THE EFFECT OF CARBOHYDRATES ON THE TRYPTOPHANASE ACTIVITY OF BACTERIA[1]

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Reports from several laboratories (Happold and Hoyle, 1936; Epps and Gale, 1942) have demonstrated that tryptophanase activity is reduced when cells are harvested from a medium containing glucose and that this effect is not entirely due to the increased hydrogen ion concentration resulting from the utilization of the carbohydrate by the microorganisms. Dawes and Happold (1949) reported data suggesting that apotryptophanase formation is inhibited when growth takes place in the presence of glucose.

The present studies were initiated because of our previous work on the effects of carbohydrates on the deaminases of bacteria (Boyd and Lichstein, 1951, 1953), and the results obtained emphasize that the formation of tryptophanase is reduced markedly when bacterial cells are grown in the presence of glucose or other carbohydrates. In addition this report is concerned with the nutritional factors required for the adaptive formation of tryptophanase.

MATERIALS AND METHODS

The organisms employed were strains carried in the stock culture collection of this laboratory. The growth medium was composed of 0.1 per cent each of K_2HPO_4, KH_2PO_4, and NaCl; 0.07 per cent $MgSO_4$; 0.4 per cent $(NH_4)_2SO_4$; 0.05 per cent sodium citrate; 0.02 per cent DL-tryptophan; and 1 per cent vitamin-free acid hydrolyzed casein (Difco). Additions of the desired concentrations of carbohydrates were made from sterile 2 M solutions to the basal medium. The casein hydrolyzate used in the growth medium and in the resting cell experiments was free of tryptophan as determined by microbiological assay (Green and Black, 1944).

The bacterial cells were grown for 16 to 18 hours at 30 C, harvested by centrifugation, washed once, and resuspended in distilled water to give a cell concentration of 0.3 to 1.5 mg of nitrogen per ml. Tryptophanase activity was measured at pH 8.3 (M/10 phosphate buffer), 37 C using L-tryptophan as substrate. After suitable incubation periods the reaction was terminated by the addition of trichloracetic acid, and indole was determined colorimetrically (Klett-Summerson photoelectric colorimeter) employing the technique described by Wood, Gunsalus, and Umbreit (1947). In all cases where parallel studies were made, equivalent masses of cells were used. The procedure described by Lichstein and Christman (1949) was employed to determine deaminase activity.

RESULTS AND DISCUSSION

The effect of carbohydrates on tryptophanase. These experiments were designed to gain additional information on the inhibitory action of glucose by employing several strains of microorganisms, different carbohydrates, and noncarbohydrate media of initial pH simulating the final pH of carbohydrate containing media. The results of such studies are given in table 1.

It is clear that for the five strains of *Escherichia coli* studied all exhibited significant reduction in tryptophanase activity when grown in media containing glucose, lactose, xylose, or maltose. The effect of maltose was considerably smaller with the Texas strain than with the other four strains of *E. coli*. That this effect was not entirely due to the reduction in pH as a result of fermentation may be concluded from the fact that the activity in noncarbohydrate media of initial pH 5 was reduced from 14 to 40 per cent with the exception of *E. coli* (Gratia) where the reduction was approximately 70 per cent. In

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TABLE 1

Effect of presence of carbohydrates in growth medium on tryptophanase activity of bacteria

Organism	μg Indole Produced*					
	Basal	Basal + glucose†	Basal + lactose	Basal + xylose	Basal + maltose	Basal pH 5
Escherichia coli (Crookes)	47.1	2.3	3.0	2.4	2.8	33.2
(0.07 mg cell N/tube)	(6.5–6.5)‡	(6.5–5.0)	(6.5–5.0)	(6.5–5.1)	(6.5–5.1)	(5.0–5.6)
Escherichia coli (Gratia)	46.8	3.0	2.8	2.7	1.8	13.7
(0.04 mg cell N/tube)	(7.0–7.0)	(7.0–5.0)	(7.0–5.3)	(7.0–5.1)	(7.0–5.3)	(5.0–5.4)
Escherichia coli (Texas)	56.9	0.6	6.3	1.5	34.4	48.5
(0.05 mg cell N/tube)	(6.8–6.8)	(6.8–5.1)	(6.8–5.3)	(6.8–5.4)	(6.8–5.9)	(5.0–5.5)
Escherichia coli (mutabilis)	21.9	0.0	8.9	0.9	2.4	13.2
(0.05 mg cell N/tube)	(6.7–6.7)	(6.7–4.7)	(6.7–5.7)	(6.7–5.0)	(6.7–5.0)	(5.0–5.4)
Escherichia coli (Davis)	30.8	1.5	2.3	1.5		19.8
(0.11 mg cell N/tube)	(7.0–6.8)	(7.0–4.6)	(7.0–4.8)	(7.0–5.1)		(5.0–6.0)
Aerobacter aerogenes (Tenn.)	41.4	4.8	43.9	21.9		26.6
(0.05 mg cell N/tube)	(7.0–6.6)	(7.0–4.8)	(7.0–6.2)	(7.0–5.7)		(5.0–5.7)

* 60 min, pH 8.3, 37 C.

