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Fructose-1,6-Diphosphatase and Acid Hexose Phosphatase of *Escherichia coli*[1]

D. G. FRAENKEL AND B. L. HORECKER
Department of Molecular Biology, Albert Einstein College of Medicine, New York, New York

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ABSTRACT

FRAENKEL, D. G. (Albert Einstein College of Medicine, New York, N.Y.), AND B. L. HORECKER. Fructose-1,6-diphosphatase and acid hexose phosphatase of *Escherichia coli*. J. Bacteriol. **90**:837-842. 1965.—The conversion of fructose-1,6-diphosphate to fructose-6-phosphate (fructose-1,6-diphosphatase activity) is essential for growth of *Escherichia coli* on glycerol, acetate, or succinate, but is unnecessary for growth on hexoses or pentoses. It has sometimes been assumed that fructose-1,6-diphosphatase activity is due to a nonspecific acid hexose phosphatase. We have now obtained a number of one-step mutants which have lost the ability to grow on glycerol, succinate, or acetate, but which grow normally on hexoses; these mutants are deficient in a fructose-1,6-diphosphatase which can be assayed spectrophotometrically in the presence of Mg^{++} and low concentrations of substrate. These mutants still possess the nonspecific acid hexose phosphatase, which does not require Mg^{++} and is active only at much higher concentrations of fructose-1,6-diphosphate. Evidence is presented to support the hypothesis that the newly described activity is the physiological fructose-1,6-diphosphatase. The acid hexose phosphatase is a different enzyme whose function remains unknown.

Fructose-1,6-diphosphatase catalyzes the dephosphorylation of fructose-1,6-diphosphate to yield fructose-6-phosphate and inorganic phosphate (Gomori, 1943). The formation of fructose-1,6-diphosphate from fructose-6-phosphate requires adenosine triphosphate (ATP) and is catalyzed by phosphofructokinase (Ostern, Guthke, and Terszakowec, 1936; Krebs and Kornberg, 1957). The relative rate of these two reactions determines whether carbohydrate metabolism is glycolytic or gluconeogenic, and there has been much work on the physiological mechanism of control of these enzyme activities (Mansour, 1963; Passonneau and Lowry, 1962; McGilvery and Pogell, 1964).

Fructose-1,6-diphosphatases have been purified from spinach leaves (Racker and Schroeder, 1958), rabbit liver (Pogell, 1962; Pontremoli et al., *in press*), rat liver (Bonsignore et al., 1963), pig kidney (Mendicino and Vasarhely, 1963), *Euglena gracilis* (Smillie, 1964; App and Jagendorf, 1964), and *Pseudomonas saccharophila* (Fossitt and Bernstein, 1963). The purified enzymes have been found to be highly specific for fructose-1,6-diphosphate (although the enzyme from mammalian liver also splits sedoheptulose-1,7-diphosphate), to have *p*H optima above 7 and K_m values of 10^{-4} M or lower, and, in general, to require magnesium or manganese ion.

In prototrophic bacteria like *Escherichia coli*, fructose-1,6-diphosphatase would presumably be essential for cells during growth on carbon sources such as succinate, acetate, or glycerol, since the formation of hexose monophosphate, which is necessary for the biosynthesis of pentose, aromatic amino acids, and polysaccharide, would require the hydrolysis of fructose diphosphate. Fructose-1,6-diphosphatase should not be necessary for growth on hexoses or pentoses. With this hypothesis in mind, Gotto and Pogell (1962) examined the ability of sonic extracts of *E. coli* B to dephosphorylate fructose diphosphate and found that the rate of hydrolysis was 10-fold greater in acetate-grown cells than in glucose-grown cells. For optimal activity this "fructose-1,6-diphosphatase" required 10^{-2} M fructose-1,6-diphosphate and a *p*H of 5. The activity was not specific for fructose-1,6-diphosphate; hexose monophosphates were split at about the same rate, and they were unable to account for the apparent lack of specificity of the induced enzyme activity.

