REPRESSION AND INHIBITION OF INDOLE-SYNTHESIZING ACTIVITY IN *NEUROSPORA CRASSA*

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ABSTRACT

LESTER, GABRIEL (Worcester Foundation for Experimental Biology, Shrewsbury, Mass.). Repression and inhibition of indole-synthesizing activity in *Neurospora crassa*. J. Bacteriol. **82**:215–223. 1961.—The possibility of repression and feedback inhibition as regulating mechanisms for the synthesis of tryptophan by *Neurospora crassa* has been examined in a tryptophan auxotroph which accumulates indole (and indoleglycerol). Indole-synthesizing activity was determined with germinated conidia suspended in medium lacking tryptophan. This activity was almost absent from cells cultured on germination medium containing more than 1.0 μmole L-tryptophan per ml, and increased with decreasing concentrations of L-tryptophan. A similar depression of the formation of indole synthesizing activity was caused by 6-methyl- and D-tryptophan, and less effectively by 5-methyltryptophan; 4-methyltryptophan was slightly stimulatory. Preformed indole synthesizing activity was inhibited by L-tryptophan, 4- and 6-methyltryptophan, and to a lesser extent by 5-methyltryptophan; D-tryptophan had no effect in this respect. The inhibition of preformed activity was partially reversed by anthranilic acid, which is a precursor of indole. However, anthranilic acid did not increase indole synthesis by cells wherein the formation of indole-synthesizing activity had been depressed by culture in the presence of high concentrations of L- or D-tryptophan. These observations indicate that regulation of tryptophan synthesis in *N. crassa* might result from the action of tryptophan as a repressor and as a feedback inhibitor. The relation of these results to other regulatory systems is discussed.

Studies on the regulation of tryptophan biosynthesis in certain bacteria have shown that tryptophan can repress the formation of various enzymes involved in the biosynthesis of tryptophan (Monod and Cohen-Bazire, 1953; Cohen and Jacob, 1959; Gibson and Yanofsky, 1960; Lester and Yanofsky, 1961). In *Escherichia coli* tryptophan exerts a repressive effect on the formation of many of these enzymes (Yanofsky, 1960), suggesting the possibility of coordinate repression (Ames and Garry, 1959) in this system. Tryptophan (and certain related compounds) also affects its synthesis by inhibiting the activity of an enzyme(s) in tryptophan biosynthesis (Trudinger and Cohen, 1956; Pardee and Prestidge, 1958; Moyed and Friedman, 1959; Moyed, 1960; Doy and Pittard, 1960). These effects of tryptophan are most marked, and almost complete repression and inhibition of enzyme activity can be achieved.

Initial studies on the regulation of tryptophan biosynthesis in *Neurospora crassa* (Lester, 1961) have shown that the formation of the terminal enzyme in the sequence, tryptophan synthetase, is not as markedly affected by tryptophan as in bacteria. At best, only a threefold variation in tryptophan synthetase levels in *N. crassa* was obtained. However, it was noted that trytophan markedly reduced the accumulation of anthranilic acid by a mutant strain, and when this strain was grown in the presence of high levels of tryptophan it showed a poor capacity for anthranilic acid synthesis. These observations suggested the possibility of a strong repressive and inhibitory action of tryptophan on the function and formation of an enzyme(s) preceding tryptophan synthetase in the biosynthesis of tryptophan. This possibility has been examined with respect to indole formation by a mutant strain of *N. crassa*, and the data to be presented indicate that tryptophan and certain of its analogues can exert strong repressive or inhibitory effects in the biosynthetic sequence leading to tryptophan.

MATERIALS AND METHODS

Organism. N. crassa strain Td_2-23 was obtained from C. Yanofsky. This strain is unable to

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produce tryptophan synthetase which can convert indole-3-glycerol phosphate or indole (plus serine) to tryptophan (but produces an antigenically closely related protein (Suskind, Yanofsky, and Bonner, 1955)) and, consequently, indole and indole-3-glycerol accumulate in its cultures. Strain Td_2-23 was chosen for two reasons: (i) indole or indole-3-glycerol could serve as an indicator of any repressive or inhibitory effects preceding the final step in tryptophan biosynthesis, and (ii) it could also be used for future studies on the formation of the altered tryptophan synthetase.

