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[25] The composition per liter volume is as follows: galactose, 10 gm.; casein digest, 8 gm.; yeast extract, 1 gm.; sodium chloride, 5 gm.; K_2HPO_4, 2 gm.; eosin Y, 0.4 gm.; methylene blue, 0.065 gm. and agar 15 gm.; 20 to 25 ml. was used for a Petri plate, according to Lederberg, J., *Methods in Medical Research* **3**, 52 (1950).

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[30] This enzyme was purified, crystallized, and kept in this form:[12] a small part of the crystalline suspension was dissolved in acetate buffer (0.15 *M*, pH 5.5) shortly before use. Such a solution remained active without the addition of glucose-1,6-diphosphate for about one week.

[31] Unpublished methods by K. Kurahashi and A. Sugimura.

[32] It is necessary to set up a zero time control for galactokinase activity and the value for Gal-1-P formed was corrected for the zero time control. This is especially important if liver transferase was utilized as indicator enzyme, since it is contaminated by small amounts of galactokinase.[13]

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*HEREDITARY DEFECTS IN GALACTOSE METABOLISM IN ESCHERICHIA COLI MUTANTS, II. GALACTOSE-INDUCED SENSITIVITY**

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The present study of hereditary galactose sensitivity in bacteria was prompted by the existence of such a pathological state in humans, so-called congenital galactosemia (more correctly, hereditary galactosemia). As is well known, the latter state manifests itself by disease symptoms if the subject receives galactose; later permanent tissue lesions ensue. It was shown [1,2] that the basis for abnormal sensitivity toward galactose is due to a defect in a single enzyme, galactose-1-phosphate

uridyl transferase.[3] The activities of galactokinase and 4-epimerase are within normal range.[4] The biochemical basis for the cellular lesions remains obscure although the accumulation of galactose-1-phosphate[5] is probably an essential feature. The molecular basis for the enzyme defect is totally unexplored.

Hereditary galactose sensitivity exists also in certain mutants of *Escherichia coli*. These strains are unable to metabolize galactose as a sole carbon source, i.e., they are "galactose negatives."

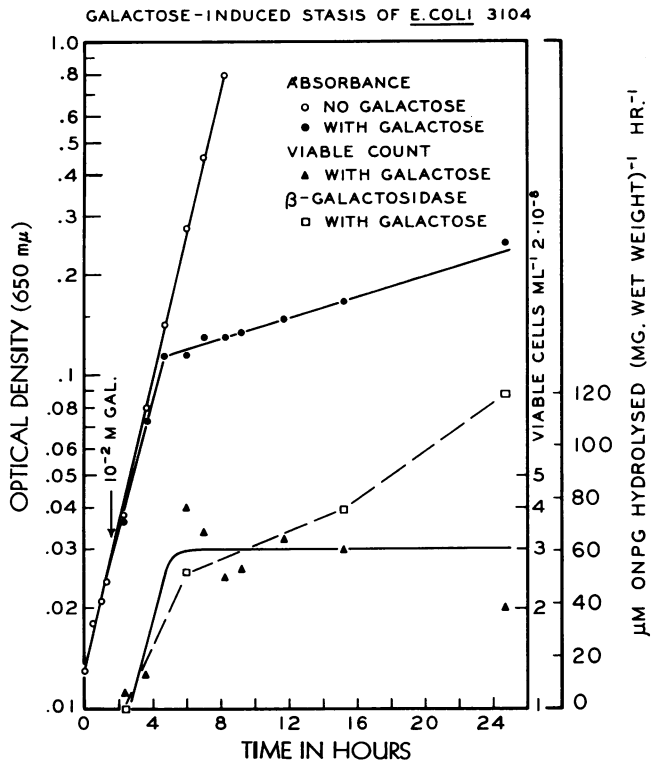

FIG. 1.—Galactose-induced stasis of *E. coli* W 3104. Cells grown in shake culture at 37° in medium A containing 1 per cent glycerol as carbon source. Galactose at a final concentration of 10^{-2} *M* added at the arrow. Viable cells calculated from median values of triplicate plate counts on Difco nutrient agar. β-galactosidase determined on washed toluenized cells by hydrolysis of the chromogenic substate *o*-nitrophenyl-β-D-galactoside.

