TRYPTOPHANASE-TRYPTOPHAN SYNTHETASE SYSTEMS IN *ESCHERICHIA COLI*

II. Effect of Glucose

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ABSTRACT

Freundlich, Martin (University of Minnesota, Minneapolis) and Herman C. Lichstein. Tryptophanase-tryptophan synthetase systems in *Escherichia coli*. II. Effect of glucose. J. Bacteriol. **84**:988–995. 1962.—The effect of glucose and other compounds on the formation of tryptophanase and tryptophan synthetase in *Escherichia coli* was examined. Although most of these compounds were potent inhibitors of the synthesis of tryptophanase, they invariably increased the formation of tryptophan synthetase. The severity of tryptophanase inhibition depended upon the degree of utilization of the compound by the growing bacterial cells. It was found that high levels of tryptophan overcame by 40% the repression caused by glucose. The stimulatory effect of glucose on tryptophan synthetase formation in *E. coli* 9723E could be duplicated by indole-3-propionic acid. A study of the amino acid pool of *E. coli* 9723E revealed no free tryptophan in cells harvested from the basal medium containing glucose. In contrast, cells grown in the absence of glucose possessed a measurable amount of this amino acid. The possible mechanisms of the effect of glucose and related compounds on tryptophanase and tryptophan synthetase formation, as well as the relationship of these effects to the metabolic control of tryptophan metabolism, are discussed.

The well-known phenomenon of glucose inhibition of the synthesis of numerous inducible enzymes in bacteria (Gale, 1943) has been reexamined recently in light of current theories of metabolic control (Magasanik, 1957). It has been suggested that intermediary compounds formed from glucose dissimilation act to prevent the formation of enzymes whose products are the same as those metabolites produced from glucose (Magasanik, 1961). Thus, the glucose effect is thought to be a manifestation of the general pattern of physiological control by repression (Magasanik, 1961), where a metabolic product inhibits the synthesis of an enzyme involved in its formation (Pardee, 1959).

The enzyme tryptophanase, which degrades tryptophan to indole, pyruvic acid, and ammonia, is repressed by glucose (Happold and Hoyle, 1936). Initial studies on the glucose inhibition of tryptophanase (Freundlich and Lichstein, 1960) have shown that glucose does not inhibit the permeation of the inducer, tryptophan, into the cell. The present paper reports the results of further investigations of glucose repression of tryptophanase, as well as an examination of the effect of this carbohydrate and related compounds on the formation of tryptophan synthetase (indole + serine → tryptophan), the final enzyme in the biosynthesis of tryptophan.

MATERIALS AND METHODS

Organisms. The strains of *Escherichia coli* largely employed in these studies were Crookes (ATCC 8739) and ATCC 9723E.

Culture. The basal medium and other details of cultivation have been described previously (Freundlich and Lichstein, 1960, 1962). For maximal tryptophan synthetase activity, the cells were grown in the presence of 10^{-1} M glucose; cells harvested for the assay of tryptophanase were grown in basal medium plus 2.5×10^{-3} M DL-tryptophan.

Chemicals. The chemicals used were obtained commercially.

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Tryptophanase and tryptophan synthetase assays. The procedures for these assays were described previously (Freundlich and Lichstein, 1960, 1962). Whole cells were employed routinely for the assay of both systems.

RESULTS

Effect of glucose on the formation of tryptophanase and tryptophan synthetase. Although the inhibitory effect of glucose on tryptophanase formation is well known, only preliminary data have been reported on its effect on tryptophan synthetase (Freundlich and Lichstein, 1961). Titration of the carbohydrate revealed that all levels tested produced cells of *E. coli* 9723E with increased synthetase activity (Table 1). At high glucose levels, the enzyme was increased sixfold. Moreover, a close relationship was found between the initial level of the sugar in the growth medium and the degree of inhibition of tryptophanase and stimulation of synthetase.

Since strain variations are common among bacteria, the effect of glucose on the formation of tryptophan synthetase in several strains was inspected. All the organisms examined produced increased levels of the enzyme when grown in the presence of 10^{-1} M glucose (Table 2). Stimulation ranged from ninefold, in the case of *E. coli* (Tennessee), to about 20% in *E. coli* (Crookes). The one strain of *Aerobacter aerogenes* investigated produced seven times the level of synthetase when grown with glucose.