Production and germination of conidia. The media and other details of these procedures have been described previously (Lester, 1961). The medium for conidia production was supplemented with 100 μg L-tryptophan per ml. Cultures for conidia production were incubated at 35 C, which resulted in consistently better yields of conidia than incubation at 30 C.

Chemicals. The chemicals used in these studies were obtained through commercial sources.

Indole (or indole-3-glycerol)-synthesizing activity. Germinated conidia were harvested by centrifugation, washed three times with large volumes of sugarless germination medium, and resuspended in the same medium. The cells were pipetted into flasks, and glucose to a final concentration of 1.5% and other supplements were added. The flasks were incubated at 30 C with agitation. At intervals, 3.0-ml samples were drawn and placed in chilled tubes containing 1.0 ml of 0.1 M Na_2HPO_4, at pH 7.8, in 0.2 M K_2CO_3; the samples were stored at −20 C until assayed. Indole determinations were made shortly after the last sample was taken. Usually samples were taken at 1.5 and 3.0 hr after the beginning of incubation, and the values obtained for indole were corrected for the small amount of indole sometimes present at zero time. The specific activity of the indole-synthesizing system is designated as mμmoles indole produced per mg cell dry weight per 3.0 hr (all specific activities were based on the dry weight of cells at the beginning of incubation).

Indole-3-glycerol determinations were made on the day following the incubation, and usually only the last samples drawn were analyzed. For purposes of comparison, the specific activity for indole-3-glycerol synthesis is also designated as mμmoles indole-3-glycerol per mg cell dry weight per 3.0 hr. Determinations of indole-3-glycerol were not made in every experiment, since the assay for indole was more sensitive and rapid. However, there was a reasonably proportional correspondence between the values for indole and indole-3-glycerol.

Indole and indole-3-glycerol assays. The samples were thawed and vigorously shaken with 6.0 ml of toluene. Since the samples had been made alkaline, interfering acidic materials, such as indoleacetic acid or anthranilic acid, and neutral materials, such as indoleglycerol or tryptophan, were not extracted. The extraction mixture was briefly centrifuged to clarify the toluene phase, and samples of the toluene extract were assayed for indole (Yanofsky, 1955).

After removing the toluene layer, 0.1 ml of 4 N HCl was added to neutralize the potassium carbonate previously added, and the aqueous suspension was centrifuged. Samples of the supernatant were assayed for indole-3-glycerol by the periodate oxidation method of Yanofsky (1956); this method will also measure indole-3-glycerol phosphate.

It can be noted here that similar values for indole or indole-3-glycerol were obtained whether or not the samples were heated before assay. Thus, there was either no significant intracellular accumulation of these compounds, or the toluene-extraction step caused their release.

RESULTS

Indole synthesis by germinated conidia. The rationale for ascribing a specific activity value to the indole-synthesizing system is demonstrated in Table 1. These data show that the production of indole is directly proportional to the initial cell weight and to the duration of incubation. If glucose is omitted from the assay medium, the rate of indole synthesis is lowered and tends to fall off with time. Consequently, in all subsequent experiments to be reported here, the indole-synthesizing activity of germinated conidia was determined in the presence of glucose.

It should be pointed out that in the presence of glucose the dry weight of the cells increases by 25 to 40% during a 3-hr incubation period. However, similar increases in weight have been noted (*unpublished observations*) if the cells are incubated in a medium consisting of only phosphate buffer and sugar. Also, in some experiments, nitrogen determinations were made on

TABLE 1. *Effect of various factors on the indole-synthesizing activity of germinated conidia**

Flask no.†	Initial cell wt	Incubation	Indole synthesis	
	mg/ml	*hr*	*mμmoles/ml*	*mμmoles/mg*
1	1.9	1	20	10.5
		2	42	22
		3	64	33.5
2	3.8	1	41	10.7
		2	83	21.7
		3	122	31.9
3	5.7	1	63	11.0
		2	124	21.6
		3	180	31.4
4	4.3	1	38	8.8
		2	71	16.3
		3	100	23.2

* The germination medium contained 0.125 μmole L-tryptophan per ml.

