosphorylation of gal-1-P, inhibits phosphoglucomutase, *in vivo*,[5] in the same manner as it has been shown to act *in vitro*.[6] The level of UDPG is also markedly lowered in late stasis, and the addition of glucose to the medium restores the level to normal.[3, 7]

We wish to report an interesting *in vivo* effect of gal-1-P in galactose-sensitive mutants, which serves to underline the complex nature of the bacteriostatic effect of galactose in these mutants. The addition of galactose to the medium causes a marked lowering in the level of the inducible enzyme, glycerokinase, a key enzyme in the utilization of glycerol by *E. coli*.[8] This effect of galactose is confined to mutants known to accumulate gal-1-P and is more clearly elicited, when glycerol serves as a supplemental rather than as the sole source of carbon.

Materials and Methods.—The K-12 strain which was used stemmed from the collection of Drs. E. and J. Lederberg and was generously donated to us.

Galactose essentially free of glucose was obtained from Sigma Chemical Company, as were the following reagents: α-glycerophosphate dehydrogenase (2X crystallized 10 mg/ml suspension), α-galactose-1-phosphate (dipotassium salt), ATP (dipotassium salt), and o-NPG.

The growth conditions in general were those described previously[2] using tryptone broth (1%) or ammonia mineral medium[9] with 1% glycerol as sole carbon source.

Extracts for enzyme assays were prepared by suspension of the washed cells in 1–2 ml of 0.1 *M* phosphate buffer, pH 7.5 and subjected to sonication in a 10 KC Raytheon sonicator for 3 min. The sonicate was spun at 10,000 $\times$ *g* for 15 min, and the supernatant used immediately for enzyme assay.

Enzyme assays: Epimerase was assayed by the two-step procedure of Kalckar *et al.*[10] β-Galactosidase assay was carried out on sonicated extracts with o-NPG as substrate.[11] Glycerokinase assay was carried out essentially according to Kennedy.[12] The increment in O.D. at 366 mμ was followed in a Cary recording spectrophotometer. The reaction was linear for at least 5 min after addition of the substrate, glycerol. Proportionality to increasing enzyme concentration was observed up to a Δ O.D. of 0.02/min. One unit of the enzyme was taken as the amount of glycerokinase which caused an O.D. increment of 0.001/min.[12] Specific activity was expressed as units per mg protein.

Protein was estimated on the extracts by a microbiuret procedure[13] after precipitation with 0.35 *M* TCA.[11]

Results.—Glycerokinase levels in T^- cells grown in glycerol-galactose medium: Figure 1 presents the growth curve and the levels of glycerokinase, epimerase, and β-galactosidase at various time intervals following the addition of galactose to the transferaseless strain W 3104. Glycerol served as the sole carbon source in these experiments. Galactose ($10^{-2}M$) was added to the culture at an O.D. of 0.2. Epimerase activity was followed as a measure of the induction of the enzymes of galactose metabolism.

The following points, evident from the figure, are of interest: (1) epimerase is induced without an appreciable lag period following addition of galactose to the medium; (2) β-galactosidase induction shows some lag period, but the kinetics and the levels reached are not conspicuously different from what can be observed in other K-12 strains induced with galactose;[10] (3) the specific activity of glycerokinase shows an appreciable decrease one hr after the addition of galactose. There is no

