INHIBITORY EFFECT OF GLUCOSE ON TRYPTOPHANASE[1]

MARTIN FREUNDLICH AND HERMAN C. LICHSTEIN

Department of Bacteriology, University of Minnesota, Minneapolis, Minnesota

Received for publication April 6, 1960

Earlier studies from this and other laboratories have shown that the presence of glucose inhibits the formation of certain inducible enzymes in bacteria (Boyd and Lichstein, 1955; Magasanik, 1957). This phenomenon is not specific to glucose, but rather is shared by other carbohydrates utilized by the bacterial cells. Although several mechanisms have been proposed to explain this carbohydrate effect, the basic nature of the inhibition is still unknown.

The present studies were initiated in an attempt to elucidate the mechanism of the inhibitory effect of glucose on the formation of tryptophanase in *Escherichia coli*.

MATERIALS AND METHODS

Two strains of *E. coli* were employed, namely Crookes (ATCC 8739) and ATCC 9723E. The growth medium was identical to that used in previous studies (Boyd and Lichstein, 1955) and contained 5×10^{-3} M DL-tryptophan. The vitamin-free acid hydrolyzed casein (Difco) employed in the medium was free of tryptophan as determined by microbiological assay. Additions of the desired concentrations of carbohydrates were made from sterile solutions to the basal medium.

The bacterial cells were grown under stationary and aerobic (shaker) conditions for the desired period of time at approximately 27 C in 200 ml of media contained in 500-ml Erlenmeyer flasks. The cells were harvested by centrifugation, washed once, and resuspended in distilled water to a concentration of 0.03 to 0.29 mg of nitrogen per ml.

Tryptophanase activity was measured at 37 C and pH 7.8 (2×10^{-2} M phosphate buffer) using 2.5×10^{-3} M L-tryptophan as substrate. The reaction was terminated by the addition of trichloroacetic acid and indole was determined colorimetrically (Klett-Summerson photoelectric colorimeter) employing the technique described by Wood, Gunsalus, and Umbreit (1947). Tryptophan synthetase activity was measured at 37 C and pH 7.0 (1×10^{-2} M phosphate buffer) using 1×10^{-2} M L-serine and 0.05 per cent indole as substrates. Pyridoxal hydrochloride (5 μg/ml) was added to the reaction tubes. Control tubes contained no serine. After suitable time periods, the reaction was stopped by the addition of trichloroacetic acid and the amount of indole left was determined colorimetrically. Synthetase activity was calculated by subtracting the amount of indole remaining in the control from that left in the experimental tubes.

Glucose was determined by the method of Folin and Malmros, as described in Umbreit, Burris, and Stauffer (1957). Interference by tryptophan was circumvented by the removal of the amino acid by adsorption on 1 per cent Norit A at pH 8.0. In some studies, glucose was measured by use of the enzymatic reagents (glucostat) available from Worthington Biochemical Corp., Freehold, N. J.

RESULTS AND DISCUSSION

The initial studies were designed to learn the influence of the age of the bacterial cells on tryptophanase activity. Both aerobic and stationary conditions of cultivation were employed. It may be seen (figure 1) that the maximum level of enzyme activity was reached between 10 and 12 hr of growth under both conditions of incubation. However, tryptophanase synthesis proceeded only after a lag of about 4 to 6 hr in the aerobically grown cells. Of interest was the observation that while the activity of the stationary grown cultures leveled off after maximal enzyme synthesis, the shaker grown bacterial cells manifested a marked decrease in activity after approximately 10 hr. The explanation of this phenomenon appears to be the fact that whereas enzyme synthesis stops between 10 and 12 hr after growth initiation, the growth of the

[1] This work was supported in part by a contract between the Office of Naval Research, Department of the Navy, and the University of Minnesota (NR103-250).

