AN ANALYSIS OF THE KINETICS OF RAT LIVER TRYPTOPHAN PYRROLASE INDUCTION: THE SIGNIFICANCE OF BOTH ENZYME SYNTHESIS AND DEGRADATION

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Received February 11, 1964

The fact that the levels of numerous enzymes in mammalian tissues can be increased by the administration of specific substrates and hormones is well established (Knox and Mehler, 1951; Greengard and Feigelson, 1961; Lin and Knox, 1957; Schimke, 1962; Conney and Gilman, 1963). Such increases superficially resemble bacterial enzyme induction, but may in fact result from different mechanisms. Previous studies from this laboratory (Schimke et al., 1963) have indicated that a decrease in the rate of enzyme degradation, i.e., enzyme stabilization,is an important control mechanism for rat liver arginase. In this paper an analysis of the kinetics of increases in rat liver tryptophan pyrrolase produced by corticosteroids and tryptophan is presented. The results provide further evidence that in mammalian tissues the level of a specific enzyme is controlled by the rate of enzyme degradation, as well as by the rate of enzyme formation.

Formulation of a Kinetic Model: The presence of an extensive, continuous replacement of liver protein, i.e. turnover (Buchanan, 1961; Swick, 1956), and the universal finding in animal tissues that enzyme activity falls to basal levels after removal of an inducing agent, indicate the need to consider the process of degradation in any mechanism of changing enzyme levels. The loss of enzyme activity upon removal of an inducing agent(s) follows first order kinetics (see Fig. 1). Hence the simplest model for a change in the tissue content of an enzyme is:

$$dP/dt = K - kP \qquad (1)$$

where P is the content of enzyme per unit weight of tissue, K the rate constant for enzyme synthesis, and k the first order rate constant for degradation. On integration this gives:

$$(2) \qquad P = \left(1 - \frac{K}{k}\right) e^{-kt} + \frac{K}{k}$$

From equation (2) it is evident that P will increase when there is an increased rate of synthesis (K) and/or a decreased rate of degradation (k). An increase in K, denoted as K', will result in an increase in P along an exponential time course to a new level $\frac{K'}{K}$ times the original, when k remains constant. A decrease in k, approaching its lower limit, zero, will result in an increase in P along a linear time course at a rate determined by K to a theoretically infinite level, i.e., $\lim_{k \to 0} \left(\frac{K}{k}\right)$.

Tryptophan pyrrolase was chosen as a suitable enzyme for a kinetic analysis of enzyme "induction" based on the above model because of the separate effects of corticosteroids and tryptophan in increasing enzyme activity (Knox and Auerbach, 1955; Greengard and Feigelson, 1962; Greengard et al., 1963). Both the corticosteroid and tryptophan mediated enzyme increases result from an increased content of newly synthesized protein, rather than activation of an inactive or less active enzyme, as indicated by studies using inhibitors of protein synthesis (Goldstein et al., 1963; Greengard et al., 1963) and specific immunologic reactions (Feigelson and Greengard, 1962). Hence in the present study it is assumed that a measurement of enzyme activity is a valid measure of the content of enzyme protein.

Experimental: Male, adrenalectomized rats, weighing 140 - 150 g each, were used. Tryptophan pyrrolase activity (TP) was assayed in liver homogenates in the presence of added hematin (Feigelson and Greengard, 1961).

Fig. 1 presents a decay curve of TP that had been increased to levels 20-fold those of control animals by repeated administrations of hydrocortisone and tryptophan. The decay follows an exponential time course with a half-life of 2 - 2.2 hours.

