REGULATION OF EARLY REACTIONS IN THE BIOSYNTHESIS OF TRYPTOPHAN IN *NEUROSPORA CRASSA*

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ABSTRACT

LESTER, GABRIEL (Reed College, Portland, Ore.). Regulation of early reactions in the biosynthesis of tryptophan in *Neurospora crassa*. J. Bacteriol. **85**:468–475. 1963.—The regulation of the biosynthesis of tryptophan was examined in *Neurospora crassa*, strain ylo-tryp-la, which accumulates anthranil compounds. The block in this strain appeared to be in the conversion of 1-(*o*-carboxyphenylamino)-1-deoxyribulose-5-phosphate to indole-3-glycerol phosphate, since the dephosphorylated form of the former compound, the anthranilic ribonucleoside, and the anthranilic acid were found. Cells cultured on levels of L-tryptophan greater than 0.1 μmole per ml were almost devoid of anthranilate-synthesizing activity, whereas cells cultured on low levels of tryptophan (e.g., 0.025 μmole/ml) could produce anthranilate at a rate of 125 mμmoles per mg (dry wt) per hr. A repressive effect was also caused by D-, 5-methyl-DL-, and 6-methyl-DL-tryptophan, but none of these compounds was as effective a repressor as L-tryptophan. Neither 4-methyl-DL-tryptophan, tryptamine, nor indole-3-acetic acid repressed the formation of anthranilate-synthesizing activity. Preformed activity was strongly inhibited by L-tryptophan, and to a lesser extent by 4-, 5-, and 6-methyl-DL-tryptophan; D-tryptophan, tryptamine, or indole-3-acetic acid did not inhibit preformed anthranilate-synthesizing activity. These results are indicative of the operation of repression and feedback-inhibition mechanisms early in the biosynthetic sequence leading to tryptophan. The relation of these results to those concerned with other aspects of tryptophan biosynthesis is discussed.

The quantitative expression of biosynthetic sequences has been variously and often shown to be regulated by the end product of such sequences acting either as an inhibitor of the first reaction unique to a sequence (Umbarger, 1961) or as a repressor of the formation of several or all of the enzymes in a sequence (Vogel, 1961). Although the experimental basis for these phenomena of feedback inhibition and repression has been largely developed with bacteria, there is mounting evidence for the occurrence of such regulatory mechanisms in higher organisms as well.

Studies of tryptophan synthesis in *Neurospora crassa* have indicated that at least the later steps in the biosynthetic sequence are subject to regulation by tryptophan. The formation of the terminal enzyme in the sequence, tryptophan synthetase, is repressed by tryptophan, although the repressive effect of tryptophan is not great and only about three- to fivefold variations in activity have been observed, with a relatively high level of tryptophan synthetase remaining under the maximal conditions of repression yet obtained (Lester, 1961*a*; Matchett and DeMoss, 1962). By contrast, tryptophan synthetase in bacteria can be made to vary over a wide range in response to tryptophan, and only negligible amounts of enzyme are observed under conditions of maximal repression (Monod and Cohen-Bazire, 1953; Cohen and Jacob, 1959; Lester and Yanofsky, 1961). Consequently, it could have appeared that tryptophan biosynthesis in *N. crassa* was not grossly subject to end-product regulation.

However, an examination of indole (and indole-3-glycerol) accumulation by a tryptophan auxotroph of *N. crassa* showed that tryptophan and certain of its analogues markedly repressed the ability of this strain to form indole-synthesizing activity, and strongly inhibited preformed activity (Lester, 1961*b*). This suggested that tryptophan could play a decisive role in regulating its own synthesis, and it became of interest to determine the site(s) in the biosynthetic sequence where tryptophan might act. The studies on indole synthesis suggested that trypto-

phan repressed the formation of enzymes between anthranilic acid and indole, but that the site of feedback inhibition was at a point prior to anthranilic acid in the sequence. Other cursory observations (Lester, 1961*a*) also suggested that tryptophan inhibited the activity and repressed the formation of enzymes in tryptophan biosynthesis prior to anthranilic acid. This paper examines more closely the influence of tryptophan on the biosynthesis of anthranilic acid, and lends further support to the suggestions made above.