† Carbohydrates employed at a final conc of 0.02 M.

‡ Initial and final pH of growth medium.

contrast, the presence of carbohydrates resulted in reduced activities in the order of 60 to 90 per cent. The results with *Aerobacter aerogenes* revealed that the effect of glucose was much greater than that obtained with lactose or xylose. This may have been due to the fact that the final pH in media containing either lactose or xylose was considerably higher than with glucose, suggesting either partial utilization of these carbohydrates or different end products. The rise in pH noted in noncarbohydrate media at acid initial pH may have been due to the formation of basic amines as a result of amino acid decarboxylation. In some respects these data differ from the results reported by Evans, Handley, and Happold (1942) and may reflect strain variations.

The nature of the reduction of tryptophanase activity by carbohydrate. The enzyme assays demonstrating reduced tryptophanase activity as a result of growth in the presence of certain carbohydrates gave no information on the nature of the effect. The experiments described in this section were designed to determine whether the reduced activity was concerned primarily with the cellular content of holoenzyme, apoenzyme, or coenzyme. A mixture of vitamins and salts was used as a source of cofactors, and vitamin-free acid hydrolyzed casein was employed as a source of amino acids. The data obtained (table 2) revealed that the addition of hydrolyzed casein to resting cell suspensions of glucose grown cells restored partially the reduced tryptophanase activity whereas minerals or vitamins had no effect when added alone or in combination with hydrolyzed casein. The effect of graded concentrations of hydrolyzed casein is plotted in figure 1. It is manifest from these data that the lost activity as a result of growth in carbohydrate media was almost completely restored by the addition of suitable concentrations of hydrolyzed casein to the resting cell suspensions. It appears likely therefore that glucose utilization resulted in prevention of tryptophanase formation directly or indirectly by causing a deficiency in an essential nitrogen source concerned in enzyme synthesis. Certainly the results with resting cell suspensions derived from carbohydrate containing media demonstrate that such cells have not lost their ability to synthesize tryptophanase.

Since vitamin-free acid hydrolyzed casein in suitable concentration restored the tryptophanase activity of glucose grown cells, it was desirable to determine if the same effect could be duplicated with a mixture of amino acids, single amino acids, or inorganic nitrogen. A mixture of amino acids less tryptophan known to be present in casein was prepared according to the concentrations given by Williamson (1944). In addition, the 17 amino acids were divided into 4 groups and also tested singly for activity. The

TABLE 2

Effect of hydrolyzed casein, vitamins, and minerals on the tryptophanase activity of glucose grown cells

Conditions of Cultivation	Additions to Resting Cell Suspensions	μg Indole Produced*	
		E. coli (Crookes)	*E. coli* (mutabilis)
Basal without glucose (initial pH 7.0)	none	54.3 (7.0–7.3)†	47.7 (7.0–7.5)
Basal without glucose (initial pH 5.0)	none	43.8 (5.0–5.7)	40.2 (5.0–6.3)
Basal + 0.02 M glucose (initial pH 7.0)	none	11.9 (7.0–4.7)	8.6 (7.0–4.7)
	hydrolyzed casein,‡ 0.5 mg	26.9	19.2
	minerals§	10.1	8.3
	vitamins‖	10.4	9.8
	minerals + hydrolyzed casein, 0.5 mg	27.3	18.9
	vitamins + hydrolyzed casein, 0.5 mg	25.8	17.7

* pH 8.3, 37 C, 180 min, 0.04 mg cell N/tube.

† Initial and final pH of growth medium.

‡ Acid hydrolyzed vitamin-free casein (Difco).

§ Concentration of minerals per reaction tube: 0.1 mg each of $MgSO_4$, NaCl, $FeSO_4$, and $MnSO_4$; 50 μg each of $ZnSO_4$, H_3BO_3, $MnCl_2$, and $FeCl_3$; and 5 μg each of $CuSO_4$ and KCl.

