*REPRESSION OF ENZYME SYNTHESIS AT THE TRANSLATIONAL LEVEL AND ITS HORMONAL CONTROL**

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Communicated by Alexander Hollaender, October 14, 1965

The tyrosine transaminase of rat liver is elevated in the livers of adrenalectomized rats treated with glucocorticoid hormones.[1, 2] The steroids are similarly effective in rats with intact adrenals, but in these animals, the transaminase level can also be elevated by treatment with a variety of agents which cause severe stress.[2] In view of the well-known response of the pituitary-adrenal system to stress, transaminase induction[3] in response to stressing agents can reasonably be interpreted as being due to steroid elaboration under these circumstances.[2] Hydrocortisone is known to increase the enzyme level by virtue of an increase in the rate of enzyme synthesis[4, 5] which follows an increased rate of synthesis of ribosomal, transfer, and "DNA-like" RNA's.[6, 7] Induction is prevented by moderate doses of actinomycin D, an inhibitor of RNA synthesis.[8] This enzyme undergoes an unusually rapid turnover ($t^1/_2$ = *ca.* 2.5 hr[5, 9, 10]), but the rate of enzyme degradation is essentially unchanged by steroid treatment.[10] These observations are consistent with the interpretation that the induction of tryrosine transaminase by glucocorticoids represents an hormonal alteration of transcriptional events, the nature of which is as yet undefined.

The recent experiments of Garren, Howell, Tomkins, and Crocco[11] point to a second mechanism controlling the level of hepatic tyrosine transaminase (and that of the similarly inducible tryptophan pyrrolase). These authors have postulated the existence of repressor(s) capable of selectively inhibiting enzyme synthesis at the translational level, based on their observation that inhibitors of RNA synthesis can prolong the steroid-induced increase in enzyme synthesis, if the inhibitors are introduced at carefully chosen time intervals after the induction is initiated. In the present report we present direct evidence for the existence of a mechanism for selective repression of hepatic enzyme synthesis, confirming the suggestion of Garren *et al.*[11] Repression appears to operate on the translation process, and the involvement of a factor of pituitary origin is indicated.

Materials and Methods.—Male rats from the Charles River Breeding Laboratories (some hypophysectomized) weighing 150–200 gm were usually adrenalectomized 48–72 hr, and always fasted 18–24 hr before experiments. Adrenalectomized animals were given 0.9% NaCl as drinking water. All injections were by the intraperitoneal route. Celite (a diatomaceous earth, Johns-Manville Corp.) and L-tyrosine were administered to initiate stress; at the dose levels used (60 mg per 100 gm) these materials are effective inducers of tyrosine transaminase in rats with intact pituitary-adrenal systems.[2] Actinomycin D (a generous gift from Merck and Co.) was given at 100 μg per 100 gm, a dose which inhibits RNA synthesis about 50%[12] and blocks transaminase induction almost completely.[8]

Enzyme assays: Livers were homogenized in 4 vol of cold 0.15 *M* KCl–0.001 *M* EDTA, and the homogenates were filtered through several layers of cheesecloth. Homogenates were assayed directly for tyrosine-α-ketoglutarate transaminase activity as described before,[13] and for tryptophan pyrrolase by the method of Knox,[14] modified by supplementing reaction mixtures with hematin (10^{-6} *M*).[15]

Immunochemical analysis: The rate of synthesis of tyrosine transaminase was measured immunochemically by an amalgam of the methods of Kenney,[5] Segal and Kim,[16] and Schimke,

Sweeney, and Berlin.[17] Animals were stressed or treated with hydrocortisone 3 hr and 20 min before a single injection of C^{14}-leucine (30 μc; 240 mc/mmole), and they were killed 40 min after the isotope was given. Controls were given isotope in the same fashion, and were otherwise untreated or were given saline in the first injection. Homogenates were centrifuged at 105,000 $\times$ g for 60 min and the clear supernatant fractions used for analyses. Labile proteins were removed by incubation at 37° for 30 min after addition of α-ketoglutarate (5×10^{-3} M) to protect the transaminase from denaturation. After centrifugation to remove denatured protein, identical aliquots were taken for precipitation with antiserum prepared against a highly purified transaminase preparation.[18] Protein content of these aliquots was essentially constant, but the enzyme content varied according to the treatment given (1,200–24,000 units). Unlabeled carrier enzyme (specific activity 34,400 units/mg protein, purified through the calcium phosphate gel step in the scheme described previously[18]) was added to each to bring the enzyme level to a constant value (25,000 or 60,000 units). An equivalent of antibody was added, and the mixture was incubated at 37° for 30 min and then at 3° overnight. Precipitates were collected, washed, and counted as described previously.[4] After removal of the precipitate the supernatant fractions contained less than 5% of the original enzyme activity. To correct for nonspecific precipitation of radioactivity[17] a second incubation was carried out, in which the enzyme level of the same supernatants was again brought to 25,000 or 60,000 units by addition of carrier enzyme. After precipitation with antibody as before, the precipitate was collected and its radioactivity was determined. Radioactivity of the second precipitate was subtracted from the value obtained in the first precipitation, the difference being the actual extent of transaminase labeling. We found the radioactivity of the second precipitate to be nearly constant in a given experiment, and subtracted an average value. Synthesis of the total soluble proteins of the liver was monitored by counting an aliquot that was pipetted onto filter paper disks and washed as described before.[4]