TABLE 1. *Effect of glucose on tryptophanase and tryptophan synthetase in Escherichia coli 9723E*

Glucose in growth medium	Tryptophanase activity[a]	Tryptophan synthetase activity[b]
M		
0	42.2	12.0
1×10^{-1}	0.2	76.4
1×10^{-2}	0.2	68.8
4×10^{-3}	20.4	34.8
2×10^{-3}	24.6	27.2
5×10^{-4}	40.8	14.0

[a] Expressed as μg of indole produced in 60 min by 0.025 mg of bacterial cell nitrogen/ml; age of cells, 14 hr.

[b] Expressed as μg of indole removed in 40 min by 0.11 mg of bacterial cell nitrogen/ml; age of cells, 20 hr.

TABLE 2. *Effect of glucose on tryptophan synthetase formation in several strains of bacteria*

Strain	Tryptophan synthetase activity[a]	
	Basal medium	Basal medium + glucose (10^{-1} M)
Escherichia coli Tennessee	3.6	32.4
E. coli Mutabilis (ATCC 9980)	19.2	52.8
E. coli Texas (ATCC 10586)	8.0	30.0
E. coli ATCC 9723E	16.0	78.4
E. coli Crookes (ATCC 8739)	22.8	27.6
Aerobacter aerogenes Tennessee	2.4	17.6

[a] Expressed as μg of indole removed in 90 min by 0.056 mg of bacterial cell nitrogen/ml; age of cells, 18 hr.

Effect of a variety of carbohydrates and products of carbohydrate dissimilation on tryptophanase and tryptophan synthetase. Although glucose normally exhibits the most marked inhibition of tryptophanase, other compounds have been reported to be capable of repressing the enzyme (Freundlich and Lichstein, 1960). The present studies made known that all of the carbohydrates and products of carbohydrate dissimilation that inhibited tryptophanase stimulated the formation of tryptophan synthetase (Table 3). Moreover, many of these compounds were as effective as glucose. It is pertinent that there appeared to be a quantitative relationship between the ability of most of these compounds to repress tryptophanase and to increase synthetase. For example, melibiose or trehalose inhibited tryptophanase about 10-fold and increased tryptophan synthetase 5-fold, while glucosamine repressed tryptophanase 3.5-fold and stimulated synthetase 2-fold.

Effect of glucose and pyruvate on the formation of tryptophan synthetase during growth. Tryptophan synthetase formation during growth was examined to determine whether the stimulatory effect of carbohydrates was exerted during a particular phase of growth only. *E. coli* 9723E was grown under shaker conditions in basal medium with and without the addition of 10^{-1} M glucose or pyruvate. Culture samples were re-

TABLE 3. *Effect of carbohydrates and products of carbohydrate dissimilation on tryptophanase and tryptophan synthetase formation in Escherichia coli 9723E*

Compound (10^{-1} M)	Tryptophanase activity[a]	Synthetase activity[b]
None	21.6	3.8
Glucose	0.8	16.8
Fructose	0.8	16.8
Lactose	1.2	17.2
Maltose	1.4	15.8
Ribose	4.4	16.8
Xylose	1.4	7.8
Trehalose	2.4	19.4
Melibiose	1.6	19.6
Mannitol	1.0	16.4
Sorbitol	3.0	15.0
Glucosamine	6.6	7.6
Pyruvate	7.8	17.6
Ethanol	17.6	4.2
Lactate	6.4	14.2
Malate	5.2	13.8
Succinate	14.6	13.4
Glycerol	8.6	10.2
L-Arabinose	0.9	15.0

[a] Conditions: 0.44 mg of N/ml; reaction time, 20 min; age of cells, 15 hr.

[b] Conditions: 0.044 mg of N/ml; reaction time, 60 min; age of cells, 16 hr.

