TRYPTOPHANASE-TRYPTOPHAN SYNTHETASE SYSTEMS IN *ESCHERICHIA COLI*

I. Effect of Tryptophan and Related Compounds

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Received for publication June 14, 1961

ABSTRACT

FREUNDLICH, MARTIN (University of Minnesota, Minneapolis) AND HERMAN C. LICHSTEIN. Tryptophanase-tryptophan synthetase systems in *Escherichia coli*. I. Effect of tryptophan and related compounds. J. Bacteriol. **84**:979-987. 1962.—The effect of tryptophan and related compounds on tryptophanase and tryptophan synthetase formation in *Escherichia coli* was determined. Several of these compounds stimulated the formation of tryptophanase while concomitantly decreasing the production of synthetase. A number of tryptophan analogues were found to inhibit growth. The possible mode of action of these substances was examined further. 5-Hydroxytryptophan greatly inhibited the formation of synthetase and also reduced growth. Its inhibitory action on growth was attributed, at least partially, to the false feedback inhibition of anthranilic acid formation. Tryptamine was found to be a potent inhibitor of the activity of synthetase, as well as of the enzyme(s) involved in the synthesis of anthranilic acid from shikimic acid. However, growth reduction was only partially reversed by tryptophan. Indole-3-acetic acid and indole-3-propionic acid decreased growth and increased the formation of synthetase six- to eightfold. The action of these compounds was ascribed to their ability to block the endogenous formation of tryptophan.

Current studies on the control of metabolism in bacteria indicate that small molecules can regulate physiological processes by adjusting the enzymatic constitution of the cell (Pardee, 1959). This control is accomplished by the stimulation or inhibition of enzyme activity or formation (Davis, 1961). Many enzymes concerned with degradative and synthetic pathways have been investigated in attempts to discover the mechanisms involved in these control systems. However, little data have been reported on the regulation of enzymes involved in both the synthesis and dissimilation of a single physiologically important compound.

The present studies were designed to elucidate some of the mechanisms involved in the physiological control of tryptophan metabolism in *Escherichia coli*. To this end, investigations were initiated on those factors affecting the formation and activity of tryptophanase (tryptophan $\rightarrow$ indole + pyruvate + NH_3), the major enzyme involved in tryptophan degradation, and tryptophan synthetase (indole + serine $\rightarrow$ tryptophan), the final enzyme in the pathway of the biosynthesis of this amino acid.

The presence of tryptophan during growth markedly affects the formation of both tryptophanase (Fildes, 1938) and tryptophan synthetase (Monod and Cohen-Bazire, 1953), and the influence of this compound on the two enzymes was studied first.

MATERIALS AND METHODS

Organisms. The two strains of *E. coli* employed in most of these studies were Crookes (ATCC 8739) and ATCC 9723E. The tryptophan auxotroph, *E. coli* T-24, which accumulates anthranilic ribonucleoside, was kindly supplied by John Spizizen.

Culture. The basal medium employed consisted of KH_2PO_4, K_2HPO_4, and NaCl (1 g each); $MgSO_4 \cdot 7H_2O$, 0.7 g; $(NH_4)_2SO_4$, 4 g; Na citrate, 0.5 g; acid-hydrolyzed casein (Difco), 10 g; and deionized water to 1 liter. The pH prior to

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autoclaving was adjusted to 6.8 to 7.0. For maximal 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 5×10^{-3} M DL-tryptophan. The tryptophan auxotroph was grown in the basal medium containing 0.2% glucose and 15 μg/ml of indole. Growth was measured in a Klett-Summerson photoelectric colorimeter at 420 mμ. Other procedural details have been described previously (Freundlich and Lichstein, 1960).

Chemicals. The chemicals used in these studies were obtained commercially.