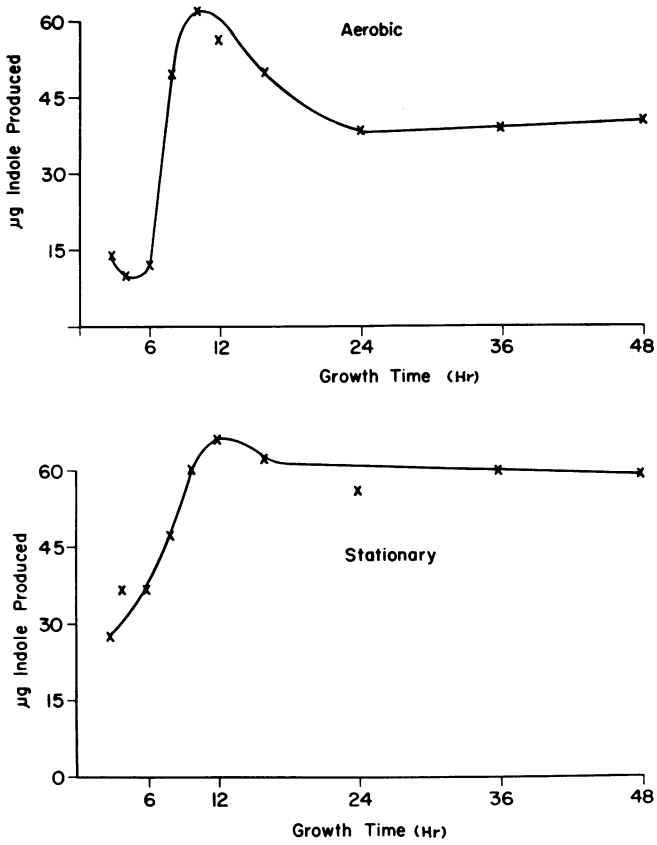

Figure 1. Effect of age of culture on tryptophanase activity of *Escherichia coli* (Crookes strain) incubated under stationary and aerobic conditions (0.095 mg bacterial nitrogen per tube; reaction time, 60 min).

bacterial cells continues. Hence, a dilution effect is noted. This is more clearly depicted in the data as plotted in figure 2. During the period from 10 to 24 hr, tryptophanase activity declined about 42 per cent while the total bacterial cell nitrogen increased by about 42 per cent.

The influence of the presence of glucose during growth was studied next. Titration of the carbohydrate revealed that the extent of inhibition varied with the cultural conditions (table 1). The concentration of glucose required for essentially complete inhibition of tryptophanase formation was approximately 10-fold greater when aerobic cultivation was employed. The reason for this difference appears to be the greater rate of glucose utilization by the bacterial cells when grown under aerobic conditions. Assuming the same initial concentration of glucose, the amount of residual sugar at any given time was always substantially lower in the aerobic cultures. For example, starting with an initial concentration of 1×10^{-2} M and using stationary conditions of cultivation, the μg of glucose per ml of medium remaining was 1000 after 10 hr, 565 after 12 hr and 410 after 13 hr. Employing aerobic conditions of cultivation only 167 μg remained after 10 hr and the glucose was exhausted by 12 hr. Also pertinent from the data given (table 1) is the fact that the effect of glucose was essentially the same whether intact bacterial cells or cell-free extracts were assayed.

The lag in enzyme formation exhibited by the aerobically grown bacterial cells (figure 1) permitted an inspection of the influence of glucose on tryptophanase synthesis when the sugar was added to the cultures at varying periods of time. To this end, glucose was added to individual flasks of media at zero time, and after 6, 9, and

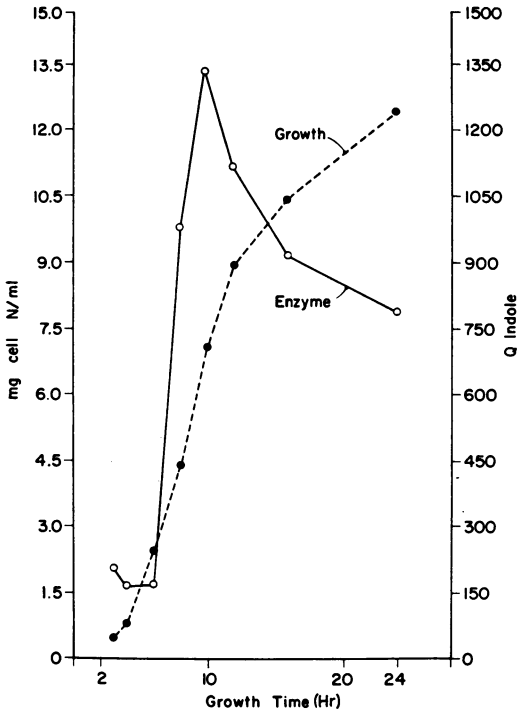

Figure 2. Growth and tryptophanase activity of *Escherichia coli* (Crookes strain) incubated under aerobic conditions. Conditions as for figure 1. The Q_{indole} is defined as the μg indole produced per mg bacterial cell nitrogen per hour. Abscissa = growth time without glucose.