† Flasks 1, 2, and 3 contained glucose at a concentration of 1.5%; no sugar was added to flask 4.

digests of cells and at best only a 5% increase in nitrogen was observed. Consequently, the increases in cell weight probably represent mainly an accretion of polysaccharide. If there is a change in protein content it probably is not related to indole-synthesizing activity which, per unit volume of suspension, remains constant for several hours. Thus, the initial dry weight appears to be a reasonable basis for comparing indole-synthesizing activities of germinated conidia.

Effect of L-tryptophan on the formation of indole-synthesizing activity. Conidia were germinated in media containing various concentrations of L-tryptophan and the dry weights and indole-synthesizing activities were determined. Table 2 shows that the yield of germinated conidia is practically proportional to the concentration of L-tryptophan, reaching a maximal value at about 0.25 μmole per ml. In the absence of L-tryptophan, there is a small but significant increase in weight which is equivalent to the growth increment expected from a concentration of 0.01 μmole L-tryptophan per ml, and might represent carryover of tryptophan from the medium used for conidia production.

TABLE 2. *Effect of L-tryptophan concentration in the germination medium on the formation of indole-synthesizing activity*

L-Tryptophan	Germinated conidia	Indole synthesis		Increase*		
				Growth	Indole synthesis	
μmoles/ml	*mg/ml*	*mμmoles/ml*	*specific activity*	*mg/ml*	*mμmoles/ml*	*specific activity*
0	1.08	21.7	20.1	0.17	17.2	100
0.025	1.53	34.3	22.4	0.62	29.8	48
0.05	2.05	50.8	24.8	1.14	46.3	41
0.10	3.03	82.5	27.2	2.12	78.0	37
0.25	3.31	21.8	6.6	2.40	17.3	7.2
0.5	3.41	13.3	3.9	2.50	8.3	3.3
1.0	3.30	2.0	0.6	2.39		
2.0	3.18	Nil	Nil	2.27		
Inoculum	0.91	4.5	5			

* The values for the dry weight and activity of conidia were subtracted from similar values for germinated conidia to estimate the activity formed during germination.

Conidia incubated in the absence of L-tryptophan show a fourfold increase in indole-synthesizing activity over nongerminated conidia, and with increasing concentrations of L-tryptophan there appears to be a gradual increase in activity up to a level of 0.1 μmole per ml. With further increases in concentration, the indole-synthesizing activity decreases markedly and cannot be detected in cells grown in a medium containing 2.0 μmoles L-tryptophan per ml. If indole-synthesizing activity is assessed on the basis of the relationship of increase in activity to increase in cell mass during germination, then it is observed (last three columns of Table 2) that the specific activity falls steadily with increasing L-tryptophan concentration. Thus, it appears that L-tryptophan has a severe repressive effect on the formation of indole-synthesizing activity.

Specificity of repression by L-tryptophan. The indole-synthesizing activities of germinated conidia obtained from media containing various analogues, precursors, and compounds derived from tryptophan are shown in Table 3. Only 6-methyltryptophan and D-tryptophan exert repressive effects comparable to L-tryptophan; however, at lower concentrations D-tryptophan is less effective than L-tryptophan. At high concentrations 5-methyltryptophan and, to a lesser

TABLE 3. *Effect of various compounds on the formation of indole- and indole-3-glycerol-synthesizing activities*

Addition to germination medium*	Expt 1			Expt 2		
	Indole synthesis	Indole-3-glycerol synthesis	Indole-3-glycerol/indole	Indole synthesis	Indole-3-glycerol synthesis	Indole-3-glycerol/indole
	specific activity	*specific activity*		*specific activity*	*specific activity*	
None	17.8	21.3	1.20	21.0	23.5	1.12
L-Tryptophan	0.4	Nil		1.0	Nil	
D-Tryptophan				3.5	Nil	
4-Methyl-DL-tryptophan	27.7	29.6	1.07	28.0	39.2	1.40
5-Methyl-DL-tryptophan	14.5	17.0	1.17	7.2	8.9	1.24
6-Methyl-DL-tryptophan	2.1	Nil		1.4	Nil	
Indole	19.2	20.8	1.08	13.9	15.4	1.11
Indole-3-acetic acid	21.1	25.7	1.22	20.2	27.5	1.36
Tryptamine				20.0	27.8	1.39
Anthranilic acid				24.8	35.4	1.43
Niacinamide				22.5	26.7	1.19

