Table 1. Specific activity of various sulfur-containing compounds obtained from roots, bleeding sap, and shoots after feeding $S^{35}O_4^{--}$ to the roots of nodulated plants of field pea. (Specific activities are expressed as counts per minute per microgram of sulfur.)

Compound	Hours after supplying $S^{35}O_4^{--}$		
	1	24	48
Roots			
Protein methionine	81.8	222	294
Protein cysteine	111	201	208
Free methionine	12.7	33.4	199
*Bleeding sap**			
Free methionine	1710	1159	428
Free glutathione			(10.1)
Free cysteine		(6)	
Shoots			
Protein methionine		242	472
Protein cysteine		307	644
Free methionine		(10.2)	31.6

* From 0.19 to 0.36 percent of the total S^{35} of the sap was recovered in the organic compounds, methionine, glutathione, and cysteine. These together constitute less than 2 percent of the total nitrogen of the bleeding sap and represent a concentration of 2 to 8 μg of sulfur per milliliter. Cysteine is present in smaller amounts than glutathione and methionine.

samples. Total radioactivity of the shoot increased throughout the 2 days of the experiment, while that of roots and sap decreased. Organic compounds of sulfur were labeled in the sap and roots within 1 hour, but in shoots not until after 4 hours. The specific activities of certain organic compounds of the root, shoot, and sap were determined by combining data from an amino acid analyzer with measurements of the radioactivity of relevant fractions of eluate from the analyzer made with a liquid scintillation counter. The data are presented in Table 1. After 1 hour, sulfur-containing amino acids were labeled in the protein and water-soluble fractions of the root, and methionine of high specific activity was recovered from the sap. At this time the S^{35} had not exchanged with organic compounds of sulfur in the shoot, so that the labeling of cysteine (cystine) and methionine of the root must have been implemented by its own reductase system.

The data suggest that the processes leading to a release of organic sulfur to the xylem are separate from the general metabolism of the root. Thus, the specific activity of the methionine of the sap is always many times greater than that of either the soluble phase or the protein of the root. Furthermore, the total S^{35} label recovered in methionine of the sap decreased over the 2 days of the experiment, while that of the body of the root increased. These findings suggest that the methionine released to the sap is produced in tissues and cellular compartments adjacent to the xylem, and that its synthesis is closely connected with the transport of sulfate across the root. At the same time, release of organic compounds of sulfur to the xylem is clearly a selective process, since although many compounds of sulfur are present in the soluble phase of the root, only a few of these carry sulfur to the shoot system.

Out of every thousand atoms of S^{35} leaving the root only two to four atoms were bound to organic compounds (Table 1). Judging from rates of bleeding of roots, this contribution of organic sulfur must be of little significance to the nutrition of the whole shoot system, and it certainly cannot be held responsible for the large increases in the labeling of shoot protein which took place throughout the experiment. However, when radioactive sulfate is supplied externally to the root only one element of the complex of transport activities within the whole plant is revealed. For instance, it fails to determine whether circulation of free sulfate occurs between phloem and xylem pathways and, if such a circulation does occur, whether it is accompanied by further reduction in the roots or translocation of organic sulfur from the shoots. Similarly, it provides no explanation as to why S^{35} exchanged readily with the cysteine and glutathione of the roots, but only extremely slowly with these same compounds in the bleeding sap.

Essentially similar results were obtained when $S^{35}O_4^{--}$ was supplied to roots of field pea, with nitrate being used as a source of nitrogen. The assimilation of sulfate can therefore be considered as an integral part of the normal metabolism of the root rather than a specialized activity of the root nodules. Parallel studies of the synthesis of organic compounds of nitrogen in the field pea have shown that both nodulated roots and roots relying on nitrate function effectively in synthesizing certain of the amides and amino acids required for protein synthesis and the establishment of soluble reserves of nitrogen in the shoot (*7*, *8*). It would be interesting to see how these functions of the root are related to the metabolism of sulfate.