‖ Concentration of vitamins per reaction tube: 100 μg each of nicotinamide, *p*-aminobenzoic acid, riboflavin, pantothenic acid, thiamin, folic acid, pyridoxal, inositol, and choline; 5 μg biotin; and 2.5 μg vitamin B_{12}.

data presented (table 3) demonstrate that for 3 strains of organisms the simulated casein hydrolyzate was essentially equivalent to the natural product in its ability to stimulate tryptophanase activity of glucose grown cells, whereas little or no effect was observed on preformed enzyme present in cells grown without glucose at an initial pH of 5 or 7. Of the 4 groups of amino acids tested only 2 were active, and studies with the single amino acids revealed only serine and glutamic acid to possess activity. Both the D and L forms of serine were active, whereas only the L-isomer of glutamic acid stimulated. Inorganic nitrogen as $(NH_4)_2SO_4$ was inactive. Although not included in the table it was found that the corresponding keto acids, namely α-ketoglutaric acid and pyruvic acid, failed to stimulate enzymic activity when employed either singly or in conjunction with ammonium ions. Moreover, equimolar concentrations of both serine and glutamic acid increased the activity of glucose grown cells, whereas no stimulation was observed when tryptophan was added in concentrations exceeding that amount employed as substrate. It would appear, therefore, that amino acids are

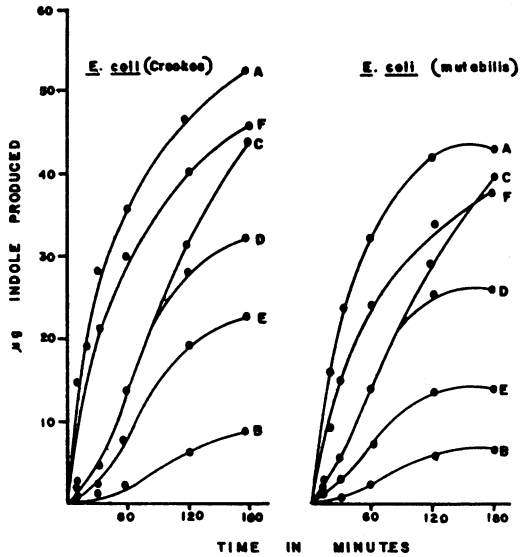

Figure 1. Stimulatory effect of acid hydrolyzed vitamin-free casein on tryptophanase activity of resting cell suspensions (0.07 mg cell N/tube).

A = nonglucose grown, pH 7.0
B = glucose grown
C = glucose grown + 5 mg casein hydrolyzate/tube
D = glucose grown + 2.5 mg casein hydrolyzate/tube
E = glucose grown + 0.5 mg casein hydrolyzate/tube
F = nonglucose grown, pH 5.0.

TABLE 3

Effect of hydrolyzed casein, simulated hydrolyzed casein, and amino acids on tryptophanase activity of resting bacterial suspensions

Conditions of cultivation	Additions to Resting Cell Suspensions	μg Indole Produced*		
		E. coli (Crookes)	*E. coli* (mutabilis)	*A. aerogenes* (Tenn.)
	mg/ml			
Basal without glucose (initial pH 7.0)	none	48.5 (7.0–6.8)†	48.9 (7.0–7.1)	60.6 (7.0–6.8)
	hydrolyzed casein‡	56.7	54.9	60.6
	hydrolyzed casein, 0.5	51.5	54.9	59.9
	simulated hydrolyzed casein,§ 5	48.5	54.9	59.9
	simulated hydrolyzed casein, 0.5	48.5	49.7	60.1
Basal without glucose (initial pH 5.0)	none	39.6 (5.0–5.6)	41.0 (5.0–5.9)	40.4 (5.0–5.8)
	hydrolyzed casein, 5	50.4	39.5	41.3
	simulated hydrolyzed casein, 5	47.4	41.0	38.9
Basal + 0.02 M glucose	none	6.5 (7.0–4.9)	8.3 (7.0–4.8)	6.5 (7.0–4.5)
	hydrolyzed casein, 5	36.6	38.7	36.8
	hydrolyzed casein, 0.5	22.2	10.2	21.8
	simulated hydrolyzed casein, 5	38.7	34.2	42.8
	simulated hydrolyzed casein, 0.5	18.9	10.7	18.8
	group I amino acids‖	17.7	13.5	19.4
	group II amino acids‖	19.5	19.5	32.9
	group III amino acids‖	9.0	8.3	7.3
	group IV amino acids‖	8.7	8.3	8.0
	$(NH_4)_2SO_4$, 0.4	6.6	8.8	6.7
	D-serine, 0.25	15.4	12.6	14.7
	L-serine, 0.25	14.0	13.2	13.8
	D-glutamic acid, 0.25	6.6	8.3	6.5
	L-glutamic acid, 0.25	16.9	23.0	26.0
	L-glutamic acid, 0.25; + DL-serine, 0.25	23.3	27.9	36.6

* pH 8.3, 37 C, 180 min, 0.07 mg cell N/tube.

† Initial and final pH of growth medium.