Among the galactose negatives there are two types of galactose sensitivity: bacteriostasis and bacteriolysis. The sensitivity is undoubtedly dependent on the induced biosynthesis of galactose permease and galactokinase, galactose being the inducer.

Methods and Techniques.—Culture conditions and enzyme assays are described in the preceding article.[6]

Galactose-Induced Bacteriostasis.—A number of galactose-negative K-12 mutants have been shown to lack the enzyme galactose-1-phosphate uridyl transferase[7] and to be inhibited in their growth by the presence of galactose.[8] This inhibition is

very pronounced in a simple minimal medium, such as a mineral ammonia medium, using glycerol as a carbon source, upon the further addition of galactose. In strain 3096[9] grown in glycerol ammonia mineral medium (see previous article[6]), concentrations of galactose as low as 10^{-4} molar suffice to give a strong inhibition of growth.[10]

We want to report some microbiological observations of a galactose-sensitive K-12

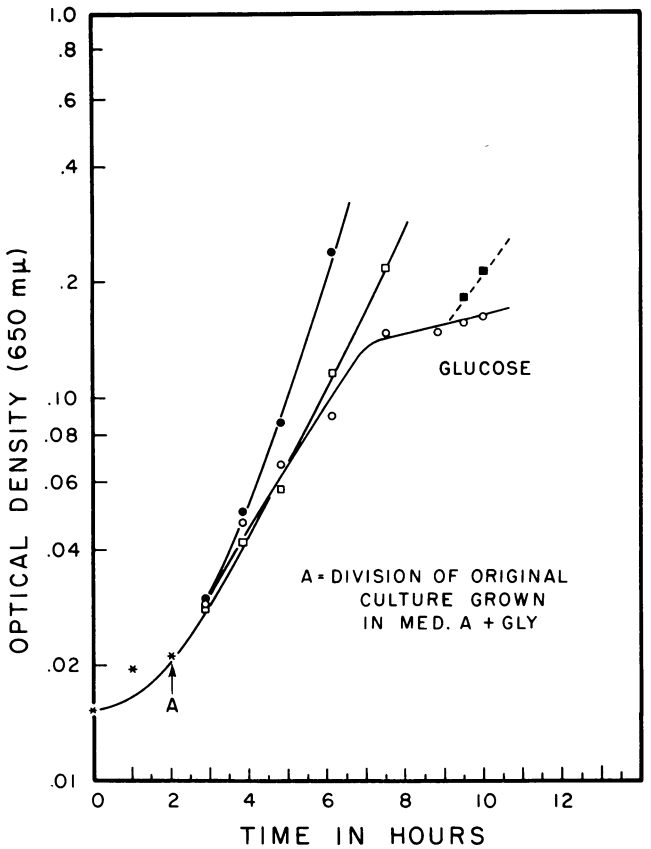

FIG. 2.—Effect of glucose on galactose induced stasis. A culture of W 3104 in medium A with glycerol as a carbon source was started (shake culture, 37°C). At point A the culture was divided into 3 parts. The first culture (●—●) received glucose (10^{-2} *M*); the second culture (□—□) was left unaltered; the third culture (○—○) received galactose (10^{-2} *M*). After 4 hr additional growth the third culture showed stasis. After 2 to 3 hr this culture was divided between two flasks. One flask was left unaltered; to the other flask (■—■) was added glucose (10^{-2} *M*).

strain, 3104. Addition of galactose to this strain brings about an abrupt growth inhibition after about two cell divisions (see Fig. 1).

The inhibition of growth is brought about specifically by galactose; related compounds such as glucose, mannose, and galactosamine or thio-galactosides (which are not hydrolyzed by β-galactosidase) are not inhibitory. The phenomenon is characteristic of the group of mutants blocked at, but not prior to, the transferase. The production of induced galactokinase is probably a prerequisite for growth in-

hibition by galactose in as much as single galactokinase-less as well as the doubly defective galactokinase-less and transferase-less strains do not show any pronounced growth inhibition after addition of galactose.[11]

The galactose inhibition of the transferase-less 3104 was studied in more detail. Viable counts taken at various times during galactose inhibition of growth (Fig. 1) reveal that galactose exerts a bacteriostatic rather than bacteriocidal action.