Materials and Methods

Organism. The data to be reported were obtained with a tryptophan auxotroph of *N. crassa*, strain ylo-tryp-l(10575)a, which is also characterized by the production of yellow rather than the usual orange conidia. This strain, supplied by D. Stadler, has the same genetic block in tryptophan synthesis as strain 10575 (which can grow on either indole or tryptophan), which was reported to accumulate anthranilic acid (Tatum, Bonner, and Beadle, 1944). However, as will be indicated subsequently, there is reason to believe that the block might occur after anthranilic acid, and that 1-(*o*-carboxyphenylamino)-1-deoxyribulose (anthranilic ribonucleoside) is the primary compound accumulated. Experiments similar to those reported here with strain ylo-tryp-la were performed with strain 10575A and similar results were obtained.

Production and germination of conidia. The details of these procedures have been described elsewhere (Lester, 1961*a*); the medium for conidia production was supplemented with 0.25 μmole/ml of L-tryptophan or indole. Washed conidia, at a concentration of 1.8×10^7/ml, were cultured 14 to 16 hr in half-strength Fries salts containing 0.5% sucrose and other supplements to be indicated; the cultures were incubated at 24 C with vigorous agitation on a rotary shaker.

Anthranilate-synthesizing activity. Germinated conidia were harvested by centrifugation, washed three times with large volumes of sugarless germination medium, and resuspended in the same medium. Measured volumes of the suspension of cells were pipetted into flasks, and glucose to a final concentration of 1.5% and other supplements were added. The flasks were incubated with agitation in a water bath at 30 C. At intervals, 3-ml samples were drawn and filtered through Whatman no. 2 paper, which retained the cells. The filtrates were assayed fluorometrically, using a Turner fluorometer equipped with a 360-mμ primary filter and a narrow-pass secondary filter at 405 mμ. A standard curve was prepared with anthranilic acid. The specific activity of the anthranilate-synthesizing system is designated as mμmoles of anthranilate produced per mg (dry wt) of cells per hr. (Specific activities are based on the dry weight of cells at the beginning of incubation.)

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

Results

Nature of the compound(s) accumulated by strain ylo-tryp-l(10575)a. In the course of these studies, filtrates from germination cultures and assay systems, with high levels of activity, were analyzed chromatographically. The filtrates were acidified and extracted with ethyl acetate; the extracts were condensed and chromatographed as described previously (Lester and Yanofsky, 1961). Two fluorescent spots were observed, one of which appeared in the same position as anthranilic acid. The other, slower-moving spot ran at the same rate as the anthranilic ribonucleoside accumulated by *Escherichia coli* strain T-26 grown in the presence of anthranilic acid (Lester and Yanofsky, 1961). The qualitative identification of the slower-moving spot as anthranilic ribonucleoside was based on procedures described by Doy and Gibson (1959), which, in addition to its chromatographic behavior and fluorescence, included a positive test for ribulose (Borenfreund and Dische, 1957) and a rapid reaction with alkaline triphenyltetrazolium (Smith and Yanofsky, 1960). Eluates of fluorescent areas giving these positive presumptive tests were hydrolyzed in alkaline solution, and when chromatographed again showed a fluorescent spot at a point corresponding to anthranilic acid. Thus, it is likely that, under the conditions employed for germination of conidia and subsequent assay, both anthranilic acid and the anthranilic ribonucleoside are produced. Because of these results, the fluorescence measured in the course of assay is considered to be a consequence of "anthranilate" rather than anthranilic acid-synthesizing activity on the part of ylo-tryp-la (although anthranilic acid was used as a reference standard). The