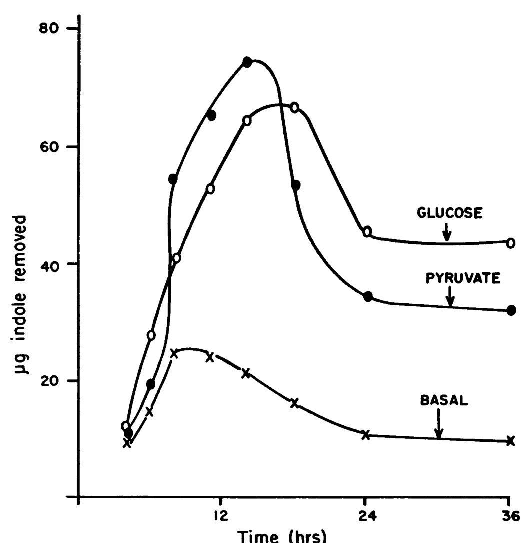

FIG. 1. *Effect of glucose and sodium pyruvate on formation of tryptophan synthetase during growth of Escherichia coli 9723E. Cell concentration, 0.06 mg of N/ml; reaction time, 60 min; pyruvate and glucose added at zero time at a concentration of* 10^{-1} *M.*

moved at intervals, and growth and synthetase activity determined. The stimulation of tryptophan synthetase by glucose or pyruvate was evident during the entire growth period of 4 to 36 hr (Fig. 1). However, increase in enzyme was most marked during the period of maximal growth of the cells (8 to 14 hr).

Mechanism of action of glucose on tryptophanase. Although the glucose inhibition of inducible enzymes is presently considered as a manifestation of metabolic control (Magasanik, 1961), the mechanism of the inhibition is not known. An earlier report from this laboratory demonstrated that the glucose repression of tryptophanase is not due to inhibition of factors necessary for the permeation of tryptophan into the cell (Freundlich and Lichstein, 1960; also Boezi and DeMoss, 1961). If, as the repressor hypothesis suggests, glucose, or derivatives of glucose, are precursors of an internal repressor which specifically inhibits the synthesis of inducible enzymes by competing with the inducer at the enzyme-forming site, then (i) carbohydrates used most rapidly should produce the most severe inhibition and (ii) increased inducer should overcome the repression (Cohn and Horibata, 1959). To test the first assumption, *E. coli* (Crookes) was grown in basal medium under shaker conditions, harvested by centrifugation, and washed once. The harvested cells were added, at a final concentration of 0.011 mg of N/ml, to 100 ml of the basal medium with 10^{-3} M L-tryptophan and 2×10^{-3} M of the carbohydrate to be tested. The cells, contained in 250-ml Erlenmeyer flasks, were placed on a rotary shaker, and samples of the cultures were removed at intervals to be assayed for tryptophanase activity and residual carbohydrate. Glucose was measured by use of the enzymatic reagents (Glucostat, Worthington Biochemicals Corp., Freehold, N.J.); the utilization of L- and D-arabinose was followed by the method of Mejbaum, as described by Umbreit, Burris, and Stauffer (1957). Under these conditions, only glucose completely inhibited tryptophanase formation (Fig. 2). L-Arabinose, which fully inhibits enzyme production at high concentrations (Table 3), permitted about 15% induction. D-Arabinose inhibited tryptophanase by only 30%, while α-methyl-α-D-glucoside had no effect on enzyme formation. An explanation

of these results may be found in the data on carbohydrate utilization (Fig. 2). Glucose appeared to be metabolized immediately and at a rapid rate. L-Arabinose was consumed more slowly than glucose initially, but both sugars were utilized at about the same rate after 3 hr of growth. In contrast, D-arabinose was used very slowly, while α-methyl-α-D-glucoside was not metabolized (as indicated by the absence of growth when the glucoside was used as sole carbon source).

The second prediction of the glucose-repressor hypothesis, namely, that inducer should reverse the inhibition, was examined next. Uninduced cells of *E. coli* (Crookes) were placed in test tubes (13 by 100 mm) containing 2×10^{-2} M phosphate buffer (pH 7.8), 10^{-3} M or 10^{-2} M glucose, varying concentrations of L-tryptophan, 1% acid-hydrolyzed casein (Difco), and deionized water to 2 ml. Control tubes contained no glucose. The reaction was terminated by the addition of 0.2 ml of 25% trichloroacetic acid, and the tubes were assayed for indole. The results revealed that, at high levels of L-tryptophan, enzyme repression by 10^{-3} M glucose was overcome by 30% (Fig. 3). At the higher glucose level, alleviation of the inhibition by tryptophan was still evident, although not as marked.