12 hr of incubation. Enzyme activity was measured in control bacterial cells grown without glucose for 6, 9, 12, and 24 hr, respectively. The glucose grown cells were all harvested and tested for tryptophanase activity at 24 hr. The results of this experiment are recorded in figure 3. The incorporation of glucose into the culture media prior to the initiation of enzyme synthesis (0 to 6 hr) caused complete inhibition of tryptophanase formation. The 6 hr control value probably reflects the synthesis of tryptophanase during the time required to harvest the bacterial cells, since the rate of enzyme synthesis at this time is rapid. When the sugar was added during the period of maximal enzyme synthesis (6 to 10 hr), further formation of tryptophanase appeared to be stopped. Glucose addition after 12 hr of growth was without effect, presumably because the level of tryptophanase in the cells had reached a maximum and no further synthesis was occurring.

The data presented in figure 4 reveal a positive

TABLE 1

Effect of glucose concentration on tryptophanase activity of intact cells and cell-free extracts of Escherichia coli (Crookes strain) grown under aerobic and stationary conditions

Molar Concn of Glucose in Growth Medium	Tryptophanase Activity			
	Stationary grown		Aerobically grown	
	Cells*	Extracts	Cells*	Extracts
0	35.4†	33.1	25.2	20.1
1×10^{-1}	0.8	0.5	2.0	0.0
1×10^{-2}	2.6	3.2	11.4	11.1
5×10^{-2}	8.4	7.0	14.4	13.5

* Age of cells, 24 hr.

† Micrograms indole produced in 60 min by 0.04 mg bacterial cell nitrogen per tube; extracts prepared by 20 min treatment in a 9-kc sonic oscillator.

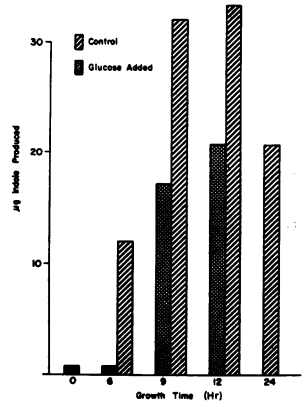

Figure 3. Effect of time of addition of glucose to aerobic cultures of *Escherichia coli* (Crookes strain) on tryptophanase activity of harvested bacterial cells. Glucose added at a concn of 1×10^{-1} M; control cells harvested and assayed at times given; glucose grown cells harvested and assayed at 24 hr; 0.029 mg bacterial nitrogen per tube; reaction time 60 min.

correlation between the amount of residual glucose in the culture medium and the level of tryptophanase in aerobically grown bacterial cells. It is clear that the initial introduction of 0.1 M glucose into the culture medium prevented tryptophanase synthesis throughout the period of 14 hr. This observation correlates well with the fact that a high level of residual glucose was maintained throughout the growth period. On the other hand, it is manifest that tryptophanase

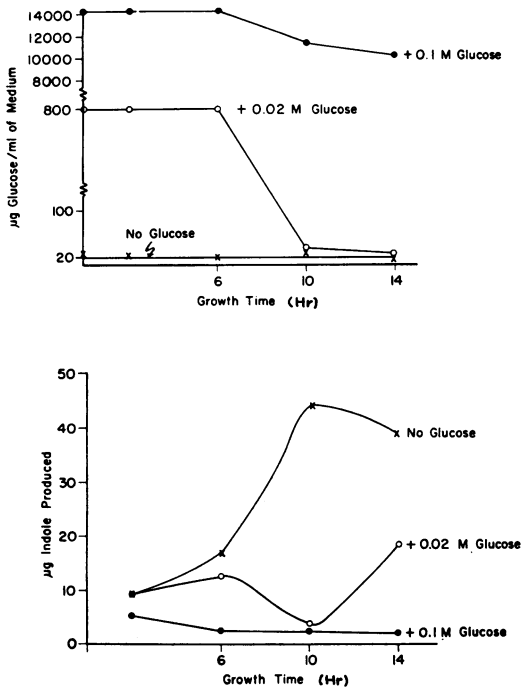

Figure 4. Comparison between tryptophanase activity of aerobically grown *Escherichia coli* (Crookes strain) and residual glucose in culture media (0.029 mg bacterial nitrogen per ml; reaction time 60 min; glucose added at zero time).

formation was initiated after 10 hr in the culture containing 0.02 M glucose initially. An inspection of the glucose concentration at this time revealed complete exhaustion of the sugar, thus permitting the initiation of tryptophanase synthesis. These results also explain those given in table 1, in which it was noted that the degree of enzyme inhibition depended on the initial concentration of glucose as well as the condition of incubation (aerobic cells utilizing glucose at a faster rate than their stationary grown counterparts).