TABLE 1. *Influence of D-tryptophan on the time course of anthranilate synthesis by germinated conidia*

L-Tryptophan in germination medium	Anthranilate synthesis					
	Incubation time	Cell concn*	No D-tryptophan		With D-tryptophan†	
μmoles/ml	*min*	*mg/ml*	*mμmoles/ml*	*mμmoles/mg*	*mμmoles/ml*	*mμmoles/mg*
0.04	30	1.42	45	32	45	32
	60		90	63	85	60
	120		190	133	190	133
	180		294	207	286	201
0.10	30	1.88	2	1	0.6	0.3
	60		35	18	1.4	0.7
	120		128	68	11.5	6.1
	180		480	255	60	32
0.25	30	2.15	0.6	0.3	0.6	0.3
	60		1.0	0.5	1.2	0.6
	120		6.6	3.1	2.0	0.9
	180		30	14	4.7	2.2

* Dry wt of germinated conidia at 0 time.
† D-Tryptophan, 0.6 μmole/ml.

significance of these observations for the site of the biochemical block in tryptophan synthesis will be discussed later.

Anthranilate synthesis by germinated conidia. One difficulty encountered in the estimation of anthranilate-synthesizing activity was that the rate of accumulation of anthranilate in the assay medium increased with time. Table 1 (see column under "no D-tryptophan") shows that this lack of linearity was most marked when the concentration of L-tryptophan in the germination medium was nearly sufficient for maximal growth (0.10 μmole/ml) under the conditions employed, but was much less apparent when limiting or excess L-tryptophan was used. In an attempt to minimize the formation of anthranilate-synthesizing activity during its assay, D-tryptophan was added to the assay medium, since previous studies (Lester, 1961*b*) showed that D-tryptophan repressed indole-synthesizing activity in *N. crassa* but did not inhibit preformed activity. Table 1 shows that D-tryptophan had little effect on the linear rate of anthranilate production by germinated conidia from a medium with a low tryptophan supplement, indicating that D-tryptophan does not inhibit preformed anthranilate-synthesizing activity. However, D-tryptophan reduced the increase in the rate of anthranilate synthesis by germinated conidia grown on higher levels of L-tryptophan, providing a nearly constant rate of anthranilate accumulation in the assay medium for the first 2 hr of incubation. Consequently, D-tryptophan, at a level of 0.6 μmole/ml was incorporated into the assay medium as a routine procedure, and samples for analysis were taken at 45 and 90 min.

The genetic block in strain 10575 is incomplete (Bonner, Yanofsky, and Partridge, 1952) and, consequently, it is likely that strain ylo-tryp-la also can produce tryptophan from the constituents of the minimal medium. It seemed desirable, then, for an evaluation of the effects of exogenously added compounds on the formation of anthranilate-synthesizing activity, to reduce the endogenous synthesis of tryptophan. Previous studies (Lester, 1961*b*) showed that 4-methyltryptophan did not inhibit the formation of indole-synthesizing activity, but did inhibit preformed activity. This suggested that the presence of 4-methyltryptophan in the germination medium might reduce the endogenous formation of tryptophan. Since this would reduce the total amount of tryptophan available to germinating conidia, there should be less repression, and more anthranilate-synthesizing activity should be observed. The data in Table 2 support this supposition, insofar as germinated conidia grown in a medium containing 4-methyltryptophan show about 20% more anthranilate-synthesizing activity than when this compound is not included in the medium. It may also be noted that the yield of germinated conidia was reduced by about 15% by 4-methyltryptophan, which would be consistent with a reduction of endogenous tryptophan synthesis. Consequently, 4-methyl-DL-tryptophan at a concentration of 0.5 μmole/ml was routinely added to the germination medium.