The activity described by Gotto and Pogell (1962) differed from the specific fructose-1,6-diphosphatases of other sources and resembled more the "acid phosphatase" of *E. coli*, as de-

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scribed by Rogers and Reithel (1960) and by Neu and Heppel (1964). The latter authors found the "acid phosphatase" to be one of the enzymes selectively removed from whole cells by successive treatment with sucrose-ethylenediaminetetraacetate (EDTA) and cold water. Other enzymes removed by this treatment included alkaline phosphatase, cyclic phosphodiesterase, 5′-nucleotidase, a ribonuclease, and a deoxyribonuclease. These enzymes are possibly involved in the provision of inorganic phosphate and the breakdown of exogenous nucleic acids; none is known to have a role in normal intermediary metabolism.

We have now obtained evidence for a true fructose-1,6-diphosphatase in *E. coli* K-12 and have isolated mutants lacking this enzyme. These mutants are unable to grow on acetate, glycerol, or succinate, but grow on hexoses and pentoses. They still possess the activity described by Gotto and Pogell (1962), which we will call the acid hexose phosphatase. The fructose-1,6-diphosphatase is not removed from the cells by the sucrose-EDTA and water treatment of Neu and Heppel (1964), which does extract the acid hexose phosphatase.

MATERIALS AND METHODS

Acid hexose phosphatase was assayed by essentially the same procedure employed by Gotto and Pogell (1962) for "fructose-1,6-diphosphatase" and by Neu and Heppel (1964) for "acid phosphatase." The reaction mixture (0.25 ml) contained 0.12 M sodium acetate buffer (*p*H 5), 0.008 M substrate (sodium fructose diphosphate or sodium glucose-6-phosphate, both from the Sigma Chemical Co., St. Louis, Mo.), and *E. coli* extract. The mixture was incubated at 37 C for 20 to 60 min, and the reaction was stopped by the addition of 0.25 ml of 0.5 N trichloroacetic acid; the precipitates were removed by centrifugation, and inorganic phosphate in the supernatant solution was measured by the method of Fiske and SubbaRow (1952). Fructose-1,6-diphosphatase was measured spectrophotometrically. (These conditions were employed by O. Rosen of this laboratory.) The reaction mixture (1.0 ml) contained 0.045 M tris(hydroxymethyl)aminomethane (Tris)-HCl buffer (*p*H 7.8); 2×10^{-4} M nicotinamide adenine dinucleotide phosphate (NADP), 10^{-2} M $MgCl_2$, 5×10^{-4} M EDTA, 1 μg of glucose-6-phosphate dehydrogenase (C. F. Boehringer & Soehne GmbH., Mannheim-Waldhof, Germany), 1 μg of phosphohexose isomerase (C. F. Boehringer & Soehne), and 1.2×10^{-4} M sodium fructose diphosphate. The reduction of NADP at room temperature was followed in a Gilford recording spectrophotometer at 340 mμ. There was generally an increase in rate during the first few minutes as hexose-monophosphate accumulated. The values reported were calculated from the maximal rates, usually attained at about 12 min. β-Galactosidase was measured by following the hydrolysis of *o*-nitrophenylgalactoside (ONPG; Lederberg, 1950). The cuvettes contained 0.022 M potassium phosphate (*p*H 7.6), 5×10^{-4} M ONPG, and extract. The change in absorbance at 420 mμ was followed, and the rates were calculated by use of the molar absorbancy coefficient for *o*-nitrophenol of 3,120.

The primary strain used in these experiments was *E. coli* Hfr 3,300 (obtained from J. Monod, Institut Pasteur, Paris, France), a K-12 strain constitutive for β-galactosidase, but otherwise possessing the wild-type phenotype (Fraenkel, Falcoz-Kelly, and Horecker, 1964). Cells were grown in minimal medium buffered with 0.1 M phosphate (Fraenkel and Horecker, 1964), and supplemented with 4 mg/ml of the appropriate carbon source and with 1 μg/ml of thiamine.