Although the initiation of tryptophanase synthesis correlates extremely well with the level of residual glucose in the culture medium, it is of great interest that the bacterial cells were able to synthesize large amounts of the enzyme at a time when enzyme formation by the control cells had reached a minimum. This intriguing phenomenon is under investigation at the present time and may have some relation to preferential synthesis of enzymes as discussed by Cohn and Horibata (1959) for the β-galactosidase system of *E. coli.*

TABLE 2

Effect of products of glucose dissimilation on tryptophanase activity of Escherichia coli (Crookes strain)

Additions to Growth Medium (1×10^{-1} M)	Tryptophanase Activity* (μg Indole Produced)		Final pH†	
	Stationary	Aerobic	Stationary	Aerobic
None	46.4	50.8	6.8	7.4
Glucose	1.6	2.2	5.1	4.7
Pyruvate	4.0	8.6	6.2	6.9
Malate	37.6	36.6	6.6	7.1
Succinate	40.0	35.2	6.7	7.5
Lactate	38.4	46.4	6.8	7.3
Ethanol	43.6	46.8	6.9	7.4
Acetate	44.0	54.0	6.8	7.3

* Reaction time 60 min; 0.04 mg cell nitrogen per tube.

† Initial pH 6.8.

It is pertinent also that inhibition of tryptophanase activity was manifest prior to any demonstrable disappearance of glucose and that this suppression was most pronounced at the higher concentration of the sugar (figure 4). These findings are in agreement with those of Cohn and Horibata (1959) who found a weak direct effect of high concentrations of glucose on the β-galactosidase system of *E. coli.* The nature of this effect is not at all clear.

The effect on tryptophanase activity of various products and intermediates of glucose dissimilation are compared under both stationary and aerobic conditions of cultivation (table 2). Of the several compounds tested, only pyruvate was almost as effective as glucose in inhibiting the formation of tryptophanase under both conditions of cultivation. An inspection of the slight pH changes resulting from the incorporation of pyruvate into the growth medium should rule out the early consideration that reduction in pH is a major factor in enzyme suppression by carbohydrates (Gale, 1943). Relatively smaller but nevertheless significant reductions in enzyme activity were observed when malate or succinate was present under both aerobic and stationary conditions. The utilization of these compounds caused only a slight reduction in the growth pH under stationary conditions of growth. Lac-

tate had a slight but definite effect only when present under stationary conditions.

The marked inhibitory activity of pyruvate is in keeping with the theory that glucose is metabolized to an inhibitor compound which is responsible for enzyme repression (Magasanik, 1957). The relatively low activity of the other compounds tested would presumably reflect the fact that they are not as efficiently converted to an inhibitor of tryptophanase. It is pertinent in this regard that the addition of pyruvate to preadapted resting cells caused a marked suppression of tryptophanase activity (Boyd, 1954).

Finally, it was considered desirable to determine whether inhibition of a tryptophan permease was involved in the glucose inhibition of tryptophanase. To this end, parallel studies of tryptophanase and tryptophan synthetase were initiated. *E. coli* strain ATCC 9723E was employed because it possessed a higher level of synthetase than did the Crookes strain. The experimental design was based on the fact that while tryptophan is an obligatory inducer for the formation of tryptophanase, the presence of this amino acid inhibits the formation of the synthetase system (Monod and Cohen-Bazire, 1953). The results of this experiment are given in figure 5. As already suggested, the presence of tryptophan during growth is essential for tryptophanase formation and markedly inhibitory to the formation of the synthetase system. It is also apparent that while glucose inhibited completely the synthesis of tryptophanase, the synthetase system was increased markedly. Moreover, when the bacterial cells were grown with glucose and tryptophan the synthetase system was present in extremely low concentration. It is reasonable to conclude from these data that glucose does not inhibit either the production or activity of a tryptophan permease, since tryptophan must have entered the bacterial cell, as shown by the prevention of synthetase formation in the cells grown with glucose and tryptophan. However, on the basis of studies with Neurospora, Gross (1959) has postulated that enzymes are compartmentalized in these cells and that glucose can prevent the entrance of a compound into a particular compartment while not affecting its passage into another. If the assumption is made that tryptophanase and tryptophan synthetase are so separated in *E. coli*, then it is possible that glucose (or a metabolite produced from