In a second group of experiments, exponentially growing cells under shaker conditions in basal medium were harvested by centrifugation, washed, and placed in fresh medium containing 2×10^{-3} M glucose as well as varying amounts of L-tryptophan. Samples were removed hourly to determine the extent of tryptophanase induction. At the highest level of L-tryptophan used, repression by glucose was reduced by more than 40% (Fig. 4).

Mechanism of action of glucose on tryptophan synthetase. During the study of factors controlling the formation of tryptophan synthetase, two general types of compounds were found to increase the production of the enzyme. These compounds were either carbohydrate derivatives (Table 3), or substances that appeared to reduce the endogenous formation of tryptophan, i.e.,

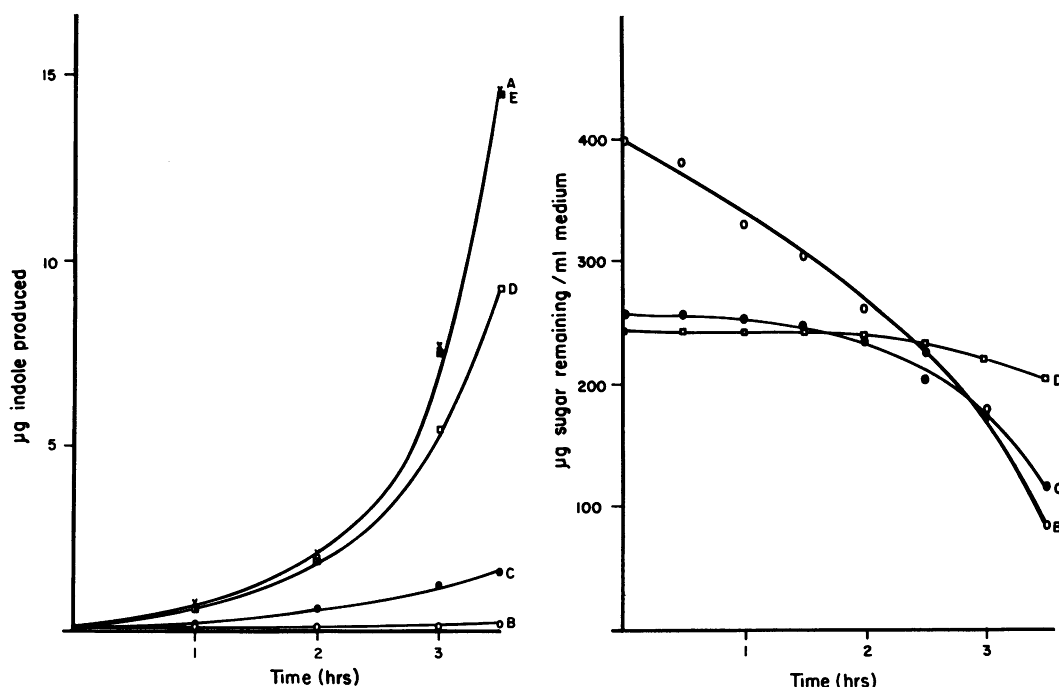

FIG. 2. *Comparison between carbohydrate utilization and tryptophanase induction in washed-cell suspensions of Escherichia coli (Crookes) under optimal growth conditions. Cell concentration, 0.02 mg of N/ml; growth time, 17 hr; assay time, 15 min; washed cells added at a concentration of 0.011 mg of N/ml; carbohydrate (2×10^{-3} M) added at zero time.* A = *basal medium;* B = *plus glucose;* C = *plus L-arabinose;* D = *plus D-arabinose;* E = *plus α-methyl-α-D-glucoside.*