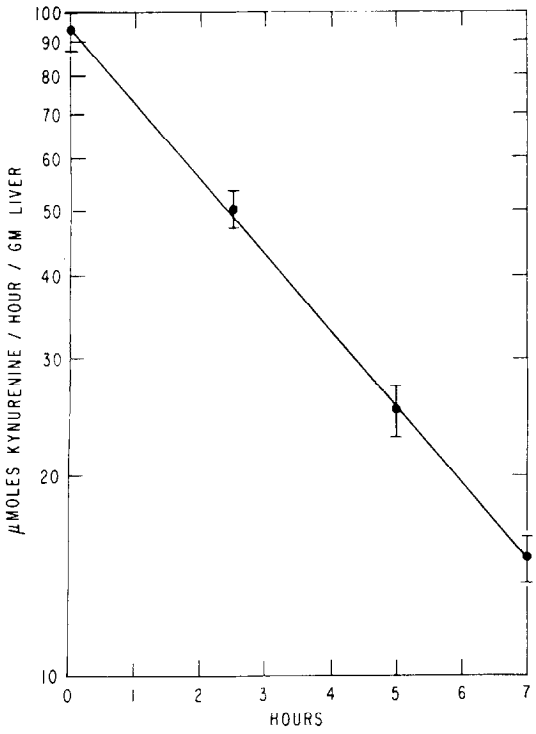

Fig. 1. Decay of Induced Rat Liver Tryptophan Pyrrolase: Rats were given 4 series of intraperitoneal injections of 5 mg hydrocortisone 21-phosphate and 100 mg tryptophan in 12 ml of 0.9% NaCl at 4 hr intervals. 5 hours after the last injections, and thereafter at 2.5 hour intervals the livers of 4-5 animals were assayed for TP. Brackets indicate 2 standard errors of the mean.

Fig. 2 depicts the increases in TP produced by repeated administrations of hydrocortisone and/or tryptophan in doses found to produce maximal effects on TP. Tryptophan alone results in a linear increase in TP, reaching levels 6 times those of control animals in 16 hours. Hydrocortisone alone results in an exponential increase, reaching a maximum 7 to 8 times control levels. The simultaneous administration of hydrocortisone and tryptophan produces a linear increase in TP at a rate 7 times that produced by tryptophan alone, resulting in 40 to 50 fold increases.

These findings are consistent with the theoretical model for "induction" kinetics described above, in which maximal doses of tryptophan decrease the rate of degradation (k) to zero, and maximal doses of hydrocortisone increase the rate of synthesis (K) 6 to 7 fold without altering the rate of degradation (k). The greatest increases in TP, then, result from both an increased rate of enzyme formation and a decreased rate of enzyme

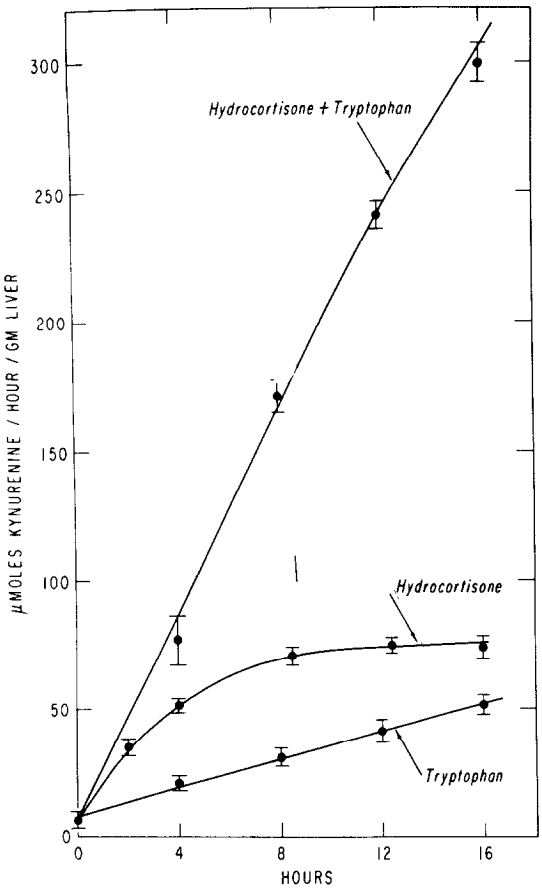

Fig. 2. Kinetics of Tryptophan Pyrrolase Induction: Rats were given injections as follows every 4 hours: 150 mg tryptophan in 12 ml of 0.9% NaCl intraperitoneally; hydrocortisone 21-phosphate, 5 mg subcutaneously. At zero time an additional 5 mg of hydrocortisone was given intraperitoneally. Every 4 hours the livers of 4 animals were assayed for TP. Brackets indicate 2 standard errors of the mean.