The apparent stasis might alternatively be explained by assuming that the population remains at all times heterogeneous with respect to its sensitivity to galactose,

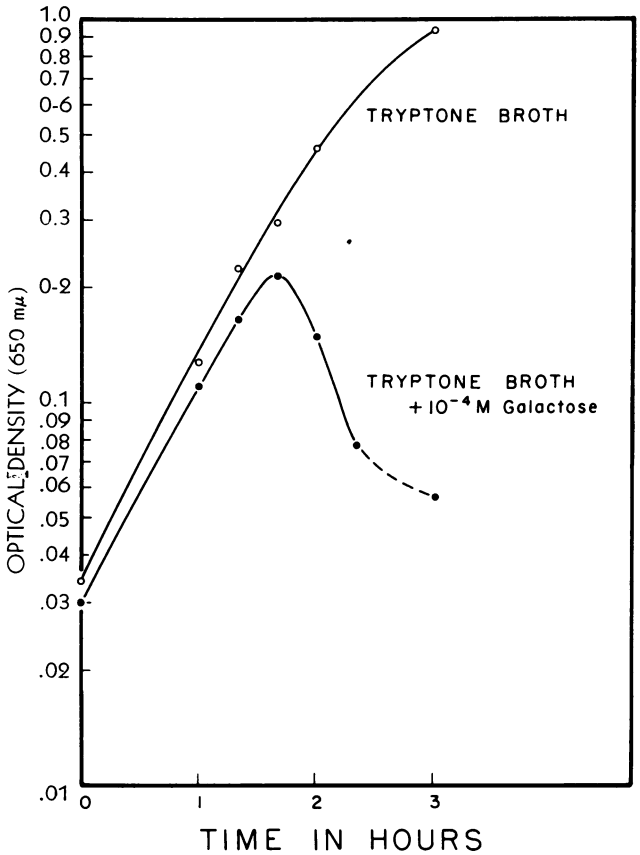

FIG. 3.—The effect of addition of galactose to a culture of *E. coli* strain C7M, growing in tryptone broth (without glucose). The galactose used was D (+) galactose, C. P., Pfanstiehl.

approximately equal proportions being unaffected and being killed, respectively. The following evidence speaks strongly against this hypothesis. The growth-death hypothesis predicts that after very few hours of "stasis" the majority of cells present should become non-viable. Since the absorbance of a culture in "stasis" does not increase rapidly, the non-viable cells must largely fail to contribute to the total turbidity, presumably by virtue of lysing. Lysed cells should release their content of soluble enzymes into the medium. Among these enzymes β-galactosidase, well induced at the onset of "stasis," should be readily detectable. In the experiment of

Figure 1, even after 20 hr of "stasis" the β-galactosidase activity present in the medium amounted to less than one-hundredth the amount found in the cells. Addition of β-galactosidase to the culture showed that the conditions of incubation did not lead to appreciable enzyme inactivation. The foregoing considerations justify the use of the term "galactose-induced bacteriostasis." It is interesting to note that the cellular levels of β-galactosidase increase during the period of stasis (Fig. 1). This is a regular phenomenon in these mutants.

As can be seen from Figure 2 the addition of glucose permits an almost immediate resumption of growth. Within half an hour the generation time goes down from 20