Table 2 also shows that the production of anthranilate is directly proportional to the initial concentration of germinated conidia in the assay system. Thus, although there is an increase in dry weight during the assay, the initial dry weight appears to be a reasonable basis for comparing the anthranilate-synthesizing activities of germinated conidia. These results, together with those of Table 1 showing a linear production of anthranilate with time, permit the

TABLE 2. *Effect of addition of 4-methyl-DL-tryptophan to the culture medium on the subsequent production of anthranilate by germinated conidia*

Culture*		Assay		
4-Methyl-DL-tryptophan	Germinated conidia	Cell concn	Anthranilate synthesis	
			Amt†	Amt‡
μmoles/ml	*mg/ml*	*mg/ml*		
0	1.95	0.96	49	51
		1.92	98	51
		2.88	143	50
0.5	1.56	0.85	54	63
		1.70	105	62
		2.55	153	60

* Germination medium contained 0.04 μmole of L-tryptophan per ml.

† Expressed as mμmoles per ml per hr.

‡ Expressed as mμmoles per mg per hr.

normalization of anthranilate-synthesizing activities in terms of specific activity, i.e., mμmoles of anthranilate synthesized per mg of initial dry wt per hr.

Effect of L-tryptophan on the formation of anthranilate-synthesizing activity. The data in Table 1 indicate that L-tryptophan can repress the formation of anthranilate-synthesizing activity. To evaluate this effect more closely, conidia were germinated on several levels of L-tryptophan in the presence of 4-methyl-DL-tryptophan. The increase in yield of germinated conidia, over the inoculum, was almost proportional to the concentration of L-tryptophan, reaching a maximal value at about 0.20 μmole/ml (Table 3). If this data were plotted graphically, the growth curve could be extrapolated to the ordinate at a point equivalent to 0.25 mg (dry wt); this would represent a weight increase in the absence of added tryptophan equivalent to the growth increment expected from a concentration of 0.0125 μmole of L-tryptophan per ml. In the absence of 4-methyltryptophan, more growth is obtained at lower levels of L-tryptophan, with a growth increment equivalent to 0.025 μmole of L-tryptophan per ml with no addition, and maximal growth at a level of added tryptophan of about 0.06 μmole/ml. Thus, again, it would appear that 4-methyltryptophan inhibits the endogenous synthesis of tryptophan.

TABLE 3. *Effect of the concentration of L-tryptophan in the germination medium on the formation of anthranilate-synthesizing activity*

Culture*		Anthranilate synthesis		Increase†		
L-Tryptophan	Germinated conidia	Amt‡	Specific activity	Germinated conidia	Anthranilate synthesis	
					Amt‡	Specific activity
μmoles/ml	*mg/ml*			*mg/ml*		
0.025	1.19	90	76	0.70	87	124
0.05	1.62	104	64	1.13	101	89
0.075	2.10	34	16	1.61	31	19
0.10	2.46	11	4.5	1.97	8	4
0.20	2.81	2.3	0.8	2.32	Nil	Nil
0.40	2.84	2.7	0.9	2.35	Nil	Nil
Inoculum	0.49	3.0	6			

* Germination medium also contained 0.5 μmole of 4-methyl-DL-tryptophan per ml.

† Values for the dry weight and activity of the inoculum were substracted from similar values for germinated conidia.

‡ Expressed as mμmoles per ml per hr.

With increasing levels of added L-tryptophan, anthranilate-synthesizing activity decreases, becoming barely detectable at levels of L-tryptophan above 0.10 μmole/ml. Thus, it appears that L-tryptophan has a marked repressive effect on the formation of anthranilate-synthesizing activity.