Results.—Specific repression of enzyme synthesis in stressed adrenalectomized rats: In previous experiments designed to study the effects of stressing agents on *induction* of tyrosine transaminase, it was found that agents (such as tyrosine or Celite) found to be effective inducers in intact rats actually *depressed* the transaminase level when administered to adrenalectomized animals.[2] Similar results have been reported by Rosen and Milholland.[19] The time course of the stress-induced decline in transaminase activity is presented as a semilog plot in Figure 1. The data indicate that the decay in enzyme activity follows first-order kinetics after a lag of about 40 min. The half life determined from the linear portion of the curve is $2^1/_2$ hr, in agreement with previous estimates of the rate of degradation of this enzyme *in vivo*.[5, 9, 10] The response appears to be due to nonspecific stress, as either of the highly insoluble materials injected was equally effective.

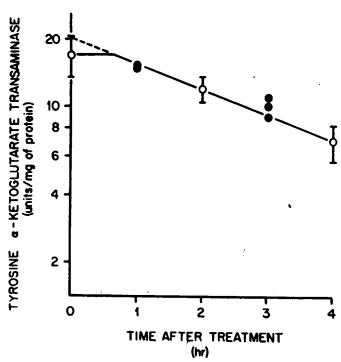

FIG. 1.—Time course of the repression of tyrosine transaminase in adrenalectomized rats. Open circles and brackets indicate the mean ± standard deviation of groups of 10–20 animals treated with either tyrosine or Celite. Closed circles represent individual animals treated with Celite.

Loss of enzyme activity at a rate equal to the known rate of enzyme degradation indicates that the rate of enzyme synthesis has been reduced essentially to zero. Direct confirmation of this conclusion was obtained by immunochemical analyses of the rate of transaminase synthesis. In these experiments the extent of incorporation of C^{14}-leucine into transaminase protein in a pulse of 40 min duration is employed as a measure of the rate of transaminase synthesis; the validity of equating in-

TABLE 1

IMMUNOCHEMICAL ANALYSIS OF TRANSAMINASE SYNTHESIS*

Expt. no.	Treatment	Transaminase activity (units/mg protein)	Radioactivity Incorporated into Transaminase (cpm)	Radioactivity Incorporated into Total soluble proteins (cpm × 10^{-6})
1	None	20	122	1.07
	Hydrocortisone	176	1,373	1.03
	Tyrosine	10	7	1.01
2	NaCl	12	113	1.23
	Hydrocortisone	115	869	1.15
	Tyrosine	5	2	1.39

* Rate of synthesis is determined as the total radioactivity in tyrosine transaminase, measured as described in *Materials and Methods*. Nonspecific precipitation of radioactive protein (2nd precipitation) averaged 237 cpm in expt. 1 and 110 cpm in expt. 2; these values have been subtracted to arrive at the values reported for transaminase radioactivity. The numbers are the average value for three or four similarly treated animals in each experiment. All the animals were adrenalectomized.

corporation of isotope with rates of synthesis in experiments of this type has been discussed extensively by Segal and Kim[16] and by Schimke *et al.*[17] Table 1 presents the data from two experiments wherein the effect of stress on transaminase synthesis was analyzed. Animals in which the transaminase was induced by hydrocortisone are included for comparison. In each experiment the steroid effected a seven- to tenfold increase in the enzyme level, which reflects a corresponding increase in the rate of enzyme synthesis, as measured by transaminase radioactivity. Stress (i.e., tyrosine treatment) reduced the enzyme level approximately 50 per cent, and this can be seen to be due to essentially complete cessation of transaminase synthesis. In neither the steroid-treated nor the stressed animals was there a marked change in total protein synthesis, as measured by incorporation of isotope into the total soluble proteins of the liver. These experiments thus provide direct evidence for selective alterations of the rate of transaminase synthesis, this synthesis being stimulated by hydrocortisone and virtually completely blocked in stressed animals lacking functional adrenals.