degradation. The extent of increase in TP reported here is 4 to 5 times greater than that previously reported (Rosen et al., 1963) and does not necessarily represent the maximal attainable level.

If stabilization of TP is the basis of the tryptophan effect, tryptophan administration should prevent the decay of TP of Fig. 1. Table 1 shows the effects of various agents on the decay of TP. It is evident that tryptophan does indeed abolish this loss of TP and that histidine and hydrocortisone do not. The maintained high level of TP resulting from tryptophan administration is not due to an extremely high rate of enzyme synthesis, since the data of Fig. 2 show that the administration of tryptophan and/or hydrocortisone at no time results

TABLE 1

Effect of Various Agents on Loss of Induced TP Activity

Tryptophan pyrrolase activity was induced to high levels as described in Fig. 1. 5 hours after the last series of injections(0 time of table) 6 animals were assayed for TP, and 4 animals in each group were administered the following compounds in 12 ml of 0.9% NaCl intraperitoneally: tryptophan 200 mg; histidine 200 mg; hydrocortisone 21-phosphate 5 mg; saline only. 4.5 hrs. later the animals were killed and livers assayed for TP.

Time	0	4.5 hours	
	μmoles kynurenine/gm/hr		
Pretreatment with hydrocortisone and tryptophan. §	193 ± 21*	Tryptophan	191 ± 20
		Saline	50 ± 11
		Histidine	50 ± 18
		Hydrocortisone	68 ± 10
		Excised Liver‡	164 ± 21

* 2 Standard errors of mean.
§ Untreated animal TP levels were 7 ± 2 μmoles kynurenine/gm/hr.
‡ Excised at 0 time, incubated at 37° in a moist atmosphere.

in sufficient accumulation of TP within a 4.5 hr. time interval to balance the rapid fall in TP that occurs in control animals (saline treated). It is of interest that excised liver incubated at 37° does not show the rapid loss of TP that occurs in the intact liver *in vivo*. The significance of this latter finding for the mechanism of enzyme degradation is being investigated.

Discussion: A number of implications follows from this kinetic analysis:

(1) Whereas in bacteria enzyme induction involves an increase in the rate of enzyme synthesis produced by specific inducers (Jacob and Monod, 1961), the accumulation of newly synthesized enzyme protein in mammalian tissues may result from two general mechanisms: (a) alterations in rates of enzyme synthesis, perhaps generally mediated by direct hormonal action, (b) stabilization of existing enzyme by substrates, metals, cofactors, etc. Enzyme stabilization *in vivo* would be consistent with known *in vitro* stabilizing effects of substrates on many enzymes, and has been suggested

to account for the in vivo increase in rat liver thymidylate kinase produced by thymidine administration (Bojarski and Hiatt, 1961).

(2) The fact that any agent, whether it be hormonal or dietary, causes an increase in the activity of one enzyme relative to total protein or another enzyme does not necessarily indicate a specific effect on that enzyme. From equation (2) it is evident that the time course of increase in a specific protein is an exponential function of the rate of degradation (k) while only a linear function of the rate of synthesis (K). Since k can be assumed to vary for every protein, the kinetics of increasing activity for each enzyme will vary, even if the rate of synthesis of all proteins is increased to the same extent.

(3) Any condition which reduces the stabilization of an enzyme, either by directly labilizing it, or by removing a stabilizing factor, could result in decreasing enzyme levels in a manner that would mimic enzyme repression as found in bacteria.

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