Specificity of repression by L-tryptophan. The activities of germinated conidia obtained from media containing various analogues and a precursor of tryptophan are shown in Table 4. Only indole shows a repressive effect comparable to L-tryptophan, and this is probably due to its conversion to tryptophan as indicated by the higher yield of germinated conidia in the presence of indole. Both D-tryptophan and 6-methyl-DL-tryptophan show moderate repressive effects, and 5-methyl-DL-tryptophan is repressive to a small extent. Neither indole-3-acetic acid nor tryptamine have any significant effect on the formation of anthranilate-synthesizing activity. In general, the pattern of effects shown here are similar to those obtained previously (Lester, 1961*b*) with the same compounds on the formation of indole-synthesizing activity, but with

TABLE 4. *Effect of various compounds on the formation of anthranilate-synthesizing activity*

Culture additions*		Germinated conidia	Anthranilate synthesis (specific activity)
Compound	Amt		
	μmoles/ml	*mg/ml*	
None		1.40	69
L-Tryptophan	0.10	2.61	0.6
D-Tryptophan	0.25	1.59	40
5-Methyl-DL-tryptophan	0.50	1.49	55
6-Methyl-DL-tryptophan	0.50	1.51	45
Indole	0.10	2.88	1.3
Indole-3-acetic acid	0.50	1.40	63
Tryptamine	0.50	1.41	64

* Germination medium also contained 0.04 μmole of L-tryptophan + 0.5 μmole of 4-methyl-DL-tryptophan per ml.

TABLE 5. *Effect of concentrations of L-tryptophan on preformed anthranilate-synthesizing activity*

L-Tryptophan	Anthranilate synthesis* (specific activity)
μmoles/ml	
0	75
0.015	21
0.03	6
0.06	2.1
0.10	1.7
0.20	1.7

* Germinated conidia were obtained from a medium containing 0.04 μmole of L-tryptophan + 0.5 μmole of 4-methyl-DL-tryptophan per ml.

some quantitative differences to be discussed subsequently.

Effect of L-tryptophan on preformed anthranilate-synthesizing activity. Germinated conidia from a low L-tryptophan medium were examined for activity in the presence of various concentrations of L-tryptophan (Table 5). It is seen that the anthranilate-synthesizing system is extremely sensitive to L-tryptophan, with a level of 0.015 μmole/ml causing about 70% inhibition. This inhibition is probably minimal, since it is likely that some of the L-tryptophan supplied was used for growth. These results suggest that tryptophan biosynthesis in *N. crassa* is subject to regulation by a negative feedback effect of tryptophan on a reaction in the biosynthetic sequence prior to

TABLE 6. *Effect of various compounds on preformed anthranilate-synthesizing activity*

Addition		Anthranilate synthesis* (specific activity)
Compound	Amt	
	μmoles/ml	
None		74
L-Tryptophan	0.1	1.9
4-Methyl-DL-tryptophan	0.5	39
5-Methyl-DL-tryptophan	0.5	68
6-Methyl-DL-tryptophan	0.5	43
Indole	0.2	2.3
Indole-3-acetic acid	0.5	74
Tryptamine	0.5	74

* Germinated conidia were obtained from a medium containing 0.04 μmole of L-tryptophan + 0.5 μmole of 4-methyl-DL-tryptophan per ml.

anthranilate synthesis. The synthesis of anthranilate in this strain is more sensitive to tryptophan than the previously reported (Lester, 1961*b*) synthesis of indole.

Specificity of inhibition of preformed anthranilate-synthesizing activity. Table 6 shows that, as in the case of repression, only indole (of the compounds examined) approximates the inhibitory effect of L-tryptophan on preformed activity. As suggested earlier, 4-methyl-DL-tryptophan also inhibits activity, and a similar effect is produced by 6-methyl-DL-tryptophan. The slight inhibition of activity by 5-methyl-DL-tryptophan is barely significant, and neither indole-3-acetic acid nor tryptamine have any effect. It has already been shown (Table 1) that D-tryptophan does not inhibit preformed anthranilate-synthesizing activity.