‡ Acid hydrolyzed vitamin-free casein (Difco).

§ Substituted amino acids less tryptophan contained in the given amount of casein.

‖ Group I = L-tyrosine, DL-alanine, glycine, L-proline, L-glutamate as contained in 5 mg casein.
Group II = DL-aspartate, DL-serine, DL-cystine, L-arginine as contained in 5 mg casein.
Group III = DL-phenylalanine, DL-leucine, DL-isoleucine, L-histidine as contained in 5 mg casein.
Group IV = DL-lysine, DL-threonine, DL-methionine, DL-valine as contained in 5 mg casein.

the active agents in the stimulation of enzymic activity possibly by acting as a reservoir for the synthesis of apotryptophanase.

General considerations of the inhibitory effect of carbohydrates. Several enzyme systems of diverse properties are affected adversely when assayed in bacterial cells harvested from a carbohydrate containing medium (Epps and Gale, 1942). The reduced deaminase activity found under these conditions appears to be associated primarily with the cellular level of codeaminase (Boyd and Lichstein, 1951), whereas the lowered tryptophanase activity is associated with the level of apotryptophanase. Thus, although the net effect of growth in carbohydrate containing media appears the same when studying either the deaminase or tryptophanase system, the component of the enzyme affected differs. It appeared desirable therefore to study both types of systems in parallel in the same

TABLE 4

Effect of presence of carbohydrates in growth medium on several enzyme systems of Escherichia coli (Crookes)

Medium	Basal	Basal + Glucose*	Basal + Lactose	Basal + Xylose	Basal + Maltose	Basal pH 5.0
Initial pH	6.6	6.6	6.6	6.6	6.6	5.0
Final pH	6.6	5.0	5.2	5.4	5.3	5.5
	μg Ammonia Nitrogen Produced†					
Aspartate deaminase	33.6	33.6	33.6	31.0	38.9	31.9
Serine deaminase	76.0	16.8	17.4	20.3	17.1	74.2
Threonine deaminase	62.1	3.8	3.8	5.5	5.2	50.8
	μg Indole Produced‡					
Tryptophanase	48.6	2.1	3.0	3.2	3.5	29.6

* Carbohydrates employed at a final conc of 0.02 M.
† 0.11 mg cell N/tube, pH 7, 60 min, 37 C.
‡ 0.11 mg cell N/tube, pH 8.3, 60 min, 37 C.

TABLE 5

Effect of presence of carbohydrates in growth medium on several enzyme systems of Aerobacter aerogenes (D)

Medium	Basal	Basal + Glucose*	Basal + Lactose	Basal + Xylose	Basal + Citrate	Basal pH 5.0
Initial pH	6.7	6.7	6.7	6.7	6.7	5.0
Final pH	6.6	4.8	6.2	5.7	6.8	5.7
	μg Ammonia Nitrogen Produced†					
Aspartate deaminase	36.3	5.5	44.4	25.8	24.5	32.8
Serine deaminase	75.4	49.9	65.5	76.0	59.2	82.9
Threonine deaminase	9.0	3.8	6.1	5.8	6.8	11.0
	μg Indole Formed‡					
Tryptophanase	36.8	4.3	47.4	19.5	30.6	34.1

* Carbohydrates employed at a final conc of 0.02 M.
† 0.16 mg cell N/tube, pH 7, 60 min, 37 C.
‡ 0.16 mg cell N/tube, pH 8.3, 60 min, 37 C.

microorganism. Several organisms were studied in this fashion; the results with two organisms exhibiting definite strain variation are given in tables 4 and 5. The data obtained with the Crookes strain of *E. coli* are particularly interesting since no effect of carbohydrate was observed on the aspartate deaminase system, whereas the other enzymes tested exhibited marked reduction in activity. It is possible that such differences are due to alternate pathways of carbohydrate dissimilation in closely related organisms.

It is interesting to speculate on the physiological basis of the carbohydrate effect on apotryptophanase. Growth in the presence of utilizable carbohydrate is much greater than in its absence. Hence it is possible that growth in carbohydrate containing media favors the synthesis of tryptophan rather than its breakdown due to the increased need for amino acids in the synthesis of cellular protein. Serine and glutamate may then be necessary for the synthesis of the enzymes concerned both in the production and degradation of tryptophan. Experimental evidence for this speculation is at present lacking.

SUMMARY

Bacteria harvested from carbohydrate containing media exhibited markedly reduced tryptophanase activity. Enzymic activity of such cells could be restored by the addition of vitamin-free acid hydrolyzed casein to resting bacterial suspensions. This effect of hydrolyzed casein could be duplicated by a mixture of amino acids present in casein or by serine and glutamic acid.

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