* The germination medium contained 0.15 μmole L-tryptophan per ml. The listed compounds were added at a concentration of 0.5 μmole per ml in experiment 1 (except L-tryptophan at 1.0 μmole per ml) and at a concentration of 1.0 μmole per ml in experiment 2.

extent, indole also reduce the formation of indole-synthesizing activity. The only other compound having a significant effect was 4-methyltryptophan, which afforded an increase in indole-synthesizing activity.

Table 3 shows that the synthesis of indoleglycerol parallels that of indole, with the respective ratios of these products falling between about 1.1 to 1.5. Since both indole and indoleglycerol are derived from the natural and immediate tryptophan precursor, indoleglycerol phosphate (Yanofsky, 1958), these results indicate that indole production is a reliable indicator of the over-all activity of the biosynthetic sequence of tryptophan formation preceding the final step. These results tend to negate the possibility that the repressive effects observed involve only the conversion of indole glycerol phosphate to indole. If this were the case, indoleglycerol phosphate or indoleglycerol should accumulate, and widely varying ratios of indoleglycerol to indole would be obtained.

Effect of L-tryptophan on indole-synthesizing activity. As indicated in the introduction, the regulation of tryptophan biosynthesis in bacteria is effected by the action of tryptophan as an inhibitor of early reactions in tryptophan biosynthesis, as well as its action as a repressor. The likelihood of such a dual action of tryptophan is suggested by the above results and the previous observation (Lester, 1961) of an inhibition of anthranilic acid synthesis by tryptophan. To assess its inhibitory action, the effect of L-tryptophan on preformed indole-synthesizing activity was examined. Germinated conidia obtained from a low L-tryptophan medium were examined for indole-synthesizing activity in the presence of various concentrations of L-tryptophan, and the results are given in Table 4. Here it is seen that L-tryptophan at concentrations above 0.025 μmole per ml severely inhibits indole synthesis, suggesting that tryptophan biosynthesis in *N. crassa* can be regulated by a

TABLE 4. *Effect of concentration of L-tryptophan on indole-synthesizing activity**

L-Tryptophan	Indole synthesis
μmole/ml	*specific activity*
0	28.2
0.001	28.4
0.005	28.4
0.025	19.7
0.125	3.1
0.625	1.2

* The germination medium contained 0.125 μmole L-tryptophan per ml.

TABLE 5. *Effect of various compounds on indole- and indole-3-glycerol-synthesizing activities**

Additions to assay medium†	Expt 1	Expt 2		
	Indole synthesis	Indole synthesis	Indole-3-glycerol synthesis	Indole-3-glycerol/ indole
	specific activity	*specific activity*	*specific activity*	
None	18.1	21.4	30.5	1.47
L-Tryptophan	0	0.8	Nil	
D-Tryptophan	17.7	19.6	30.2	1.54
4-Methyl-DL-tryptophan	0.9	1.0	Nil	
5-Methyl-DL-tryptophan	15.5	14.9	16.8	1.13
6-Methyl-DL-tryptophan	3.6	3.1	Nil	
Indole-3-acetic acid	19.4	20.6	31.3	1.52
Indole-3-propionic acid	16.5			
Indole-3-butyric acid	17.1			
Tryptamine	18.6	20.3	30.2	1.49
Anthranilic acid	20.9	22.1	24.3	1.10
Niacinamide		21.9	31.3	1.43

* The germination medium contained 0.15 μ mole L-tryptophan per ml.

† The compounds listed were added to the assay medium at a concentration of 0.5 μmole per ml in experiment 1, and 0.75 μmole per ml in experiment 2.

negative feedback inhibition of established enzyme activity.

Specificity of inhibition of indole-synthesizing activity by L-tryptophan. Table 5 shows the effects of various compounds on indole-synthesizing activity. Only L-tryptophan and 4- and 6-methyltryptophan markedly inhibit activity, with 5-methyltryptophan showing a moderate effect. It is of interest to note that while D-tryptophan acts as repressor it has no significant effect on indole-synthesizing activity once formed. On the other hand, 4-methyltryptophan, which stimulates formation of indole-synthesizing activity, is a potent inhibitor of this activity. Here, too, there is relatively constant proportionality between the synthesis of indole glycerol and indole.