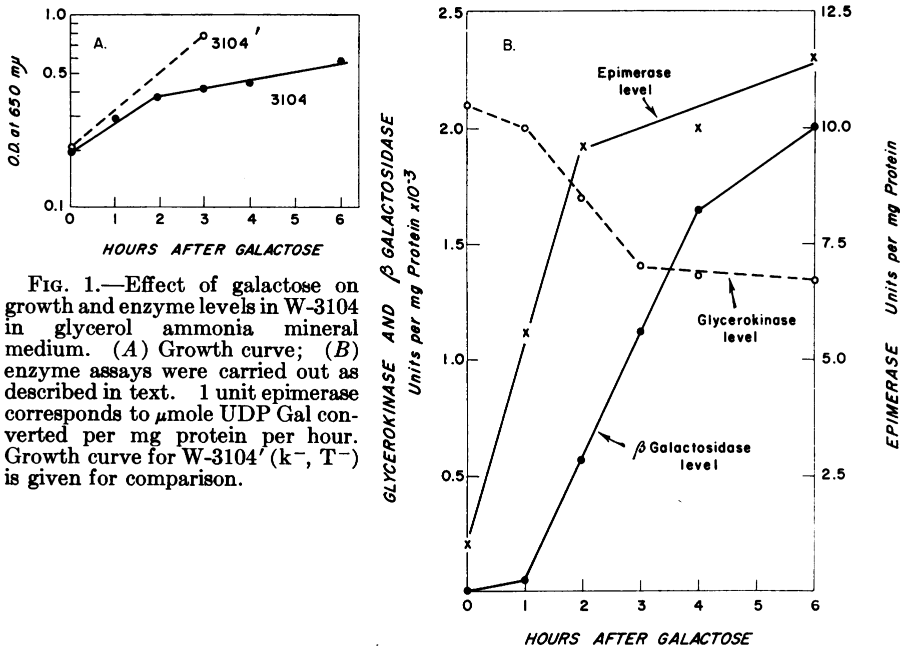

FIG. 1.—Effect of galactose on growth and enzyme levels in W-3104 in glycerol ammonia mineral medium. (*A*) Growth curve; (*B*) enzyme assays were carried out as described in text. 1 unit epimerase corresponds to μmole UDP Gal converted per mg protein per hour. Growth curve for W-3104′ (k^-, T^-) is given for comparison.

further drop in activity after the 3rd hr, by which time growth inhibition has set in. The drop in specific activity reflects a decreased synthesis of the enzyme as compared to the synthesis of the total protein.

In contrast, a galactose-resistant, kinaseless strain derived from 3104 showed no decrease in glycerokinase activity under comparable conditions, suggesting that galactose *per se* does not interfere with the induction of the kinase (Table 1).

TABLE 1

GLYCEROKINASE LEVELS IN GALACTOSE-RESISTANT KINASELESS STRAIN FROM 3104 IN GALACTOSE-GLYCEROL MEDIUM

Time after addition of galactose	O.D. 650 mμ at harvesting	Glycerokinase (units/mg protein × 10^3)	β-Galactosidase (units/mg protein* × 10^3)
0 hr	0.20	2.4	0.005
3 hr	0.78	3.6	2.01

* mμmoles o-NP liberated per min per mg protein.
Experimental conditions were the same as in Fig. 1.

Induction in broth medium: With a view to eliminate the possibility of growth inhibition as a contributory factor for the decrease in the activity of glycerokinase, the experiments were repeated using broth medium. In such a medium, galactose has little or no bacteriostatic effect. In these experiments, galactose ($10^{-2}M$) and glycerol (1 per cent final concentration) were both added at zero time to a broth culture not preinduced with either of these substrates. Galactose was withheld in control experiments. The cells were harvested in the log phase at an O.D. (Beckmann, 650 mμ) of 0.5. The results of such a study carried out with mutants of different phenotypes are presented in Table 2.

TABLE 2

Mutant	Phenotype	Glycerokinase (units/mg protein × 10^3) −Gal	+Gal
3100	$K^+T^+E^+$	1.33	1.10
3104	$K^+T^-E^+$	1.63	0.23
3104′	$K^-T^-E^+$	1.22	1.02
3096	$K^+T^-E^+$	0.81	0.07
4597	$K^+T^+E^+$ (UDPG synthetaseless)	0.95	0.10
3097	$K^+T^-E^+$	1.0	0.21
3097R	$K^+T^+E^+$	1.0	0.51

The cells were grown in 1% tryptone broth, 0.01 M $MgSO_4$—1% glycerol with or without galactose (10^{-2} M). The cells were not preinduced with either glycerol or galactose.

It is evident that galactose does not interfere with the induction of glycerokinase in the wild-type and in the kinaseless strains. The decrease in activity is very pronounced in mutants which are known to accumulate gal-1-P (80–95 per cent repression). In a revertant obtained from a transferaseless strain, there is some repression by galactose (approximately 50 %), but this is, in any case, much less than that seen in the transferase defectives or UDPG defectives.