Preparation of extracts. Bacterial cell suspensions (1 g wet weight of cells in 8 ml of 0.5 M tris(hydroxymethyl)aminomethane (tris)-succinate buffer, pH 7.0) were subjected to sonic oscillation for 5 min in a 20-kc magnetostrictive oscillator (Mullard). Cell debris was removed by centrifugation at 15,000 × *g* for 15 min. Protein was determined by the biuret method (Gornall, Bardawill, and David, 1949).

Tryptophanase and tryptophan synthetase assays. The procedure for these assays was described previously (Freundlich and Lichstein, 1960). Whole cells were used except where noted. Pyridoxal hydrochloride (5 μg/ml) was added in the synthetase assays employing intact bacterial cells, but was found unnecessary for maximal tryptophanase activity in whole cells. Pyridoxal phosphate (5 μg/ml) was added to bacterial extracts for the assay of both enzymes.

Enzymatic synthesis of anthranilic acid. The method of Srinivasan (1959) was used with modification. The reaction mixture contained the following in a final volume of 2 ml: shikimic acid (0.3 μmoles), glutamine (10 μmoles), $MgCl_2$ (8 μmoles), adenosine triphosphate (2 μmoles), diphosphopyridine nucleotide (0.7 mg), tris buffer (pH 8.2; 50 μmoles), and varying amounts of bacterial extract. The reaction was stopped by immersing the tubes in boiling water for 2 min. The contents were then brought to pH 6.0 with 1 M tris buffer and extracted in 2 ml of ethyl acetate. A 1-ml sample of the ethyl acetate layer was recovered and the solvent removed by boiling. The residue was suspended in 5.5 ml of distilled water, and the amount of anthranilic acid in the material was estimated by the method of Bratton and Marshall as modified by Rhuland and Bard (1952).

RESULTS AND DISCUSSION

Effect of tryptophan on the formation of tryptophanase and tryptophan synthetase. The formation of both enzymes was affected profoundly by the level of tryptophan in the growth medium. For example, tryptophanase was increased more than 40-fold in the presence of 5×10^{-3} M DL-tryptophan, while synthetase formation was decreased approximately 15-fold at the same concentration (Table 1). Lower concentrations of the amino acid permitted increased production of the synthetase system, whereas tryptophanase was not formed in maximal amounts. It is of interest that tryptophan levels as low as 10^{-4} M affected the formation of both enzymes. Moreover, synthetase could not be repressed completely, even by high levels of tryptophan.

Effect of compounds related to tryptophan on tryptophanase and tryptophan synthetase formation. Excellent tryptophanase activity was obtained when the bacterial cells were grown in basal media containing indole, L-, DL-, or glycyl-L-tryptophan (Table 2). Some tryptophanase formation was also evident when 5-hydroxytryptophan, anthranilic acid, shikimic acid, or indole-3-acetic acid were included in the growth medium. These compounds were then added to uninduced washed bacterial cells, to investigate more fully their ability to promote tryptophanase formation. In this case, however, 1% acid-hydrolyzed casein was added

TABLE 1. *Effect of* DL-*tryptophan on the formation of tryptophanase and tryptophan synthetase in Escherichia coli 9723E*

DL-Tryptophan	Tryptophanase[a] (μg indole produced)	Tryptophan synthetase[b] (μg indole removed)
M		
0	0.8	46.2
5×10^{-3}	37.2	2.8
2.5×10^{-3}	30.0	6.4
5×10^{-4}	11.2	12.6
2.5×10^{-4}	8.4	18.6
1×10^{-4}	4.8	39.8

[a] Conditions: 0.02 mg of cell nitrogen/tube; reaction time, 30 min; grown in basal plus 10^{-1} M glucose, 16 hr.

[b] Conditions: 0.14 mg of cell nitrogen/tube; reaction time, 40 min; growth time, 22 hr in basal medium.