Effect of anthranilic acid on inhibited and repressed indole-synthesizing activity. The preceding data have shown that relatively small amounts of L-tryptophan are sufficient to produce a large inhibition of established indole-synthesizing activity. This raises the question of whether the low indole-synthesizing activity of germinated conidia obtained from a medium high in L-tryptophan is due to a repression of enzyme formation, or to an inhibition of established indole-synthesizing activity by tryptophan accumulated during germination. The latter possibility is indirectly negated by the opposite effects of D-tryptophan and 4-methyltryptophan on the formation and functioning of indole-synthesizing activity. The low indole-synthesizing activity of cells germinated in the presence of D-tryptophan cannot be due to an inhibition of such activity by accumulated D-tryptophan since D-tryptophan does not inhibit established indole-synthesizing activity. Similarly, although 4-methyltryptophan inhibits established activity, it is apparently not accumulated in sufficient quantity during germination to affect subsequent determinations of indole-synthesizing activity.

However, a more direct examination of this problem could be made by assessing the effect of anthranilic acid on inhibited and apparently repressed indole-synthesizing activity. Since most cases of feedback inhibition of enzyme activity involve the first step in a biosynthetic sequence, it was reasonable to assume that anthranilic acid should restore indole-synthesizing activity that had been inhibited by L-tryptophan or 4-methyltryptophan. Previous observations (Lester, 1961) indicated that tryptophan and 4-methyltryptophan inhibited the synthesis of anthranilic acid. Repression, on the other hand, usually involves a decreased formation of most or all of the enzymes in a biosynthetic sequence. Thus, if the decreased indole-synthesizing activity of germinated conidia obtained from media with high concentrations of L- or D-tryptophan is due

TABLE 6. *Effect of anthranilic acid on indole synthesis*

Germination medium		Assay medium		
Addition	Concn	Addition*	Anthranilic acid	Indole synthesis
	μmole/ml			*specific activity*
L-Tryptophan	0.125	None	−	30.8
		None	+	23.1
		L-Tryptophan	−	1.0
		L-Tryptophan	+	4.6
		D-Tryptophan	−	30.8
		D-Tryptophan	+	22.9
		4-Methyl-DL-Tryptophan	−	8.2
		4-Methyl-DL-Tryptophan	+	23.3
L-Tryptophan	0.625	None	−	1.6
		None	+	1.8
L-Tryptophan + D-tryptophan	0.125 0.50	None	−	17.5
		None	+	17.1

* The L- and D-tryptophan were added at a concentration of 0.3 μmole per ml, 4-methyl-DL-tryptophan at 0.6 μmole per ml, and anthranilic acid at 0.5 μmole per ml.

to repression, rather than inhibition of activity, anthranilic acid should not restore indole-synthesizing activity.

Germinated conidia obtained from media with low or high L-tryptophan or high D-tryptophan supplements were examined for indole-synthesizing activity in the presence and absence of anthranilic acid. Also, the ability of anthranilic acid to reverse inhibition of activity by L-tryptophan and 4-methyltryptophan was examined. The results are shown in Table 6. The high concentration of L-tryptophan was such that some indole-synthesizing activity could be observed. As indicated previously, L-tryptophan and 4-methyltryptophan inhibit activity, whereas D-tryptophan has no effect; L-tryptophan appears to be a more effective inhibitor than 4-methyltryptophan. The addition of anthranilic acid causes a decrease in indole-synthesizing activity of about 25% in the absence or presence of D-tryptophan. The nature of this inhibition is not clear, although anthranilic acid has been observed to inhibit the formation of indoleglycerol phosphate in *E. coli* (Gibson and Yanofsky, 1960; Lester and Yanofsky, 1961). The inhibition of indole-synthesizing activity by L-tryptophan was partially reversed by anthranilic acid, and the inhibition by 4-methyltryptophan was reversed by anthranilic acid to give an activity equivalent to that obtained in the presence of anthranilic acid.