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References and Notes

1. G. Reuter and H. Wolffgang, *Flora* **142**, 146 (1954); N. E. Tolbert and H. Wiebe, *Plant Physiol.* **30**, 499 (1955).
2. D. Féjer, *Acta. Biol. Acad. Sci. Hung.* **9**, 159 (1959).
3. E. G. Bollard, *Ann. Rev. Plant Physiol.* **11**, 141 (1960).
4. M. D. Thomas, *Encyclopedia of Plant Physiology* (Springer, Berlin, 1958), vol. 9; B. B. Biswas and S. P. Sen, *Indian J. Plant Physiol.* **2**, 1 (1959); L. G. Wilson, *Ann. Rev. Plant Physiol.* **13**, 201 (1962).
5. J. L. Liverman and J. B. Ragland, *Plant Physiol.* **31**, vii (1956).
6. J. S. Pate, *Plant Soil* **17**, 333 (1962).
7. ———, J. Walker, W. Wallace, *Ann. Bot.*, in press.
8. J. S. Pate and W. Wallace, *ibid.* **28**, 83 (1964).
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Adaptive Enzyme Synthesis: Its Inhibition as a Possible Analogue of Immunological Tolerance

Abstract. *Substrate induction of tryptophan pyrrolase in the liver of rats is inhibited by prior treatment of very young rats with tryptophan. This inhibition seems analogous to immunological tolerance, which can be produced by prior treatment with the antigen. The findings provide support for an analogy between mechanisms of adaptive enzyme synthesis and those involved in adaptive immunity.*

The phenomena of immunological paralysis (*1*) and immunological tolerance (*2*) have been difficult to account for in current theories of antibody formation. A completely satisfactory hypothesis has not been proposed, and this necessitates further investigations into the mechanism of the induction of paralysis and tolerance. Both situations of unresponsiveness can be specifically induced by excess of antigen, and early life is the period during which animals are most susceptible to the induction of the unresponsiveness.

Immunological tolerance similar to immunological responsiveness is a property of a population of lymphoid and reticular cells which constitute the immunological system. This population consists of different cell types, and their relations to each other are only partly known. The changes in this cell population which constantly occur in response to contact with antigens severely complicate the study of the intracellular processes that lead to antibody production or unresponsiveness.

Since the required condition of a stable cell population could not be

found in the immunological system we have looked for other protein-synthesizing cells that might serve as a model for the study of antibody synthesis and the induction of unresponsiveness. The analogy between the induction of antibody by antigen and the induction of so-called adaptive enzymes by their specific substrates has often been discussed (*3*). We have investigated the question of whether the resemblance between these two processes is more than superficial by attempting to produce a paralysis of enzyme induction, using a procedure which is effective in producing immunological tolerance to antigens.

We chose for the induction tryptophan pyrrolase (TP), an enzyme which has been extensively studied (*4*). In adult rats TP activity rises rapidly after a single intraperitoneal injection of tryptophan and reaches a maximum after 3 to 4 hours (*5, 6*). This increase is, at least for the greater part, due to an increase in enzyme protein according to Feigelson and Greengard (*7*), who estimated the enzyme by an immunochemical method. This rapid response resembles the secondary antibody response in immunologically sensitized animals. A situation analogous to the primary immune response, which involves a lag period of several days to achieve peak antibody titers, could be visualized with regard to the development of TP activity in young animals.

The liver of newborn rats has no TP activity; the enzyme is formed in the course of the first 2 weeks of extrauterine life, presumably in response to tryptophan derived from extraneous sources (the food or the bacterial flora of the gut, or both) or under influence of the cortical steroids. There is an additional similarity between synthesis of this enzyme and antibody synthesis in that in early life there is a period during which synthesis cannot be induced to any significant extent (*8*). In view of possible differences owing to the rat strain and diet, we repeated these observations and found the unresponsive period to last throughout the first week of age (Fig. 1).