TABLE 2. *Effect of compounds related to tryptophan on the formation of tryptophanase and tryptophan synthetase in Escherichia coli 9723E*

Compound[a]	Tryptophanase[b]	Growth, 18 hr[c]	Synthetase[d]	Growth, 12 hr[c]
None	0.8	440	28.0	300
L-Tryptophan	14.6	425	6.4	325
DL-Tryptophan	15.0	410	6.6	325
D-Tryptophan	1.0	430	26.8	310
Glycyl-L-tryptophan	13.8	410	7.0	335
Acetyltryptophan	1.2	480	24.6	310
5-Hydroxytryptophan	4.0	192	4.2	140
5-Methyl-DL-tryptophan	0.2	130	3.4	85
Indole	13.4	385	25.2	280
Indole-3-propionic acid	1.0	152	44.6	55
Indole-3-acetic acid	4.6	365	39.4	210
Tryptamine	0.2	138	23.8	24
Anthranilic acid	5.2	350	20.4	240
3-Hydroxyanthranilic acid	1.2	410	22.6	292
Shikimic acid	4.2	395	24.8	295

[a] Added at 200 μg/ml, except tryptamine and 5-methyl-DL-tryptophan which were added at a concentration of 50 μg/ml.

[b] Expressed as μg of indole produced in 30 min; 0.035 mg of nitrogen/ml; cells harvested from basal medium.

[c] Klett-Summerson readings at 420 mμ.

[d] Expressed as μg of indole removed in 30 min; 0.07 mg of nitrogen/ml; cells harvested from basal medium plus 10^{-1} M glucose.

to permit induction, and L-tryptophan was replaced by the compound to be tested. Little or no enzyme activity was manifest unless L-tryptophan was present, suggesting that the capacity of these compounds to induce tryptophanase during growth is due to their conversion by the bacterial cells to indole or tryptophan (Table 3).

The data in Table 2 reveal that compounds which increased the level of tryptophanase decreased the formation of synthetase; L-, DL-, and glycyl-L-tryptophan were equally effective in repressing the enzyme. 5-Hydroxy and 5-methyl tryptophan diminished synthetase more effectively than did L-tryptophan. Possibly

TABLE 3. *Effect of compounds related to tryptophan on the production of tryptophanase by resting-cell suspensions of Escherichia coli 9723E*[a]

Compound	Tryptophanase activity
None	0
L-Tryptophan	34.0
5-Hydroxytryptophan	1.2
Anthranilic acid	0
Shikimic acid	0
Indole-3-acetic acid	0
Indole-3-propionic acid	1.0
Glycyl-L-tryptophan	4.8

[a] Acid-hydrolyzed casein (1%) was added to resting-cell suspensions to permit induction; cell concentration, 0.184 mg of nitrogen/tube; reaction time, 2 hr. The test compounds were added at a level of 200 μg/ml. Results are expressed as μg of indole produced.

the analogues were metabolized more slowly than the natural amino acid, thus permitting a longer period of inhibition. Both shikimic and anthranilic acids, known precursors of tryptophan (Yanofsky, 1960), were slightly inhibitory to synthetase formation, while allowing some induction of tryptophanase. This suggests that the addition of these compounds to the growth medium resulted in the production of tryptophan by the bacterial cells. However, some growth reduction was noted when the cells were grown in the presence of anthranilic acid. Lester and Yanofsky (1961) found that anthranilic acid inhibited growth of an *E. coli* mutant and increased the formation of synthetase by blocking the production of the tryptophan precursor, indole-3-glycerol phosphate. The fact that anthranilic acid decreased both growth and the level of synthetase while increasing tryptophanase in the present system points to another site of inhibition. In this connection, Burns (1961) reported that in *E. coli* anthranilic acid has the ability to protect tryptophan from the action of tryptophanase, thus permitting greater production of the inducible enzyme. Possibly anthranilic acid competes in a tryptophan reaction involved in the formation of cellular components essential for growth. Thus, tryptophan would be made available for the induction of tryptophanase and the repression of synthetase, while blocking of the hypothetical reaction would concomitantly decrease growth

TABLE 4. *Effect of 5-hydroxytryptophan on growth and tryptophan synthetase formation in Escherichia coli 9723E*[a]