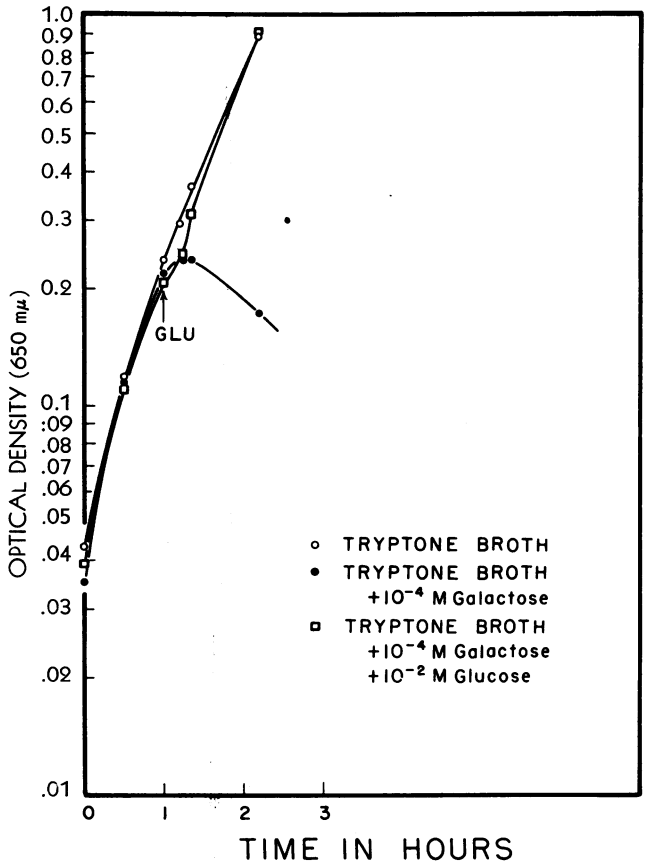

FIG. 4.—The prevention of galactose-induced lysis of *E. coli*, C7M, by glucose.

or 30 hr to about 2 hr. The growth remains logarithmic but fails to attain the rate expected on glucose plus glycerol.

Galactose-Induced Bacteriolysis.—Fukasawa and Nikaido have recently found[12, 13] that galactose causes lysis in a number of galactose-negative mutants of *E. coli* and *Salmonella enteriditis* derived from highly mutable strains (so-called "M" strains). If the cells are in hypertonic medium, spheroplast or protoplast formation ensues.

We have studied some properties of one of these *E. coli* M strains which is known as "C7M."[12–14] In this strain galactose in concentrations as low as 10^{-4} M

causes striking lysis even in tryptone broth (see Fig. 3, and ref. 12). As stated in previous communications,[6,13] the "lytic" galactose-negative strains have active galactokinase as well as galactose-1-phosphate uridyl transferase but no trace of 4-epimerase (cells collected and sonicated 1 hr after the addition of galactose but before lysis).

The presence of glucose in addition to galactose in the medium protects the cells against the effects of galactose.[12] It is possible to obtain protection by glucose if it is added 1 hour after the addition of galactose (Fig. 4). At that time, some of the enzymes of galactose-metabolism are induced; the degree of induction is not known.

Accumulation of galactose-1-phosphate takes place during induced stasis as well as prior to and at the onset of lysis.[13] Even in early stasis, levels of 2 to 5.5 μmoles of galactose-1-phosphate per gram cells (wet weight) have been encountered (Spyrides, Jordan, and Kalckar unpublished observations).

Variations in the levels of galactose-1-phosphate, uridine nucleotides and adenyl pyrophosphate compounds during normal, unbalanced, and restored growth are under current study.

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† Postdoctoral Fellow of the National Science Foundation.

‡ Predoctoral Fellow of National Institutes of Health.

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[9] It should be noted that the K-12 strains used are usually preceeded by a "W" *e.g.* W3104.

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[11] One can obtain a spontaneous galactose-negative mutant 3096′ as a galactose insensitive strain from the "galactose static" 3096 population. Strain 3096′ is a mutant because it remains insensitive even after passage through galactose-free (non-selective) media. This selected strain was found to be lacking in galactokinase as well as in transferase (cf. [6]).

In addition to these examples can be cited the following additional multiply defective strains which show no sign of galactose sensitivity under the conditions described above: (i) A galactose negative *E. coli* strain ML 32,400 defective in galactokinase and in transferase but having high epimerase; (ii) three triply defective galactose negatives, i.e., having abnormally low galactokinase, transferase and 4-epimerase.

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[13] *Ibid.*, **184**, 1168 (1959).

[14] Our thanks are due to Drs. Fukasawa and Nikaido for making the C7M strain available to us.