Effect of extracts of T^- mutants and of gal-1-P on glycerokinase: The decrease in kinase activity in these experiments could be due to repression of enzyme formation or might represent inhibition of enzyme activity by gal-1-P or some other metabolite accumulated by the cell. To resolve this question, the effect of added gal-1-P and of extracts of galactose-grown transferaseless mutants on the activity of glycerokinase was studied. Again, a decrease in kinase activity could also be due to competition for the available ATP for phosphorylation of galactose and glycerol. The results presented in Tables 3 and 4 offer answers to these questions. (1) The addition of equimolar amounts of galactose and of glycerol to the glycerokinase assay medium has no effect on glycerokinase activity even when the ATP level is reduced to a third of the level used in the usual assay. (2) The addition of gal-1-P up to 1.2 μmoles to the assay mixture does not inhibit glycerokinase; nor is there an inhibition in activity when extracts of galactose-grown T^- cells are added to extracts obtained from cells grown in the absence of galactose.

TABLE 3

LACK OF INHIBITION OF GLYCEROKINASE BY GALACTOSE AND GAL-1-P

Additions to assay mixture	ATP (0.1 M)	Glycerokinase units*
(1) Nil	30 μl	37
(2) Galactose (0.1 M) 40 μl	30 μl	38
(3) Nil	10 μl	25
(4) Galactose (0.1 M)	10 μl	22
(5) Gal-1-P- (1.2 μmoles)	30 μl	36

* The values represent activity units in 10 μl of the enzyme extract.

Conditions of assay as in text. A concentrated extract of W-3104 grown in glycerol (1%)-galactose ($10^{-2}M$)-salts medium, having a specific activity of 0.46 glycerokinase units/mg protein, was used as source of glycerokinase. Reaction was started by the addition of 40 μl of 0.1 M glycerol after leveling off of the blank reaction.

TABLE 4

LACK OF INHIBITION OF GLYCEROKINASE BY EXTRACTS OF GALACTOSE-GROWN T^- CELLS

Enzyme source	Aliquot used for assay	Glycerokinase units	Sp. activity units/mg protein
(1) W-3104 grown in glycerol	10 μl	42	12.2
(2) W-3104 grown in glycerol + galactose	10 μl	4	2.0
(3) Mixed extracts of (1) and (2)	10 μl each	40	—

Assay mixture and other conditions are as described in text. The cells were grown in glycerol minimal medium with or without galactose ($10^{-2}M$).

Glycerokinase level in T^- cells grown in the presence of glucose: Cells of W 3104 preinduced in glycerol medium were distributed into two flasks containing medium of the same composition. After 2 hr at 37°, glucose at $10^{-3}M$ concentration was added to one of the flasks while the second flask served as control. The cells were harvested at an O.D. of 0.5 and the glycerokinase activity determined on the extracts. There was a sharp decrease in the specific activity of glycerokinase in the first case, as expected from the well-known "glucose effect" (Table 5).

TABLE 5

EFFECT OF GLUCOSE ON GLYCEROKINASE LEVEL IN W-3104

Addition	Glycerokinase (units/mg protein × 10^3)
Nil	1.22
Glucose	0.23

W-3104 grown in salts medium-glycerol. After 2 hr glucose ($10^{-3}M$) was added to one of the flasks and the other served as control. Cells harvested in the log phase and assayed for glycerokinase.

Discussion.—That gal-1-P represses glycerokinase formation is evident from the following observations:

(1) In transferaseless mutants growing in glycerol medium, the addition of galactose brings about a decrease in the specific activity of glycerokinase, the decrease becoming evident when the enzymes of galactose metabolism are well induced (Fig. 1). Since such a decrease is not observed in a T^- mutant which lacks galactokinase as well, the phosphorylation of galactose seems to be essential for the drop in glycerokinase activity. Since glycerol is the only usable carbon source, one might well consider the stasis as a result of a partial starvation (like a chemostat), although this needs further examination.

(2) The interference with the induction of glycerokinase is even more marked when the cells are growing in a broth medium, without preinduction in glycerol (Table 2). With mutants known to accumulate gal-1-P, the glycerokinase levels, in the presence of galactose, are about 10–20 per cent of the value observed with galactose omitted from the medium. Galactose does not interfere with glycerokinase induction either in the wild type, which is known to metabolize gal-1-P rapidly, or in K^- cells which have lost the phosphorylating mechanism for galactose.

(3) The decrease in glycerokinase activity is not due to inhibition of enzyme activity by gal-1-P or some other metabolite of galactose (Tables 3 and 4). The results are compatible with the assumption that gal-1-P inhibits formation of the enzyme.