Extracts were prepared from cells washed once with 0.9% NaCl by grinding frozen pellets with twice their weight of alumina (Special Alumina A-305, Alcoa Aluminum Co. of America), and extracting the paste with 0.01 M Tris-HCl buffer (*p*H 8.0). The mixtures were centrifuged at 20,000 × *g* for 30 min, and the residue was discarded. Protein was measured by the method of Lowry et al. (1951).

Selection of fructose-1,6-diphosphatase-less mutants. An overnight culture of *E. coli* Hfr 3,300 was diluted to 2×10^8 cells per milliliter in minimal medium containing 0.9 mg/ml of the mutagen *N*-methyl-nitroso-*N*-nitroguanidine (Mandell and Greenberg, 1960; obtained from K & K Laboratories, Plainfield, N.Y.), and incubated for 30 min at 37 C. This treatment reduced the number of colonies formed on nutrient agar to 10^5 cells per milliliter. The treated cells were washed in 0.9% saline and allowed to grow out in minimal medium containing glucose as sole carbon source. Full turbidity was reached in 40 hr. The cells were plated onto minimal glucose-agar plates, and the resulting colonies were replicated directly to succinate-agar, glycerol-agar, and glucose-agar. Of 526 colonies which grew on glucose, 12 failed to grow on glycerol but grew on succinate, 2 grew on glycerol, but failed to grow on succinate, and 3 failed to grow on either glycerol or succinate (Q11 is one of the last type). In another experiment, a penicillin selection step (Gorini and Kaufmann, 1960) was included before the first plating on glucose agar. The survivors of the nitrosoguanidine treatment were subcultured for 4 hr in a medium containing glycerol, succinate, and acetate, treated with 2,000 units per ml of penicillin, and 2 hr later plated on glucose medium. In this experiment, of 66 survivors, 2 were succinate-negative, 1 was glycerol-negative, and 16 (including Q17) were negative for both succinate and glycerol.

RESULTS

Growth of mutants. The mutants selected for the simultaneous loss of ability to grow on

glycerol or succinate had the growth pattern expected of fructose-1,6-diphosphatase-less cells. For example, when tested for growth on solid or in liquid media, Q11 grew, as did the parent strain, on glucose, fructose, galactose, gluconate, and xylose, but failed to grow on glycerol, succinate, pyruvate, or acetate.

Enzyme levels in wild-type and mutant strains. After growth on glucose, strains 3,300, Q11, and Q17 contained similar activities of acid hexose phosphatase, but both Q11 and Q17 were deficient in fructose-1,6-diphosphatase (Table 1). The low activities in the mutant extracts were at the limit of sensitivity of the fructose-1,6-diphosphatase assay and are of doubtful significance. Fructose-1,6-diphosphatase in the parent strain 3,300 was dependent on magnesium ion; the effect of EDTA varied from one extract to another, but generally it stimulated about twofold. Thus, for example, with 350 μg of an extract of succinate-grown cells, the rates (millimicromoles per minute per milligram of protein) were: complete system, 4.1; minus EDTA, 2.2; minus magnesium chloride, 0.3; and minus substrate, 0.2.

The mutant strains Q11 and Q17, like their parent strain, were constitutive for β-galactosidase (Table 1).

Enzyme levels in various growth conditions. In agreement with the results of Gotto and Pogell (1962), we found that the level of acid hexose phosphatase depended on the carbon source used for growth. Activity was high in succinate-grown cells and low in glucose-grown cells (Table 2). The level of fructose-1,6-diphosphatase, in contrast, did not vary significantly. The level of β-galactosidase in the constitutive strain 3,300 also depended on the carbon source, as was first shown by Brown (1961). The mutants could not be grown on succinate, but, in minimal medium containing 1% nutrient broth as carbon source, some limited growth was achieved (probably due to pentose and hexose in the nutrient broth); the cells grown under these conditions contained high levels of acid hexose phosphatase, but still lacked fructose-1,6-diphosphatase (Table 2).

Separation of acid hexose phosphatase from fructose-1,6-diphosphatase. A separation of the two activities was accomplished by the sucrose-EDTA and cold water treatment of Neu and Heppel (1964; Table 3). All the acid hexose phosphatase activity was found in the water supernatant fraction after cold-water treatment, whereas the fructose-1,6-diphosphatase remained in the cells.