5-Hydroxy-tryptophan	L-Trypto-phan	Indole	Growth		Trypto-phan synthetase activity
			Basal	Basal + glucose	
μg/ml	*μg/ml*	*μg/ml*			
500	0	0	58	98	6.2
100	0	0	62	95	12.4
50	0	0	67	97	8.4
25	0	0	72	100	10.4
5	0	0	85	125	17.6
0	0	0	370	284	55.2
500	50	0	335	270	
500	5	0	245	290	
500	0	50	288	274	
500	0	5	350	246	

[a] Cells were grown for 17 hr in basal media or basal + 10^{-1} M glucose. Aeration was maintained on a reciprocal shaker; cell concentration, 0.172 mg of nitrogen/ml; reaction time, 60 min. Growth was measured in a Klett-Summerson colorimeter at 420 mμ, and is expressed as Klett unit. Tryptophan synthetase activity is expressed as μg of indole removed.

Effect of 5-hydroxytryptophan on growth and tryptophan synthetase. In these experiments, the bacterial cells were incubated on a reciprocal shaker in 10 ml of medium contained in screw-cap tubes (16 × 125 mm). All levels of 5-hydroxytryptophan (5 to 500 μg/ml) added to the media inhibited both synthetase and growth (Table 4). Growth suppression was overcome by the addition of L-tryptophan or indole in amounts as low as 5 μg/ml. Restoration of growth in the presence of indole suggested that repression of synthetase by 5-hydroxytryptophan was not the mechanism for growth inhibition. Moyed (1960*a*) reported similar effects on growth and tryptophan synthetase formation when *E. coli* was grown in the presence of 5-methyltryptophan. He also found that 5-methyltryptophan inhibits the activity of the enzyme(s) converting shikimic acid-5-phosphate to anthranilic acid.

The possibility that 5-hydroxytryptophan acts in a similar fashion was investigated. The bacterial cells were grown under aerobic conditions in basal medium with added 5-hydroxy- or 5-methyltryptophan. Growth repression by the two analogues was almost completely overcome by the addition of shikimic acid or shikimic acid plus glutamine (Table 5). Surprisingly, anthranilic acid was only slightly effective in restoring growth. Permeability of anthranilate did not appear to be a factor, since some growth inhibition was noted when this compound was added to the basal medium devoid of 5-hydroxy- or 5-methyltryptophan. Trudinger and Cohen (1956) reported that anthranilic acid enhanced the growth-inhibitory action of 4-methyltryptophan, a compound which acts like 5-methyltryptophan. Lester and Yanofsky (1961) found that growth inhibition by high concentrations of anthranilic acid cannot be overcome by tryptophan. It is probable that a reaction not leading to tryptophan synthesis is inhibited by anthranilic acid, thus preventing it from alleviating growth reduction by compounds which block the formation of intermediates in tryptophan production.

TABLE 5. *Effect of anthranilic acid and shikimic acid on growth inhibition of Escherichia coli 9723E by 5-hydroxy- and 5-methyltryptophan*[a]

Additions to media (μg/ml)			Growth		
Shikimic acid	Glutamine	Anthranilic acid	Basal medium	Basal medium + 5-hydroxy-tryptophan (50 μg/ml)	Basal medium + 5-methyl-tryptophan (25 μg/ml)
0	0	0	240	87	58
5	0	0	248	153	—
10	0	0	246	177	155
50	0	0	226	200	180
100	0	0	190	169	—
10	20	0	228	220	172
0	0	5	206	126	—
0	0	10	222	118	68
0	0	50	186	104	88
0	0	100	158	98	—

[a] Cells were grown for 11 hr in basal media with or without additions under aerobic conditions; growth was measured in a Klett-Summerson colorimeter at 420 mμ.