DISCUSSION

The pathway of tryptophan biosynthesis in coliform bacteria has been almost completely delineated (Yanofsky, 1956*a*, 1956*b*; Gibson, Doy, and Segall, 1958; Srinivasan, 1959; Smith and Yanofsky, 1960; Gibson and Yanofsky, 1960; Doy, Rivera, and Srinivasan, 1961). The reactions are schematized as follows:

$$5\text{-PS} \xrightarrow{I} \text{AA} \xrightarrow{II} \text{PRA} \xrightarrow{III}$$

$$\text{CDRP} \xrightarrow{IV} \text{IGP} \xrightarrow{V} \text{Tryptophan}$$

where 5-PS is 5-phosphoshikimic acid; AA, anthranilic acid; PRA, *N*-(5′-phosphoribosyl)

anthranilic acid; CDRP, 1-(*o*-carboxyphenylamino)-1-deoxyribulose-5-phosphate; and IGP, indole-3-glycerol phosphate. On the basis of studies with tryptophan auxotrophs, it appears likely that the same sequence of reactions occurs in *Neurospora*, and enzymatic evidence has been obtained for some of the reactions between anthranilic acid and tryptophan (Yanofsky and Rachmeler, 1958; Wegman and DeMoss, 1962).

The present work suggests that strain ylo-tryp-la is blocked at reaction IV, since the dephosphorylated derivative of CDRP (anthranilic ribonucleoside) accumulated in the conidia germination medium and in the medium used for determining anthranilate-synthesizing activity. Reaction II could be implicated as the site of the block by assuming that the accumulated anthranilic acid inhibits reaction IV, as is the case in *E. coli* (Gibson and Yanofsky, 1960; Lester and Yanofsky, 1961), and the accumulation of anthranilic ribonucleoside would be a consequence of the leakiness of this mutant. The same situation could occur if the block were at reaction III, since the lability of *N*-ribosylanthranilic acid under the acid conditions encountered (Doy, 1961) would mitigate against the accumulation of this compound and its decomposition product, anthranilic acid, would accumulate instead. Although both anthranilic acid and anthranilic ribonucleoside have almost always been visualized in the chromatograms of various samples, there are two kinds of observations which argue against the block occurring at a site other than reaction IV. First, the intensity of the fluorescence of the anthranilic ribonucleoside spots is often greater than that of anthranilic acid in the same sample; this visually estimated difference is probably minimal since the fluorescence of anthranilic acid is about 1.3 to 1.4 as great as that of anthranilic ribonucleoside on a molar basis. Also, in one experiment the relative amounts of these two compounds were determined, and the ratio of anthranilic acid to anthranilic ribonucleoside was found to increase with time of incubation in the assay system. Second, in one instance the germination medium was found to contain only anthranilic ribonucleoside; no anthranilic acid was visualized on the chromatogram. This predominance of anthranilic ribonucleoside would not be expected if its occurrence depended on the prior accumulation of anthranilic acid. The secondary appearance of anthranilic acid suggests that it might be a product of the decomposition of anthranilic ribonucleoside. Thus, it seems likely that the site of the block is at reaction IV, and strain ylo-tryp-la belongs to that class of mutants designated "indole-2" by Wegman and DeMoss (1962).

The site of the block in strain ylo-tryp-la has not been rigorously defined, but the present experimental data should have similar significance, whether the block is at reaction II, III, or IV. This is so because, first, the fluorometric assay employed would measure all the anthranil compounds involved, so that, although the measured fluorescence did not indicate which or how much of each compound was present, differences in fluorescence would reflect differences in anthranilate-synthesizing activity. As indicated above, the use of anthranilic acid as a standard could not have given apparent values which differed from the real values for anthranilate by a factor of more than about 1.3. Second, anthranilic acid is the first compound to occur in this part of the sequence, and the occurrence of fluorescence is dependent on the prior synthesis of anthranilic acid, no matter which anthranil compounds are actually measured. Therefore, the following observations can be considered in terms of that part of the sequence of tryptophan biosynthesis leading to anthranilic acid.