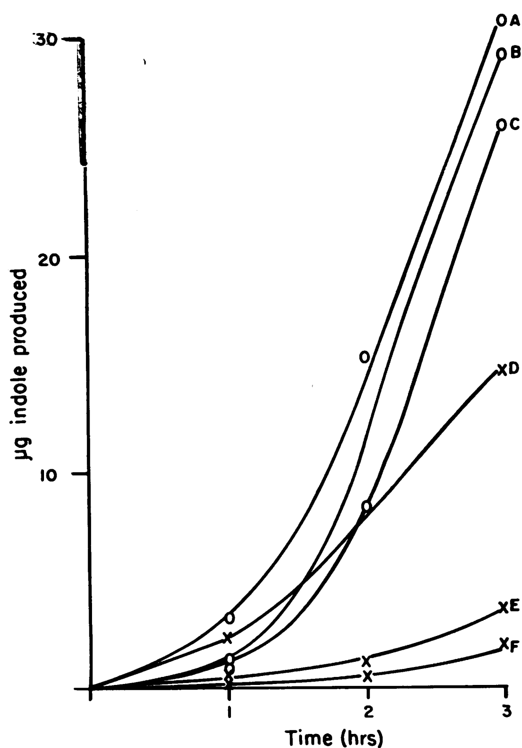

FIG. 3. *Effect of level of L-tryptophan on glucose inhibition of tryptophanase induction by Escherichia coli 9723E under growing conditions. Cell concentration, 0.02 mg of N/ml; growth time, 13 hr; assay time, 30 min; washed cells added at a concentration of 0.008 mg of N/ml; curves* A, B, *and* C *represent* 2×10^{-2}, 2×10^{-3}, *and* 2×10^{-4} *M L-tryptophan, respectively; curves* D, E, *and* F *represent the same concentration of L-tryptophan in the presence of* 2×10^{-3} *M glucose.*

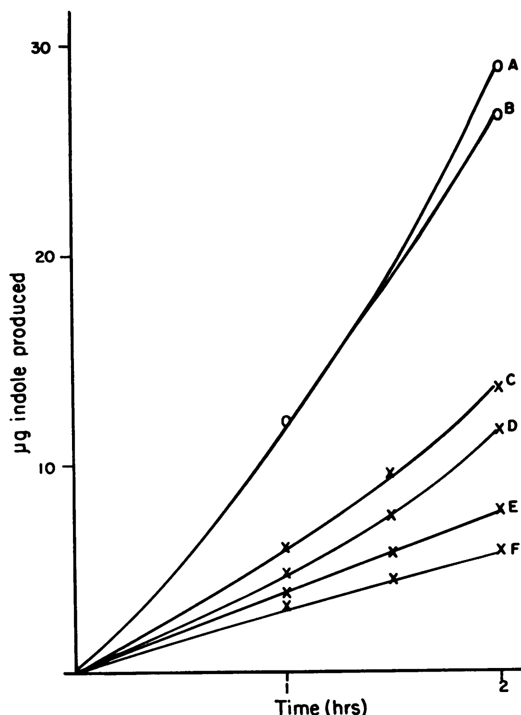

FIG. 4. *Effect of level of L-tryptophan on glucose inhibition of tryptophanase induction in washed resting-cell suspensions of Escherichia coli (Crookes). Cell concentration, 0.12 mg of N/ml; growth time, 18 hr.* A = 5×10^{-3} *M*, 2×10^{-3} *M, or* 1×10^{-3} *M L-tryptophan,* B = 2×10^{-4} *M L-tryptophan; curves* C–F *represent data in the presence of* 10^{-3} *M glucose and L-tryptophan concentrations as follows:* C = 5×10^{-3} *M*, D = 2×10^{-3} *M*, E = 1×10^{-3} *M*, F = 2×10^{-4} *M*.

indoleacetic acid or indolepropionic acid (Freundlich and Lichstein, 1962). It was important, therefore, to investigate the possible relationship between indolepropionic acid and glucose in order to discover the mechanism of the carbohydrate stimulation of tryptophan synthetase.

The activity of this enzyme in *E. coli* 9723E was increased fourfold when the cells were grown in media containing glucose, indolepropionic acid, or glucose and indolepropionic acid (Table 4). Tryptophan synthetase activity of the Crookes strain was stimulated only slightly by the addition of glucose, but was increased threefold when indolepropionic acid was added to the basal medium, and fourfold when both glucose and indolepropionic acid were present. The

TABLE 4. *Stimulation of tryptophan synthetase in Escherichia coli by glucose and indolepropionic acid*

Strain of *E. coli*	Additions to basal medium[a]	Synthetase activity[b]
9723E	None	15.2
	Glucose	58.4
	Indole-3-propionic acid	63.2
	Glucose + indolepropionate	64.6
Crookes	None	14.4
	Glucose	21.6
	Indole-3-propionic acid	44.4
	Glucose + indolepropionate	60.8

[a] Glucose concentration, 10^{-1} M; indole-3-propionic acid, 100 μg/ml. Growth time, 12 hr.