To investigate whether the analogy between the two systems of protein synthesis can be further extended, we attempted to produce unresponsiveness to induction by tryptophan by exposing young animals to an excess of the amino acid. Baby rats of a random-bred albino strain received single or repeated daily intraperitoneal injections of tryptophan (1 mg per gram of body weight) in physiological saline, and an equal number of littermates matched according to body weight were similarly treated with physiological saline. At intervals after the end of this treatment, an experimental and a control rat each received an intraperitoneal injection of tryptophan (1 mg per gram of body weight); these were the induced group. Another experimental and a control rat received saline to serve as noninduced controls. Each experiment included therefore four different animals which had been treated as follows: tryptophan treated, tryptophan induced; tryptophan treated, noninduced; saline treated, induced; saline treated, noninduced. Three hours after treatment the rats were killed by decapitation, and the liver was excised. The liver was washed carefully with 0.14*M* KCl to remove any traces of amino acid from the surface in the case of intraperitoneally injected animals, and the tissue was homogenized in seven volumes of a mixture of 0.14*M* KCl and 0.0025*N* NaOH (*6*).

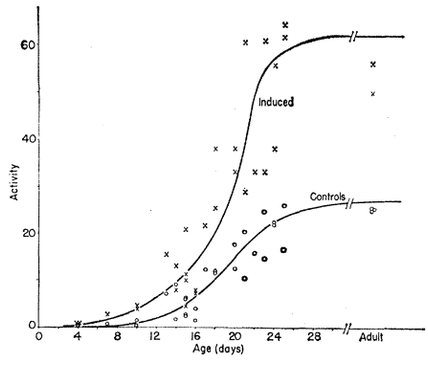

Fig. 1. "Natural" and induced tryptophan pyrrolase activity (micromoles of kynurenine per gram of protein per hour) in the liver of rats at various ages. All values are corrected for extinction of samples incubated in the absence of substrate. Rats were killed 3 hours after the inducing injection of tryptophan. Curves were drawn by eye to fit experimental points. Crosses, induced; circles, noninduced controls.

The incubation mixture had the following composition: phosphate buffer, *p*H 7.0, 50 m*M*; L-tryptophan, 2.5 m*M*; hematin, 1 μM; ethylenediaminetetraacetic acid (EDTA), 25 m*M*; and liver homogenate, 25 percent. The hematin was added to ensure saturation of the enzyme with its cofactor (*9*); EDTA was added to the reaction mixture because in the liver of young animals an inhibiter of tryptophan pyrrolase is present that is inactivated by EDTA (*10*). Control incubations were performed without tryptophan in the medium. The mixtures were shaken in 50-ml erlenmeyer flasks incubated in a water bath at 37°C; samples (4 ml) were removed at intervals (0, 30, 60, and 90 minutes) and deproteinized with 2 ml of 15-percent metaphosphoric acid.