Mapping of fructose-1,6-diphosphatase. Yu, Kaney, and Atwood (1965) employed a different procedure to isolate a series of mutants of *E. coli* K-12 which possess the same growth pattern as

TABLE 1. *Enzyme levels in parent and mutant strains**

Strain	Acid hexose phosphatase		Fructose-1,6-diphosphatase	β-Galactosidase
	FDP	G6P		
3,300	72	60	10.6	3,180
Q11	55	32	0.15	2,360
Q17	43	30	0.32	1,770

* The strains were grown on glucose, and extracts were prepared by grinding with alumina. Acid hexose phosphatase was tested with fructose-1,6-diphosphate (FDP) or glucose-6-phosphate (G6P). Results are expressed as millimicromoles per minute per milligram of protein.

TABLE 2. *Enzyme levels in various growth conditions**

Strain	Carbon source and growth phase	Acid hexose phosphatase		Fructose-1,6-diphosphatase	β-Galactosidase
		FDP	G6P		
Wild type (3,300)	Glucose				
	Logarithmic	39	39	4.5	2,560
	Stationary	72	60	10.6	3,180
	Succinate				
	Logarithmic	218	133	5.2	10,000
	Stationary	340	283	4.0	8,000
Mutant (361)†	Glucose				
	Stationary	7	7	0.0	0
	Nutrient broth				
	Stationary	206	178	0.5	—

* Results are expressed as millimicromoles per minute per milligram of protein. FDP = fructose-1,6-diphosphate; G6P = glucose-6-phosphate.

† Strain 361 is a fructose-1,6-diphosphatase-less strain of *Escherichia coli* K-12 isolated by Yu et al. (1965).

TABLE 3. *Separation of acid hexose phosphatase and fructose-1,6-diphosphatase**

Fraction	Enzyme		
	Fructose-1,6-diphosphatase	Acid hexose phosphatase	
		FDP	G6P
EDTA-sucrose supernatant solution	1	26	20
Water	0	1,150	750
Remaining in cells	47†	28	14

* Strain 3,300 was grown in minimal medium containing 1% nutrient broth. The method of fractionation was that of Neu and Heppel (1964). The cells were washed once with 0.01 N Tris-HCl (*p*H 7.8). The pellet, 1.2 g (wet weight), was suspended in 45 ml of 0.03 M Tris (*p*H 8.0), contining 5×10^{-4} M EDTA and 20% sucrose, and was stirred at room temperature for 15 min; the cells were collected by centrifugation. They were then suspended in 50 ml of ice water, and after 10 min the suspension was centrifuged. The cell pellet was frozen and extracted by alumina grinding, as usual. Results are expressed as micromoles per hour per gram (wet weight) of cells. FDP = fructose-1,6-diphosphate; G6P = glucose-6-phosphate.

† The *specific* activity of the fructose-1,6-diphosphatase in the "remaining in cells" fraction was 12 mμmoles per min per mg of protein.

our fructose-1,6-diphosphatase-less mutants, and we have shown that they also lack fructose-1,6-diphosphatase activity (see Table 2). Yu et al. (1965) mapped the fructose-1,6-diphosphatase locus by interrupted conjugation experiments with several different donors and found it to map at minute 84 according to the map of Taylor and Thoman (1964). It is interesting that the only other enzyme of intermediary carbohydrate metabolism whose gene has been mapped, the phosphoglucose isomerase of *Salmonella typhimurium* (Fraenkel et al., 1963), is close to the methionine A locus, but not cotransducible with it. The methionine A locus in *E. coli* is at minute 80. Thus, it is possible that the genes governing these two adjacent enzymes of intermediary metabolism are linked closely.