To ascertain more precisely the site of inhibition by 5-hydroxytryptophan, cells of *E. coli* (strain T-24) were subjected to sonic oscillation at 20 kc for 5 min in a magnetostrictive oscillator (Mullard). The synthesis of anthranilic acid was studied, employing these extracts. In agreement with Moyed (1960*a*), it was found that tryptophan and 5-methyltryptophan were potent

TABLE 6. *Inhibition of conversion of shikimic acid and glutamine to anthranilic acid*[a]

Compound	Concn	Inhibition
	M	%
L-Tryptophan	2.5×10^{-5}	50
5-Methyl-DL-tryptophan	2.0×10^{-4}	50
Tryptamine	5.0×10^{-4}	50
5-Hydroxytryptophan	1.5×10^{-3}	32
Indole-3-acetic acid	5.0×10^{-4}	0
Indole-3-propionic acid	5.0×10^{-4}	0

[a] Cells of *E. coli* T-24 were grown for 15 hr at 37 C under aerobic conditions. Sonic extracts prepared from these cells were added at a concentration of 5.05 mg of protein/tube. In the absence of inhibitor, 60 mμmoles of anthranilic acid were formed in 60 min.

TABLE 7. *Effect of tryptamine on tryptophan synthetase activity of sonic extracts of Escherichia coli 9723E*

Tryptamine	Tryptophan synthetase
μg/ml	
0	41.6
5	31.2
25	27.6
50	26.0
250	6.4
500	4.8

[a] Cells grown 12 hr; cell-extract concentration, 0.54 mg of protein/tube; reaction time, 60 min. Results are expressed as μg of indole removed.

inhibitors of anthranilic acid synthesis (Table 6). However, 5-hydroxytryptophan was much less effective. These results may help to explain the effect on growth of 5-hydroxytryptophan as contrasted with 5-methyltryptophan. Even at the highest level employed, growth was not completely inhibited by 5-hydroxytryptophan (Table 4), whereas complete suppression of growth occurred when 5-methyltryptophan was added at concentrations above 100 μg/ml. It is not known whether the partial inhibition of the formation of anthranilic acid by 5-hydroxytryptophan is the only reaction affected by the analogue.

Effect of tryptamine on growth and enzymes in the tryptophan pathway. As already noted, tryptamine markedly repressed the growth of *E. coli* (9723E) and decreased the endogenous formation of tryptophanase (Table 2). Further study revealed that tryptamine was a potent inhibitor of synthetase activity in whole cells and sonic extracts (Table 7), inhibition being virtually complete in the presence of 500 μg/ml. The synthesis of anthranilic acid by bacterial extracts was also strongly retarded in the presence of tryptamine (Table 6). These data suggest that tryptamine inhibits growth by blocking the biosynthesis of tryptophan. However, the addition of tryptophan only partially alleviated growth depression (Table 8). Competition between tryptophan and tryptamine for entry into the cell did not appear to be a factor, since even high concentrations of tryptophan were not very effective in restoring growth. In this connection, it has been shown (Boezi and DeMoss, 1961) that tryptamine has no effect on the uptake of tryptophan in resting-cell suspensions of *E. coli*. It is probable that the compound blocks some other reaction in addition to those involved in tryptophan biosynthesis.

Further experiments revealed that the presence of tryptamine caused a long lag period in growth (Fig. 1). Moreover, growth suppression was gradually alleviated with time so that by 24 hr growth in the presence of inhibitor was close to that obtained in the absence of tryptamine. To investigate further this effect, bacterial cells that had "recovered" from the effect of tryptamine were harvested and inoculated into fresh medium with added tryptamine (Fig. 2). The medium from which the "recovered" cells

TABLE 8. *Effect of tryptophan and indole on growth inhibition of Escherichia coli 9723E by tryptamine*

Additions to media (μg/ml)			Growth[a]	
Tryptophan	Indole	Glycyl-L-tryptophan	With tryptamine (100 μg/ml)	Without tryptamine
0	0	0	10	142
5	0	0	30	140
10	0	0	42	144
25	0	0	62	137
100	0	0	16	128
0	5	0	26	127
0	10	0	33	116
0	50	0	15	118
0	0	50	44	145

[a] After 16 hr under aerobic conditions; read at 420 mμ in a Klett-Summerson colorimeter.