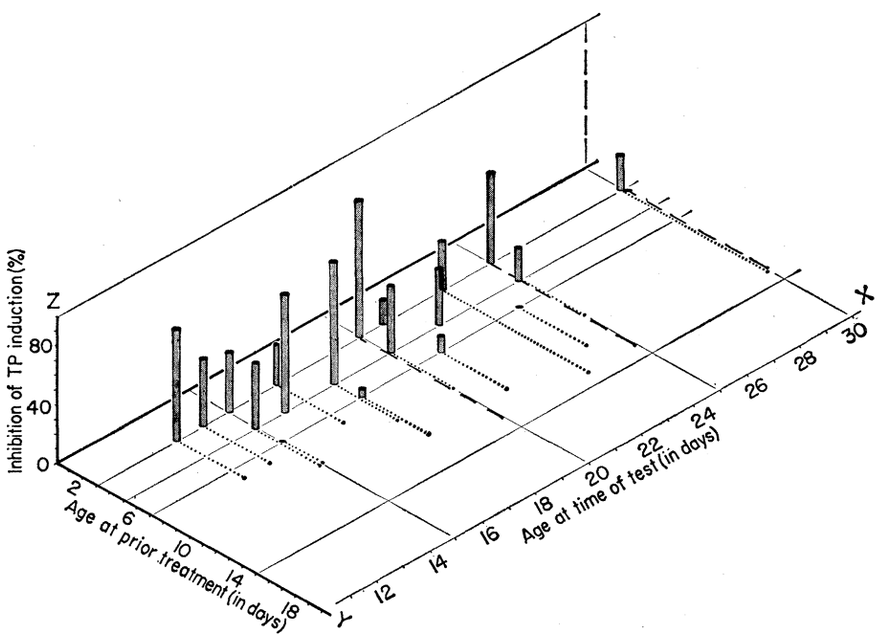

Fig. 2. Degree of inhibition of the substrate-induced tryptophan pyrrolase activity in animals treated with the substrate at an early age. X-axis: age at day of induction; y-axis: age at prior treatment (dotted lines designate periods of treatment); z-axis: percentage inhibition as compared to controls.

The precipitate was removed by centrifugation and the supernatant was neutralized with 2*N* NaOH. Kynurenine was estimated in a Beckman DK2 recording spectrophotometer by recording the extinction between 400 and 325 mμ. The values recorded at 350 mμ—representing the mean absorption peak—were used to calculate the amount of kynurenine formed, with a molar extinction of 4700 as derived from measurements with the pure compound (Sigma). Lee (*5*) used a molar extinction of 5150 at 370 mμ and Knox and Auerbach (*11*) a value of 4540 at 365 mμ.

The kynurenine values were expressed as micromoles per gram of protein in the incubation mixture. The protein content of the homogenate was estimated by a modified biuret reaction (*12*). The TP activity was calculated from the amount of kynurenine formed during the 1-hour period between 30 and 90 minutes of incubation and corrected for the extinction observed in the samples incubated without substrate. No kynurenine was formed under these conditions; these control blanks served as a correction for the presence in the samples of a slight opalescence which varied with the length of the incubation period. In the case of the measurements of induced activity by tryptophan, corrections were also made for the activity of the noninduced livers. The period between 30 and 90 minutes was chosen because the rate of kynurenine formation slowly increases and reaches a rather constant value after 30 minutes of incubation.

Table 1. The effect of prior treatment with tryptophan, at indicated intervals after birth, on tryptophan-induced tryptophan pyrrolase activity in livers of rats. The induced activity is expressed as micromoles of kynurenine per hour per gram of protein. All values are corrected for tryptophan pyrrolase activity in the livers of noninduced littermates.

Age at day of test (days)	Induced TP activity		Inhibition of TP induction by substrate (%)
	Controls	Prior treatment	
Prior treatment at 3rd day			
15	14.9	8.8	41
21	39.9	32.6	18
Prior treatment at 3rd to 8th day, daily			
13	8.4	1.9	77
14	4.1	2.2	46
Prior treatment at 3, 5, 7, 9, 11, and 13 days			
20	21.0	1.3	94
25	47.4	18.1	62
30	22.3	32.2	none
Prior treatment at 3rd to 14th day, daily			
23	18.7	12.2	34
30	33.7	25.0	26
Prior treatment at 5th day			
16	3.8	0.7	82
22	36.5	22.5	38
Prior treatment at 5th to 10th day, daily			
15	7.6	3.4	55
18	14.7	2.3	84
20	20.4	11.0	46
25	35.4	28.0	21
Prior treatment at 7th to 10th day, daily			
15	5.1	5.5	none
Prior treatment at 7th to 12th day, daily			
18	26.9	24.3	9
21	18.4	16.1	12
24	16.1	20.5	none

Table 2. Cortisone effect in "tolerant" rats.