DISCUSSION

Rogers and Reithel (1960) purified two acid phosphatases of *E. coli* ML 308 which differed in their catalytic activity; phosphatase I was a nucleoside 2′- or 3′-phosphatase, and phosphatase II hydrolyzed hexose phosphates. The latter activity was separated by hydroxyapatite chromatography into several fractions differing in their *p*H optima and in their relative activities with different hexose phosphate substrates; these were thought to be multiple forms of one enzyme. Neu and Heppel (1964) separated three distinct acid phosphatases from *E. coli* K-12: (i) a cyclic nucleotidase (Anraku, 1964*a*, *b*), which may be identical with phosphatase I of Rogers and Reithel (1960); (ii) a 5′-nucleotidase; and (iii) an "acid phosphatase," which splits phosphate from hexose-6-phosphates and hexose-α-1-phosphates (L. A. Heppel, *personal communication*). Hofsten and Porath (1961) also fractionated acid phosphatases from *E. coli*.

We have shown that the fructose-1,6-diphosphatase of *E. coli* is a different enzyme from that described by Gotto and Pogell (1962). This conclusion is based on the criteria of differential enzyme assays, uncoordinated regulation, physical separation, and mutant analysis. The enzyme described by Gotto and Pogell (1962) in *E. coli* B has the properties of a nonspecific acid hexose phosphatase, possibly identical with the "acid phosphatase II" of Rogers and Reithel (1960) from *E. coli* ML 308, and the "acid phosphatase" of Neu and Heppel (1964) from *E. coli* K-12. The possible multiple nature of "acid phosphatase" remains to be clarified, but it is likely that there exists an acid hexose phosphatase which catalyzes the dephosphorylation of several hexose phosphates. We have confirmed the observation of Gotto and Pogell (1962) that the level of the acid hexose phosphatase varied with the growth medium. It is of interest that Torriani (1960) reported that "acid phosphatase," in contrast to alkaline phosphatase, was relatively insensitive to the phosphate concentration of the medium. Hofsten (1961) later showed that this activity was high in succinate- or glycerol-grown cells and apparently repressed by glucose. These authors all employed *p*-nitrophenylphosphate as substrate, and they may have been assaying several enzymes simultaneously. Nevertheless, it appears that the level of acid hexose phosphatase is relatively sensitive to the nature of the carbon source for growth and, therefore, subject to catabolite repression (Magasanik, 1961).

The function of the acid hexose phosphatase remains unclear. It cannot replace fructose-1,6-diphosphatase, since mutants lacking fructose-1,6-diphosphatase are evidently unable to form hexose monophosphate from fructose-1,6-diphosphate, despite the presence of substantial levels of acid hexose phosphatase activity. The acid hexose phosphatase may be responsible for the growth on glucose-1-phosphate at *p*H 5 in a medium free from inorganic phosphate (Hofsten, 1961). It is of interest that Roberts and Wolffe (1951) showed that glucose-1-phosphate could

enter *E. coli* without hydrolysis (*see also* Fraenkel et al., 1964). Klungsöyr and Endressen (1964) recently implicated the acid hexose phosphatase in the utilization of fructose diphosphate by resting cells of *E. coli*, but Roberts and Wolffe (1951) found that *E. coli* does not grow in a high phosphate medium on fructose diphosphate as sole carbon source, and we have confirmed this finding. Thus, there is no clear example of a phosphorylated compound whose utilization requires the acid hexose phosphatase.

Englesberg, Watson, and Hoffee (1961) found that mutants of *S. typhimurium* lacking the glucose effect possessed elevated acid hexose phosphatase levels. Hofsten (1961) suggested that this might be a consequence of impaired glucose metabolism rather than a cause of it. Neidhardt (*personal communication*) has come to the same conclusion regarding a strain of *Aerobacter aerogenes* which lacks the glucose effect (Neidhardt, 1960), is missing a pathway of glucose oxidation (Magasanik and Boyarski, 1960), and shows elevated acid hexose phosphatase levels. Some interesting suggestions for the function of the acid hexose phosphatase include a role in the glucose permease system (Englesberg et al., 1961), and a role in regulating the internal inorganic phosphate level (Englesberg et al., 1961; H. Rickenberg, *personal communication*). Hofsten (1963) has isolated mutants of *E. coli* unable to grow on succinate but capable of growth on glucose. None of these showed altered levels of the acid hexose phosphatase. One strain (E 15/2) was able to grow on hexose or pentose, but failed to grow on succinate, glycerol, or acetate. The metabolic lesion was not established. We would suggest that this mutant may be deficient in fructose-1,6-diphosphatase.