Germinated conidia obtained from media containing high levels of L- or D-tryptophan showed reduced indole-synthesizing activities, with L-tryptophan appearing to be more effective in this respect. In contrast to the above results, anthranilic acid had no significant effect on indole synthesis by these cells, as might have been expected if the low activities observed were due to an inhibition by accumulated tryptophan. Thus, it appears that both L- and D-tryptophan can repress the formation of indole-synthesizing activity.

DISCUSSION

A rigid proof of the operation of control mechanisms such as repression and negative feedback inhibition entails direct measurements of the formation and activity of enzymes. The data presented here have been derived from studies with intact cells, and the amounts or activities of the enzymes involved in tryptophan synthesis in *N. crassa* have been inferentially estimated from the variations in the end product(s) of their action. Under such conditions it is not possible to quantitatively evaluate the actions of various compounds as repressors or inhibitors of enzyme formation or function because of such factors as differences in permeability or metabolism. Also, in using the end product of series of reactions as an indicator it is difficult to precisely designate a site(s) of action for a particular repressor or inhibitor. But these studies appear so analogous to those obtained with more rigidly defined examples of repression and negative feedback control that it is reasonable to assume that the results obtained are due to the operation of such control mechanisms in the biosynthesis of tryptophan in *N. crassa*. The following discussion is premised on this assumption and the preceding considerations.

The capacity for indole synthesis by germinated conidia is almost completely lost during growth in the presence of excess L-tryptophan, suggesting that the formation of an enzyme(s)

in the sequence leading to indole is repressed by L-tryptophan. This action of L-tryptophan is fairly specific since only 5-methyl, 6-methyl-, and D-tryptophan act as repressors, whereas the other indolyl compounds tested were inactive or stimulated the formation of indole-synthesizing activity. The observed effectiveness of these compounds could be misleading since neither their intracellular concentrations nor their metabolic fates were critically examined. However, neither indoleacetic acid nor niacin, both metabolites of L-tryptophan, affected the formation of indole-synthesizing activity. Also, preliminary studies indicated that D-tryptophan, which acts as a repressor, is not metabolized. Thus, it would appear that the basic molecular structure required for the repression of indole-synthesizing activity is that of tryptophan. However, an apparent lack of stereospecificity is indicated by the similar effects of D- and L-tryptophan; somewhat analogous situations have been described for tyrosinase in Neurospora (Horowitz et al., 1960) and tryptophan pyrrolase in rat liver (Civen and Knox, 1960) which are effectively induced by the corresponding D-amino acids. These observations raise the question of whether D-amino acids might have a unique function as regulators of enzyme formation.

The present data do not permit an identification of the enzyme(s) repressed, and, since indole synthesis is the culmination of a long sequence of reactions, individual repressors might have different sites of action, as well as unequal effects at a particular site. In this respect it can be noted of the four repressors of indole-synthesizing activity only L-tryptophan and 6-methyltryptophan repress the formation of tryptophan synthetase (Lester, 1961). In the case of indole synthesis, L-tryptophan (and probably D-tryptophan) appears to repress the formation of an enzyme(s) in the sequence from anthranilic acid to indoleglycerol phosphate. Other studies (Lester, 1961) suggest that L-tryptophan also represses the formation of an enzyme(s) in the sequence leading to anthranilic acid, as well as tryptophan synthetase. Thus, L-tryptophan seems to repress the formation of at least three enzymes involved in the biosynthesis of tryptophan. This could suggest the occurrence of coordinate repression (Ames and Garry, 1959) in the biosynthesis of tryptophan. However, the effect of L-tryptophan on the formation of indole-synthesizing activity is quantitatively quite different from its effect on the formation of tryptophan synthetase. In terms of specific activities, indole-synthesizing activity can be made to vary from about 0 to 50 and tryptophan synthetase from about 100 to 300, by varying the concentration of L-tryptophan in the germination medium. Consequently, it would appear that a coordinate repression of the entire sequence of tryptophan biosynthesis does not occur; whether coordinate repression occurs in segments of the sequence prior to tryptophan synthetase has yet to be determined.