The formation of anthranilate-synthesizing activity can be almost completely abolished by relatively low levels of L-tryptophan, which indicates that regulation of tryptophan synthesis by repression involves early as well as later reactions in the biosynthetic sequence. Similarly, preformed anthranilate-synthesizing activity is markedly inhibited by L-tryptophan, indicating that regulation by feedback inhibition probably has as its site of action the first reaction unique to tryptophan biosynthesis.

The pattern of specificity in the repression of anthranilate-synthesizing activity resembles that previously observed (Lester, 1961*b*) in the case of indole-synthesizing activity. Thus, D-tryptophan and 6-methyl-DL-tryptophan repress both activities, although not as effectively as L-tryptophan, and 5-methyl-DL-tryptophan is only weakly effective as a repressor. A different pattern of specificity is observed with respect to feedback inhibition, in that preformed indole-

and anthranilic-synthesizing activities were inhibited by 4-, 5-, and 6-methyl-DL-tryptophan (again less effectively than by L-tryptophan) but D-tryptophan had no effect on either preformed activity. Since anthranil compounds are precursors of indole (and indole glycerol), the above correspondence would be expected. However, rather marked differences of a quantitative nature have been observed with respect to the sensitivity of these systems to repressors and feedback inhibitors, as well as to the activity of these systems.

The formation of anthranilate-synthesizing activity is almost completely repressed when the germination medium contains 0.10 μmole of L-tryptophan per ml, whereas a level of 0.25 to 0.5 μmole per ml is required to produce a similar repression of indole-synthesizing activity. On the other hand, 5-methyl-DL-, 6-methyl-DL-, and D-tryptophan appear to be more efficient repressors of indole-synthesizing activity than of anthranilate-synthesizing activity. A similar situation is encountered in the case of feedback inhibition; L-tryptophan is more effective in inhibiting anthranilate synthesis, whereas analogues of tryptophan have a greater inhibitory effect on indole synthesis. The basis for these differences is unknown and might be attributed to the fact that different strains of *Neurospora* were used in these studies, and that the sensitivity of the regulatory mechanisms might be subject to variation. It is also possible that some of these differences, particularly with respect to the tryptophan analogues, might be attributed to a difference in the repression or inhibition of reaction IV, which separates the anthranil portion from the indolyl portion of the sequence. In this respect, it should be noted that the formation of tryptophan synthetase in strain 10575 is less sensitive to tryptophan than is the formation of anthranilate-synthesizing activity, and the formation of tryptophan synthetase is hardly affected at all by various analogues of tryptophan (Lester, 1961*a*). Thus, in similar strains, the sensitivity to repression is not the same for all the enzymes of the sequence; repression is not coordinate in tryptophan biosynthesis in *Neurospora*. It might be speculated that repression might exhibit greater individuality when the enzyme-determining genes for a biosynthetic sequence are distributed among different chromosomes (as is the case for tryptophan in *Neurospora*) than when all are clustered on the same chromosome (as is the case for some coordinately repressed sequences in *E. coli*).

Besides exhibiting quantitative differences in repressibility, the three systems so far examined also show differences in activity. The maximal specific activities observed, under comparable conditions and on a comparable basis, are 125 for anthranilate synthesis, 40 for indole plus indole glycerol synthesis, and about 100 for tryptophan synthetase. Thus, under conditions of minimal repression, reaction IV might be expected to be the rate-limiting reaction in the sequence, and it has been recently observed (*unpublished data*) that anthranilate accumulates in cultures of a tryptophan synthetase mutant which also accumulates indole and indole glycerol. [The nature of the anthranil compound(s) has not been determined as yet.] However, because of the differential susceptibility to repression of these systems, this lack of coordination in activity disappears in the presence of small amounts of L-tryptophan, somewhat less than 0.1 μmole/ml. Then, tryptophan synthetase exhibits the greatest activity, followed by indole-synthesizing activity, and anthranilate-synthesizing activity is the least of the three; in this situation, no precursors would accumulate. Thus, although the repression of different segments of the sequence is not coordinate, the end result of repression by tryptophan is an economical biosynthesis of tryptophan.

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