[b] Expressed as μg of indole removed in 80 min by 0.038 mg of bacterial nitrogen/ml.

possibility that indolepropionic acid or indoleacetic acid was produced by glucose-grown cells of strain 9723E and not the Crookes strain was investigated. However, these compounds could not be detected by chromatographic analysis of either culture fluids or sonic extracts of cells grown in basal medium plus glucose. Another factor mitigating against the hypothesis that bacterial cells growing in the presence of glucose produce a substance that decreases tryptophan formation is the result obtained with compounds inhibitory to both growth and tryptophan formation. In all cases, bacterial cells inoculated into a medium containing both glucose and the inhibitor produced a greater cell yield at the end of the growth period (Table 5). If a compound was produced from glucose that decreased tryptophan production, it would be assumed that growth reduction in the presence of carbohydrate and added inhibitor would be more severe.

A second hypothesis, namely, that the formation of tryptophan synthetase is increased in the presence of glucose because of a more rapid utilization of endogenously formed tryptophan, appeared more likely. This hypothesis was supported by the observation that other environmental factors that led to increased growth (i.e., temperature, aeration, and organic nitrogen) also increased the level of the enzyme (Table 6). A more direct test was the measurement of the tryptophan content in the amino acid pool of exponentially grown cells of strain 9723E. The cells were grown on a rotary shaker in 300 ml of basal medium lacking ammonium sulfate, with and without 10^{-1} M glucose, contained in 500-ml Erlenmeyer flasks. After 12 hr of growth, the cultures were harvested by centrifugation, washed once, and resuspended in deionized water at a concentration of 4.84 mg of N/ml. The amino acid pool of 5 ml of each culture was extracted by the method of Boezi and DeMoss (1961), and a Beckman/Spinco Amino Acid Analyzer was used for the analysis of the free-tryptophan content of this material. No free tryptophan could be detected in the amino acid pool formed by the bacterial cells grown with glucose, while the pool obtained from an equivalent mass of cells grown without glucose contained 0.018 μmole of tryptophan.

TABLE 5. *Effect of glucose on growth of Escherichia coli 9723E in the presence of inhibitors of tryptophan formation*

Addition to medium (μg/ml)	Growth[a]	
	Basal medium	Basal medium + glucose (10^{-1} M)
None	288	305
5-Hydroxytryptophan (100)	25	93
Tryptamine (100)	144	218
Indole-3-propionic acid (50)	48	87
Anthranilic acid (100)	218	252
5-Methyltryptophan (5)	25	47

[a] Measured in a Klett-Summerson photoelectric colorimeter at 420 mμ; growth under shaker conditions for 10 hr.

TABLE 6. *Relationship between growth and tryptophan synthetase formation in Escherichia coli 9723E*

Growth conditions[a]	Growth[b]	Enzyme activity[c]
Degree of aeration[d]		
Stationary, air	130	28.0
Stationary, CO_2	120	21.6
Shaker, air	350	41.2
Bubbling, air	465	60.8
Temperature[e]		
27 C	315	44.8
37 C	165	26.4
45 C	118	23.2
Medium[f]		
Basal + glucose	315	10.0
Basal + glucose + L-alanine	325	12.6
Basal + glucose + L-lysine, L-phenylalanine, L-methionine	350	19.8
Basal + glucose + acid-hydrolyzed casein	430	57.4

[a] Age of cells: aeration, 14 hr; temperature, 13 hr; medium, 18 hr.

[b] Measured in a Klett-Summerson photoelectric colorimeter at 420 mμ.

[c] Indole removed (μg) in 80 min by 0.029 mg of bacterial nitrogen/ml.

[d] Basal + glucose medium, 27 C.

[e] Basal + glucose medium, shaker.