Prior treatment at day:	Age at induction (days)	Inhibition of TP induction (%)	
		By substrate	By cortisone
3	15	41	None
3–8, daily	14	46	51
3, 5, 7, 9, 11, 13	25	62	51
5	16	82	23
5	22	38	28
5–10, daily	18	84	81

Prior treatment with tryptophan to attempt the production of "unresponsiveness" was usually limited to the period between 3 and 12 days of age. Figure 2 and Table 1 show that the majority of the animals that received prior treatment exhibit a significant inhibition of the induced TP activity when tested at periods varying between 5 and 18 days after the last injection. The data also indicate that the age between 3 and 5 days is the most susceptible period for the production of unresponsiveness. The state of unresponsiveness is not a stable one but tends to disappear within about 2 weeks. However, if the experiments in which tryptophan was administered on day 3 only are being compared with the experiments in which the rats received tryptophan on days 3, 5, 7, 9, 11, and 13, it seems that the unresponsiveness can be maintained by extending the period of exposure to the amino acid.

These results show a striking resemblance to the production of immunological tolerance by the administration of an excess of antigen to immunologically immature animals. Immunological tolerance also tends to disappear after exposure to the antigen is discontinued, although at a slower rate. Immunological tolerance may gradually change to responsiveness; it can be maintained by continued administration of the antigen (*13*).

The manner in which the tryptophan acts in the very young animal to produce a decreased ability to react to subsequent stimulation with the substrate is of great interest. It is known that substrate induction is inhibited by puromycin but not by actinomycin D, and therefore the induction by substrate is supposed to act at the level of protein synthesis from already existing RNA templates (*8*). On the other hand, the increased TP activity which can be produced by injecting cortisone is inhibited by both actinomycin D and by puromycin, which indicates that cortisone acts by stimulating the production of additional RNA templates (*14*).

In a number of animals that received prior treatment with tryptophan the induction of enzyme was tested by the injection of hydrocortisone (0.01 mg per gram of body weight) at the same time as the substrate induction was studied in the littermates. Table 2 shows that the decreased responsiveness to induction by substrate was accompanied in 5 out of 6 cases by a decreased response to the corticosteroid. Although the results indicate less inhibition of the cortisone-inducible activity, they provide some support for the hypothesis that unresponsiveness is produced by an action of tryptophan at the level of messenger-RNA synthesis from DNA in the immature animal.

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References and Notes

1. L. D. Felton, G. Kauffmann, B. Prescott, B. Ottinger, *J. Immunol.* **74**, 17 (1955).
2. P. B. Medawar, *Science* **133**, 303 (1961).
3. F. MacFarlane Burnet, *Enzyme Antigen and Virus* (Cambridge Univ. Press, Cambridge, 1958); H. Kröger and B. Greuer, *Biochem. Z.* **341**, 190 (1965).
4. P. Feigelson, M. Feigelson, O. Greengard, *Recent Progr. Hormone Res.* **18**, 491 (1962).
5. N. D. Lee, *J. Biol. Chem.* **219**, 211 (1956).
6. O. Greengard and P. Feigelson, *ibid.* **236**, 158 (1961).
7. P. Feigelson and O. Greengard, *ibid.* **237**, 3714 (1962).
8. V. H. Auerbach and H. A. Waisman, *ibid.* **234**, 304 (1959); O. Greengard, M. A. Smith, G. Acs, *ibid.* **238**, 1548 (1963).
9. M. V. Jago, J. F. Nelson, S. Rose, *Biochim. Biophys. Acta* **92**, 44 (1964).
10. E. Spiegel and M. Spiegel, *Exp. Cell. Res.* **36**, 427 (1964).
11. W. E. Knox and V. H. Auerbach, *J. Biol. Chem.* **214**, 307 (1955).
12. A. G. Gornall, C. J. Bardawell, M. M. David, *ibid.* **177**, 751 (1949).
13. G. J. Thorbecke, G. W. Siskind, N. Goldberger, *J. Immunol.* **87**, 147 (1961).
14. H. C. Pitot, *Perspectives Biol. Med.* **8**, 50 (1964).
15. We thank J. I. M. van Hooft for technical assistance.

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