The apparent constitutive nature of the fructose-1,6-diphosphatase of *E. coli* K-12 was surprising in view of the fact that this activity might be expected to be detrimental to cells growing on hexose. It is likely that this fructose-1,6-diphosphatase, like others, is subject to some kind of metabolic control in the cell. To study this possibility, we are now engaged in purifying the enzyme from *E. coli*.

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LITERATURE CITED

ANRAKU, Y. 1964*a*. A new cyclic phosphodiesterase having 3′-nucleotidase activity from *Escherichia coli* B. I. Purification and some properties of the enzyme. J. Biol. Chem. **239**:3412–3419.

ANRAKU, Y. 1964*b*. A new cyclic phosphodiesterase having 3′-nucleotidase activity from *Escherichia coli* B. II. Further studies on substrate specificity and mode of action of the enzyme. J. Biol. Chem. **239**:3420–3424.

APP, A. A., AND A. T. JAGENDORF. 1964. Purification of alkaline fructose diphosphatase from *Euglena gracilis*. Biochim. Biophys. Acta **85**:427–434.

BONSIGNORE, A., G. MANGIAROTTI, M. A. MANGIAROTTI, A. DE FLORA, AND S. PONTREMOLI. 1963. Cleavage of sedoheptulose 1,7-diphosphate by a purified rat liver diphosphatase. J. Biol. Chem. **238**:3151–3154.

BROWN, D. D. 1961. Discussion. Cold Spring Harbor Symp. Quant. Biol. **26**:254–255.

ENGLESBERG, E., J. A. WATSON, AND P. A. HOFFEE. 1961. The glucose effect and the relationship between glucose permease, acid phosphatase, and glucose resistance. Cold Spring Harbor Symp. Quant. Biol. **26**:261–276.

FISKE, C. H., AND Y. SUBBAROW. 1952. The colorimetric determination of phosphorus. J. Biol. Chem. **66**:375–400.

FOSSITT, D. D., AND I. A. BERNSTEIN. 1963. Fructose-1,6-diphosphatase from *Pseudomonas saccharophila*. J. Bacteriol. **86**:598–599.

FRAENKEL, D. G., F. FALCOZ-KELLY, AND B. L. HORECKER. 1964. Utilization of glucose 6-phosphate by glucokinaseless and wild type strains of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **52**:1207–1213.

FRAENKEL, D. G., AND B. L. HORECKER. 1964. Pathways of D-glucose metabolism in *Salmonella typhimurium*. A study of a mutant lacking phosphoglucose isomerase. J. Biol. Chem. **239**:2765–2771.

FRAENKEL, D. G., M. J. OSBORN, B. L. HORECKER, AND S. M. SMITH. 1963. Metabolism and cell wall structure of a mutant of *Salmonella typhimurium* deficient in phosphoglucose isomerase. Biochem. Biophys. Res. Commun. **11**:423–428.

GOMORI, G. 1943. Hexose diphosphatase. J. Biol. Chem. **148**:139–149.

GORINI, L., AND H. KAUFMANN. 1960. Selecting bacterial mutants by the penicillin method. Science **3**:604–605.

GOTTO, A. M., AND B. M. POGELL. 1962. Induction of fructose 1,6-diphosphatase activity in *Escherichia coli*. Biochem. Biophys. Res. Commun. **9**:381–387.

HOFSTEN, B. v. 1961. Acid phosphatase and the growth of *Escherichia coli*. Biochim. Biophys. Acta **48**:171–181.

HOFSTEN, B. v. 1963. Mutations affecting the glyconeogenesis of *Escherichia coli*. Biochim. Biophys. Acta **76**:140–142.

HOFSTEN, B. v., AND J. PORATH. 1961. Acid phosphatases in *Escherichia coli*. Acta Chem. Scand. **15**:1791–1793.