Besides acting as a repressor, L-tryptophan also markedly inhibits preformed indole-synthesizing activity. This inhibition is highly specific since, of the compounds examined, only 4- and 6-methyltryptophan were nearly as effective as L-tryptophan, whereas 5-methyltryptophan had a much smaller effect. In contrast to repression, the inhibition of indole-synthesizing activity is stereospecific as indicated by the lack of an effect by D-tryptophan. This rigid specificity supports the assumption that L-tryptophan inhibits enzymic activity involved in its biosynthesis.

At present only a rough approximation can be made as to the site of inhibition by L-tryptophan. As indicated above, L-tryptophan inhibits the synthesis of anthranilic acid, and the present work shows that the inhibition of indole synthesis by L-tryptophan (and 4-methyltryptophan) can be partially reversed by anthranilic acid. The incomplete reversal of this inhibition by anthranilic acid might suggest that L-tryptophan inhibits reactions coming after, as well as before, anthranilic acid. It might also be considered that anthranilic acid of itself is not the normal intermediate in tryptophan biosynthesis and, under the conditions employed, might not be as rapidly converted to indoleglycerol phosphate. The partial inhibition of indole-synthesizing activity by anthranilic acid alone also makes it difficult to use this compound to locate the site of inhibition by L-tryptophan. Nevertheless, these observations show that at least one site of inhibition by L-tryptophan is a reaction prior to anthranilic acid. On this basis it would appear that a negative feedback control mechanism is operative in the biosynthesis of L-tryptophan by *N. crassa*.

The concepts of repression and feedback inhibition have been developed almost exclusively, and certainly most precisely, from studies with bacteria, although examples of these regulatory phenomena have been reported in other organisms (Umbarger, 1961; Vogel, 1961). In Neurospora, aside from the present work, marked end product repression has not been observed (so far as the author could ascertain), although a situation resembling repression has been encountered (Horowitz and Shen, 1952) in the inhibition of tyrosinase formation by sulfate. Also, proline appears to have a small repressive effect on the formation of pyrroline-5-carboxylate reductase (Yura and Vogel, 1959). No repression was observed in the formation of enzymes involved in histidine biosynthesis (Ames and Garry, 1959), or those concerned with the biosynthesis of shikimic acid (Gross and Fein, 1960). On the other hand, kynureninase (Jacoby and Bonner, 1953; Wainwright and Bonner, 1959), dehydroshikimic acid dehydrase and protocatechuic oxidase (Gross, 1959), tyrosinase (Horowitz et al., 1960), and possibly β-galactosidase (Landman, 1951) appear to be inducible enzymes in *N. crassa*. If induction is considered as a reversal of repression (Vogel, 1957) then it might be assumed that repression is not uncommon in Neurospora. Feedback inhibition in *N. crassa* has not been extensively investigated, but its occurrence is indicated by the present studies and other observations of reduced accumulation of intermediates by mutants cultured in the presence of an excess of the required end product (Ames and Garry, 1959).

Variations in enzyme activities have been frequently observed during the course of growth and development of higher organisms, which might suggest the operation of repression and feedback control. The potential role of these control mechanisms in processes of differentiation have been noted often, and the occurrence of genetic alterations of these mechanisms suggests that they might be involved in speciation as well. The present studies with Neurospora indicate that the identification of repression and feedback control in complex organisms might well require the study of many or all of the enzymes in a biosynthetic sequence. Thus, a marked repression is apparent within the sequence of tryptophan biosynthesis prior to indoleglycerol phosphate, whereas only a mild repression was observed in the case of the terminal enzyme, tryptophan synthetase. A qualitative difference in the repression of these segments of the biosynthetic sequence is also indicated by the differences in repressor specificity. Another point to be considered, especially with differentiated organisms, is the stage of development; in this respect it can be noted that tryptophan synthetase and indole-synthesizing activities appear to be much lower in nongerminated than in germinated conidia. Thus, the expression or pattern of repression and feedback inhibition mechanisms in complex organisms might vary with the place in a biosynthetic sequence and the time of development. It might also be speculated that the disappearance of effective end product regulation of metabolism could be an evolutionary prelude to the development of other regulatory mechanisms, such as those exemplified by the action of hormones in higher organisms.

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