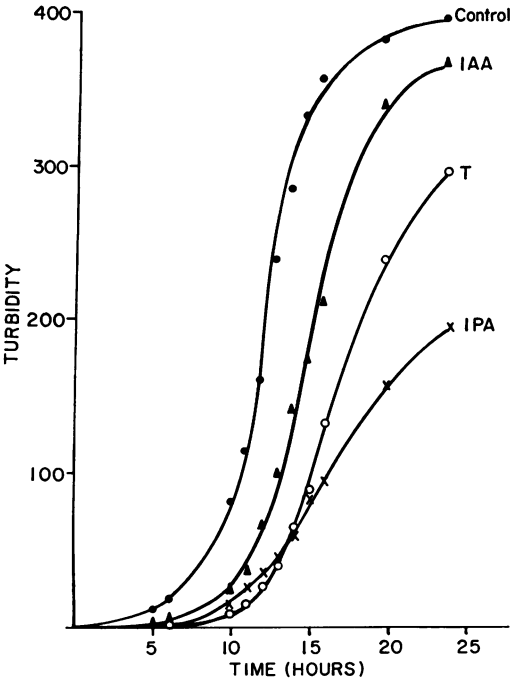

FIG. 1. *Effect of tryptophan analogues on growth of Escherichia coli 9723E. The cultures were aerated at 27 C. Analogues were added at a concentration of 100 μg/ml. IAA = indole-3-acetic acid; IPA = indole-3-propionic acid; T = tryptamine. Growth was measured in a Klett-Summerson colorimeter at 420 mμ.*

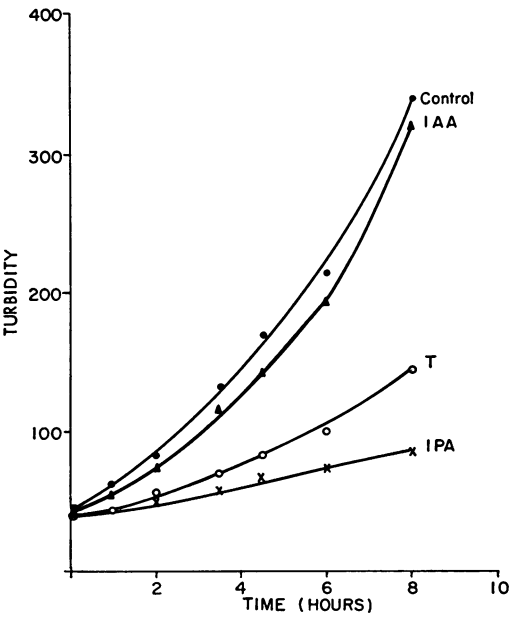

FIG. 2. *Effect of tryptophan analogues on growth of Escherichia coli 9723E previously cultured in the presence of these analogues. Cells grown in the presence of these compounds (Fig. 1) were recovered by centrifugation and reinoculated into fresh media with and without the analogues. Growth was measured in a Klett-Summerson colorimeter at 420 mμ. Other conditions as in Fig. 1.*

had grown was reinoculated with fresh cells (Fig. 3). Under these conditions, growth was reduced in the "recovered" cells but not in the flasks containing fresh cells and old medium. Furthermore, samples of old medium produced a deep pink color with the Salkowski reagent (Tang and Bonner, 1947), whereas fresh medium with added tryptamine gave no reaction. Samples of medium from which cells had recovered from growth did not inhibit the synthesis of anthranilic acid in bacterial extracts, a reaction strongly inhibited by tryptamine (Table 6). It would appear, therefore, that the rapid recovery in growth by cells grown in the presence of tryptamine is brought about by the breakdown of the inhibitor to an unidentified, nontoxic compound.