[f] Shaker, 27 C; glycine (1 mg/ml) added to basal medium (which did not contain acid-hydrolyzed casein); glucose, 10^{-1} M; alanine, 1 mg/ml; leucine, 0.2 mg/ml; phenylalanine, 0.4 mg/ml; methionine, 0.2 mg/ml.

DISCUSSION

These results demonstrate that glucose and related compounds exert a profound effect on the

formation of tryptophanase and tryptophan synthetase in *E. coli*. In general, the extent of tryptophanase inhibition or synthetase stimulation was similar for a given compound. Thus, it appears that a related phenomenon might be operative in producing the effects on the two enzymes.

The inhibition by glucose and other carbohydrates of induced enzyme formation has been attributed to repression by intermediates of carbohydrate metabolism (Magasanik, 1957). These compounds are thought to be the "metabolite moieties" of the repressor molecule which prevent enzyme synthesis by competing with the inducer at the enzyme-forming site (Levin and Magasanik, 1961). The present findings that the extent of tryptophanase inhibition depends on the rate of utilization of a particular compound by the bacterial cells and that increased inducer can overcome the inhibition by glucose to the extent of approximately 40% are consistent with the repressor hypothesis. The physiological significance of the glucose inhibition of tryptophanase becomes clearer when this is considered in conjunction with the stimulatory effect of glucose on tryptophan synthetase. The formation of many biosynthetic enzymes is controlled through repression (Pardee, 1959). In *E. coli*, the intracellular level of tryptophan has been shown to regulate the formation of tryptophan synthetase (Yanofsky, 1960), and it is probable that the mechanism of the glucose effect on this enzyme is related to the level of tryptophan in the cell. Direct measurements of the amino acid pool of *E. coli* showed that the free-tryptophan content of cells grown without glucose was considerably higher than of those grown with the carbohydrate.

Two hypotheses which can account for the depletion of tryptophan in the presence of glucose are: (i) glucose is metabolized to a compound which blocks the endogenous formation of tryptophan, and (*ii*) tryptophan is metabolized so rapidly in the presence of glucose that there is little chance for the amino acid to accumulate. Concerning the first hypothesis, indolepropionic acid could duplicate the stimulatory effect of glucose on the formation of tryptophan synthetase in *E. coli* 9723E. Moreover, other compounds stimulate tryptophan synthetase production in *E. coli* by inhibiting tryptophan formation (Lester and Yanofsky, 1961; Freundlich and Lichstein, 1962). It was found, however, that when *E. coli* 9723E was inoculated into media containing compounds inhibitory to tryptophan formation and growth the cells grew more rapidly when glucose was present. It appears improbable, therefore, that a compound further inhibiting tryptophan formation would be produced from glucose. The second hypothesis, namely, that the bacterial cells, in the presence of glucose, rapidly metabolize tryptophan, is favored and is consistent with the apparent function of repression in controlling the formation of tryptophan synthetase. This enzyme is not repressed completely, even by high levels of tryptophan (Freundlich and Lichstein, 1962). Other constitutive systems have been reported, which apparently cannot be repressed below a certain minimal level (Ames and Garry, 1959; Yates and Pardee, 1957). Thus, the function of repression in tryptophan biosynthesis appears *not* to decrease the amount of tryptophan produced in the presence of excess exogenous tryptophan, but, rather, to provide for economy (Pardee, 1959) and flexibility (Ames and Garry, 1959) in the formation of the synthetase enzyme. It is likely that cells growing in the presence of glucose would need more of an enzyme synthesizing an amino acid essential for increased protein formation. This argument is supported further by the fact that other environmental factors that increased growth also increased the level of tryptophan synthetase. The formation of other repressible biosynthetic enzymes has been reported to be stimulated in cells grown in enriched media that supported increased growth (Yates and Pardee, 1957; Schwartz, Maas, and Simon, 1959; Novick and Maas, 1961).

Thus, the role of glucose and other fermentable carbohydrates in the control of tryptophan metabolism in *E. coli* may be viewed as regulating the level of repressors of tryptophanase and tryptophan synthetase formation. In the case of the degradative enzyme, it is speculated that repressors of tryptophanase formation are produced from carbohydrate dissimilation. On the other hand, the increased metabolic rate caused by glucose and other carbon sources reduces the intracellular level of tryptophan, the repressor of tryptophan synthetase.

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