KLUNGSÖYR, L., AND A. ENDRESSEN. 1964. Intra-

cellular pH effect on phosphoglucose isomerase in *Escherichia coli*. Biochim. Biophys. Acta **92**:378–387.

KREBS, H. A., AND H. L. KORNBERG. 1957. Energy transformations in living matter. Springer-Verlag, Berlin.

LEDERBERG, J. 1950. The beta-D-galactosidase of Escherichia coli, strain K-12. J. Bacteriol. **60**: 381–392.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.

MCGILVERY, R. W., AND B. M. POGELL [ed]. 1964. Fructose 1,6-diphosphatase and its role in gluconeogenesis. American Institute of Biological Sciences, Washington, D.C.

MAGASANIK, B. 1961. Catabolite repression. Cold Spring Harbor Symp. Quant. Biol. **26**:249–254.

MAGASANIK, A. K., AND A. BOJARSKA. 1960. Enzyme induction and repression by glucose in *Aerobacter aerogenes*. Biochem. Biophys. Res. Commun. **2**:77–81.

MANDELL, J. D., AND J. GREENBERG. 1960. New chemical mutagen for bacteria, 1-methyl-3-nitro-1-nitrosoguanidine. Biochem. Biophys. Res. Commun. **3**:575–577.

MANSOUR, T. E. 1963. Studies on heart phosphofructokinase: purification, inhibition, and activation. J. Biol. Chem. **238**:2285–2292.

MENDICINO, J., AND F. VASARHELY. 1963. Renal D-fructose 1,6-diphosphatase. J. Biol. Chem. **238**:3528–3534.

NEIDHARDT, F. C. 1960. Mutant of *Aerobacter aerogenes* lacking glucose repression. J. Bacteriol. **80**:536–543.

NEU, H. C., AND L. A. HEPPEL. 1964. On the surface localization of enzymes in *Escherichia coli*. Biochem. Biophys. Res. Commun. **17**:215–219.

OSTERN, P. J., A. GUTHKE, AND J. TERSZAKOWEC. 1936. Uber die bildung des hexose-monophosphorsaure-esters und dessen umwandling in fructosediphosphorsaure-ester im muskel. Z. Physiol. Chem. **243**:9–37.

PASSONNEAU, J. V., AND O. H. LOWRY. 1962. Phosphofructokinase and the Pasteur effect. Biochem. Biophys. Res. Commun. **7**:10–15.

POGELL, B. M. 1962. Enzyme purification by selective elution with substrate from substituted cellulose columns. Biochem. Biophys. Res. Commun. **7**:225–230.

RACKER, E., AND E. A. R. SCHROEDER. 1958. The reductive pentose phosphate cycle. II. Specific C-1 phosphatases for fructose 1,6-diphosphate and sedoheptulose 1,7-diphosphate. Arch. Biochem. Biophys. **74**:326–344.

ROBERTS, I. Z., AND E. L. WOLFFE. 1951. Utilization of labeled fructose 6-phosphate and fructose 1,6-diphosphate by *Escherichia coli*. Arch. Biochem. Biophys. **33**:165–166.

ROGERS, D., AND F. J. REITHEL. 1960. Acid phosphatases of *Escherichia coli*. Arch. Biochem. Biophys. **89**:97–104.

SMILLIE, R. M. 1964. Plant fructose-1,6-diphosphatases, p. 31–41. *In* R. W. McGilvery and B. M. Pogell [ed.], Fructose 1,6-diphosphatase and its role in gluconeogenesis. American Institute of Biological Sciences, Washington, D.C.

TAYLOR, A. L., AND M. S. THOMAN. 1964. The genetic map of *Escherichia coli* K12. Genetics **50**:659–677.

TORRIANI, A. 1960. Influence of inorganic phosphate in the formation of phosphatases by *Escherichia coli*. Biochem. Biophys. Acta **38**: 460–479.

YU, M. T., A. R. KANEY, AND K. C. ATWOOD. 1965. Genetic mapping of fructose-1,6-diphosphatase mutants in *Escherichia coli*. J. Bacteriol. **90**:1150–1152.