Effect of indole-3-propionic acid and indole-3-acetic acid on tryptophan synthetase and growth. The addition of indole-3-propionic acid or indole-3-acetic acid to the growth medium increased the formation of synthetase and decreased the growth of *E. coli* 9723E (Table 2). The stimulation of synthetase by these compounds was more marked with *E. coli* (Crookes), hence this strain was employed in the following experiments. The addition of indole-3-propionic acid or indole-3-acetic acid increased synthetase approximately sixfold, while decreasing growth (Table 9). Growth inhibition was less severe in the presence of indole-3-acetic acid. Moreover, significant stimulation of enzyme without growth reduction was noted when 10 μg/ml were used. The effect of these compounds appeared to be due to an inhibition of tryptophan production, since the addition of tryptophan or indole reduced synthetase stimulation and increased growth (Table 10). However, added tryptophan did not repress synthetase completely in the presence of indole-3-acetic or indole-3-propionic acid. These results are similar to those of Lester and Yanofsky (1961), who reported that 3-methylanthranilic acid or anthra-

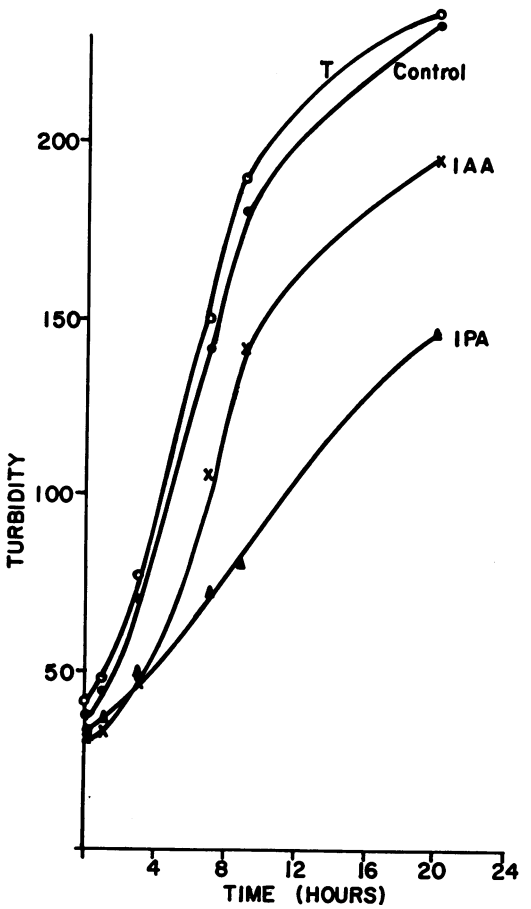

FIG. 3. *Growth of Escherichia coli 9723E in previously utilized media. The cultures were grown for 24 hr in the presence of the analogue (Fig. 1). Media were freed of bacteria by centrifugation, and reinoculated with fresh bacterial cells. Growth was measured in a Klett-Summerson colorimeter at 420 mμ. Growth conditions as in Fig. 1.*

nilic acid decreased growth and increased tryptophan synthetase by virtue of their ability to block the formation of indole-3-glycerol phosphate.

It was noted previously (Table 2) that cells grown in the presence of indole-3-acetic acid possessed a fair level of tryptophanase. The mechanism responsible for these seemingly contradictory results was studied next. In these experiments, the cells were grown at 27 C, on a rotary shaker, in 15 ml of medium contained in 300-ml flasks fitted with a colorimeter tube. Growth of the bacterial cells in the presence of

TABLE 9. *Effect of concentration of indole-3-propionic acid and indole-3-acetic acid on growth and tryptophan synthetase formation by Escherichia coli (Crookes)*[a]

Indole-3-propionic acid	Indole-3-acetic acid	Growth	Synthetase activity
μg/ml	*μg/ml*		
0	0	375	11.2
10	0	375	29.6
50	0	270	60.8
200	0	76	54.8
0	10	370	26.2
0	50	335	64.4
0	200	298	62.6

[a] Growth time, 16 hr; 0.104 mg of cell nitrogen/tube; reaction time, 40 min. Growth measured in a Klett-Summerson colorimeter at 420 mμ. Synthetase activity is expressed as μg of indole removed.