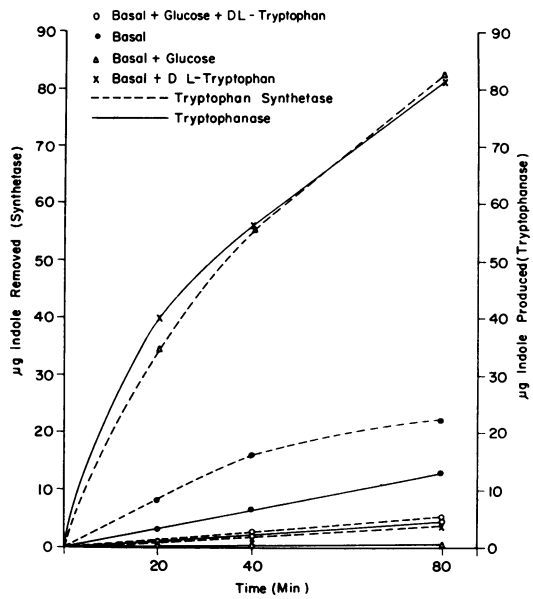

Figure 5. Effect of glucose on tryptophanase and tryptophan synthetase activity of *Escherichia coli* (9723E). Aerobic cultivation; enzyme activity measured after 23 hr incubation; 0.57 mg bacterial nitrogen per ml; reaction time 60 min.

glucose) might prevent tryptophan from reaching the tryptophanase system while having no effect on the permeation of this amino acid into the synthetase system. This is, of course, purely speculative in *E. coli*.

SUMMARY

Data have been presented concerning the inhibitory effect of glucose, when present in the growth medium, on the tryptophanase enzyme system of *Escherichia coli*. Addition of glucose to growing bacterial cells at any time prior to the initiation of enzyme synthesis prevents completely the formation of tryptophanase. Measurement of glucose revealed a close correlation between the amount of residual sugar in the cultures and tryptophanase activity, and explained the differences in results between stationary and aerobic conditions of cultivation. Although the mechanism of this glucose effect is not yet clear, two observations are particularly noteworthy in this regard; namely, the absence of a demonstrable effect of the carbohydrate on a tryptophan permease, and the marked stimulation of tryptophan synthetase by concentrations of glucose sufficient to inhibit tryptophanase.

REFERENCES

BOYD, W. L. 1954 The effect of carbohydrates on the tryptophanase and several deaminase systems of bacteria. Ph.D. Thesis, Univ. of Minnesota, Minneapolis.

BOYD, W. L., AND H. C. LICHSTEIN. 1955 The effect of carbohydrates on the tryptophanase activity of bacteria. J. Bacteriol., **69,** 584–589.

COHN, M., AND K. HORIBATA. 1959 Physiology of the inhibition by glucose of the induced synthesis of the β-galactosidase enzyme system of *Escherichia coli*. J. Bacteriol., **78,** 624–635.

GALE, E. F. 1943 Factors influencing the enzymic activities of bacteria. Bacteriol. Rev., **7,** 139–173.

GROSS, S. R. 1959 Enzymatic autoinduction and the hypothesis of intracellular permeability barriers in *Neurospora*. Trans. N. Y. Acad. Sci. Ser. II, **22,** 44–48.

MAGASANIK, B. 1957 Nutrition of bacteria and fungi. Ann. Rev. Microbiol., **11,** 221–252.

MONOD, J., AND G. COHEN-BAZIRE. 1953 L'effet d'inhibition specifique dans la biosynthése de la tryptophane-desmase chez *Aerobacter aerogenes*. Compt. rend., **236,** 530–532.

UMBREIT, W. W., R. H. BURRIS, AND J. F. STAUFFER. 1957 *Manometric techniques*. Burgess Publishing Co., Minneapolis.

WOOD, W. A., I. C. GUNSALUS, AND W. W. UMBREIT. 1947 Function of pyridoxal phosphate: resolution and purification of the tryptophanase enzyme of *Escherichia coli*. J. Biol. Chem., **170,** 313–321.