The difference, noted in these experiments, in the response of the two inducible enzymes, β-galactosidase and glycerokinase, is of interest in view of the recent observations of McFall and Mandelstam[14] to the effect that inducible enzymes do not share a common metabolite repressor and that the repressor is identical with or closely related to the end-product of reaction catalyzed by the inducible enzyme. To explain the repression of glycerokinase by gal-1-P, the following possibilities may be considered.

The effect of gal-1-P could be indirect, through its adverse effect on phosphoglucomutase[5] and aldolase[15] resulting in the accumulation of glycolytic intermediates which might act as catabolite repressors.[16] Apart from this explanation, based on gal-1-P *per se*, one should consider the consequences of the generation of large

amounts of gal-1-P at the expense of ATP and the way this affects the levels of inorganic phosphate and especially of high energy phosphates and their dephosphorylated (partially or complete) products in the cell. Spyrides and Kalckar (unpublished observations), who looked into some of these questions, could not find any changes in ATP and ADP levels by the specific firefly assay method. However, the high energy phosphate pool includes mainly other nucleotides as well as intermediates like phosphoenol pyruvate and phosphoglycerate. Any increase in the levels of dephosphorylated products of the latter metabolites might result in the repression of glycerokinase.

Finally, it would be of interest to look into the effect of gal-1-P accumulation on the formation and/or activity of the permease system for glycerol.

Whatever the mechanism underlying the repressor effect of gal-1-P, it is obvious that the growth inhibitory effect of gal-1-P is more involved than hitherto assumed. The readiness with which glucose reverses stasis makes it probable that gal-1-P inhibits growth through the inhibition of formation and/or activity of essential enzymes involved in the utilization of a poor carbon source.

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The abbreviations used are: ADP = adenosine diphosphate; ATP = adenosine triphosphate; UDPG = uridine diphosphoglucose; G-1-P = glucose-1-phosphate; G-6-P = glucose-6-phosphate; Gal-1-P = α-galactose-1-phosphate; Gal-1,6-P = galactose-1,6-diphosphate; NAD = nicotinamide adenine dinucleotide; o-NPG = orthonitrophenyl galactoside; TCA = trichloroacetic acid; K^- = defective in galactokinase; T^- = defective in uridyl transferase.

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RELATIVISTIC SURFACE DYNAMICS OF AN ISOLATED WORLD TUBE OF PERFECT FLUID

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1. The dynamics of discontinuity surfaces in general relativity[1] has been used to examine the properties of the bounding surfaces of galaxies in the Hubble E-Series.[2, 3] In view of the fact that most previous relativistic galactic models have been based on the assumption that the momentum-energy tensor interior to a galaxy has a form characteristic of a perfect fluid, it seemed appropriate to examine the surface dynamics of an isolated world tube of perfect fluid. The results show that one obtains a unique representation of all limiting quantities through simple constructions in a subsidiary three-dimensional hyperbolic-normal metric space. It is also shown that the geodesic hypothesis can be invalidated only by internal dynamic processes. If the bounding surface is not known, a consistent procedure is given for the construction of the first and second fundamental forms and the boundary data such that a bounding surface is realizable in the underlying Einstein-Riemann space.

2. Let E be an Einstein-Riemann space whose metric structure is defined by the quadratic differential form

$$ds^2 = h_{AB}\, dx^A\, dx^B \ (A, B = 0, 1, 2, 3) \tag{2.1}$$

having signature -2. We denote by Σ a regular, timelike hypersurface in E which is defined by the parametric equations

$$x^A = f^A(u^0, u^1, u^2).$$

The normal vector to Σ, defined by

$$x_\alpha{}^A N_A = 0, \ (x^A{}_\alpha \overset{def}{=} \partial f^A(u)/\partial u^\alpha)$$

can be normalized by the requirement $N_A N^A = -1$. We shall also assume that any "time-slice" of Σ yields a closed two-surface. Let $a_{\alpha\beta}\, du^\alpha\, du^\beta$ denote the first fundamental form on Σ, then

$$a_{\alpha\beta} = \bar{h}_{AB} x_\alpha{}^A x_\beta{}^B$$

where the bar is used to denote evaluation on Σ. It is evident that the four vectors N^A, $x_\alpha{}^A$ for $\alpha = 0, 1, 2$ are linearly independent on Σ in E and it can be shown that

$$a^{\alpha\beta} x_\alpha{}^A x_\beta{}^B = \bar{h}^{AB} + N^A N^B; \tag{2.2}$$