TABLE 10. *Effect of tryptophan and indole on growth and tryptophan synthetase formation by Escherichia coli (Crookes) in the presence of indole-3-acetic acid and indole-3-propionic acid*[a]

Additions to medium	Growth	Synthetase activity
None	335	10.4
Indole-3-propionic acid	66	62.4
Indole-3-acetic acid	248	78.6
Indole-3-propionic acid + L-tryptophan	305	25.2
Indole-3-acetic acid + L-tryptophan	320	14.8
Indole-3-propionic acid + indole	315	25.6
Indole-3-acetic acid + indole	315	42.2

[a] Growth time, 16 hr; 0.086 mg of cell nitrogen/tube; reaction time, 60 min; indole added at a concentration of 50 μg/ml; other compounds added at 200 μg/ml. Growth measured in a Klett-Summerson colorimeter at 420 mμ. Synthetase activity expressed as μg of indole removed.

either indole-3-propionic acid or indole-3-acetic acid was inhibited to the same degree until about 10 hr (Fig. 1). After this time, the cells grown with indole-3-acetic acid proliferated much more rapidly, and at 24 hr the total growth of such cells was comparable with that of the culture grown without inhibitors. In contrast,

cells grown with indole-3-propionic acid were inhibited to the extent of 50% at 24 hr. The increased growth after 10 hr in the presence of indole-3-acetic acid was not due to destruction of the inhibitor, since fresh cells inoculated into old medium showed growth inhibition (Fig. 3). However, cells which had recovered from inhibition by indole-3-acetic acid grew rapidly when placed in fresh medium with added inhibitor (Fig. 2). Growth inhibition by indole-3-propionic acid was not alleviated by these methods. The type of growth inhibition manifested by indole-3-acetic acid appears similar to that described by Moyed (1960*b*) for 2-thiozolealanine, a compound which blocks growth by inhibiting a reaction in the biosynthesis of histidine. Moyed (1961) showed that the initial growth inhibition by the analogue is overcome because of increased formation of the enzyme whose activity is blocked by 2-thiozolealanine. This increase in enzyme synthesis is caused by the decrease in histidine formation due to the inhibitory action of 2-thiozolealanine.

Such data may offer an explanation for the increase in tryptophanase in cells grown with indole-3-acetic acid. Thus, the increase may be attributed to a rise in formation of endogenous tryptophan after the cells had overcome the inhibition by the analogue. When growth inhibition by indole-3-acetic acid was greatest, the formation of tryptophanase was, in fact, inhibited (Table 11). However, cells harvested from the same cultures after they had overcome growth inhibition possessed higher amounts of tryptophanase, indicating that the endogenous formation of tryptophan had increased. In this regard, Lester (1961) found that indole-3-acetic acid and indole-3-propionic acid increased the formation of synthetase in *Neurospora crassa* without inhibiting growth. Moreover, these compounds did not increase synthetase by decreasing the endogenous formation of tryptophan. Although the precise site of action of indole-3-acetic acid and indole-3-propionic acid was not discovered in the present studies, it would seem likely that, at least in *E. coli*, these compounds inhibit tryptophan production.

TABLE 11. *Effect of indole-3-acetic acid on tryptophanase formation in Escherichia coli 9723E*[a]

Additions to growth medium (100 μg/ml)	Growth		Tryptophanase activity	
	11 hr	24 hr	11 hr	24 hr
None	138	430	3.2	1.4
Indole-3-acetic acid	74	400	0.8	7.2

[a] Tryptophanase activity is expressed as μg of indole produced in 60 min; 0.087 mg of cell nitrogen/tube. Growth measured in a Klett-Summerson colorimeter at 420 mμ.

ACKNOWLEDGMENT

This work was supported in part by a contract (NR 103-250) between the Office of Naval Research, Department of the Navy, and the University of Minnesota.

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