The specificity of the response to stress is apparently greater than that observed in the experiments of Garren *et al.*,[11] since the level of tryptophan pyrrolase was unchanged in the stressed animals (Fig. 2). Interpretation of this result must be made cautiously, since the level of tryptophan pyrrolase can be stabilized *in vivo* by the enzyme substrate,[17] and it is thus possible to maintain the level of this enzyme in the absence of continued enzyme synthesis. Immunochemical analysis of tryptophan pyrrolase synthesis is necessary to determine this point unequivocally.

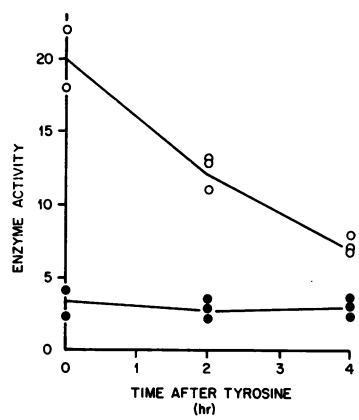

FIG. 2.—Tyrosine transaminase and tryptophan pyrrolase levels in livers of adrenalectomized rats treated with a stressing dose of tyrosine. Each point indicates a single determination; both enzymes were assayed in the same homogenates. *Open circles*, tyrosine transaminase (units per mg protein); *closed circles*, tryptophan pyrrolase (μmoles kynurenine/hr/gm protein).

Effect of actinomycin D on repression: At a dose sufficient to prevent transaminase induction by hydrocortisone, actinomycin is without significant effect on the base level of enzyme over a 4- to 6-hr period[8, 11] (Table 2). Since the enzyme is known to be undergoing rapid turnover in the noninduced

TABLE 2

EFFECT OF ACTINOMYCIN D ON TYROSINE TRANSAMINASE REPRESSION

Treatment	Transaminase activity* (units/mg protein)
None	15 ± 4.0 (7)
Tyrosine	7 ± 1.2 (5)
Tyrosine + actinomycin D	18 ± 1.6 (6)
Actinomycin D	13 ± 1.9 (7)

* Values are mean ± standard deviation; number of observations in parentheses. Actinomycin was given 4 hr 30 min, and tyrosine 4 hr, before the animals were killed. All the animals were adrenalectomized.

TABLE 3

EFFECT OF HYPOPHYSECTOMY ON TYROSINE TRANSAMINASE REPRESSION

Endocrine status*	Tyrosine treatment	Transaminase activity† (units/mg of protein)
Adx only	−	20 ± 4.9 (4)
	+	8 ± 1.2 (5)
Adx-hypox	−	18 ± 3.0 (5)
	+	18 ± 3.1 (6)
Hypox only	−	21 ± 0 (2)
	+	22 ± 2.7 (4)

* Adx, adrenalectomized; Hypox, hypophysectomized.
† Values are mean ± standard deviation; number of observations in parentheses. Assays were made 4 hr after treatment.

state, this result indicates that the templates for transaminase synthesis are stable for this period.[11] If new templates are not required to maintain the base level of enzyme, it follows that repression must operate at the level of enzyme *translation* rather than at the genetic or transcriptional level. However, inhibition of transcriptional processes by actinomycin abolishes the stress-initiated repression of transaminase synthesis (Table 2). Dependence on continued RNA synthesis suggests that the reaction to stress initiates (or stimulates) transcriptional processes leading to formation of an active repressor. Thus, whereas the presumed repressor operates at the translational level, its formation depends upon transcriptional events, and the response to stress involves alteration of these events.

Role of the hypophysis in repression: Since it is well known that the pituitary-adrenal system is of major importance in the response to stress and that the adrenal response to stress is abolished by hypophysectomy, we examined the effect of hypophysectomy on the stress-mediated repression of tyrosine transaminase. The hypophysectomized animals appeared to be incapable of forming repressor in response to the stress of tyrosine injection (Table 3). This result clearly implicates pituitary factor(s) in the repression phenomenon, and suggests the possibility that pituitary hormone(s) synthesized or released in response to stress function as hepatic repressors or stimulate the synthesis of such repressors in the liver.[20]

Discussion.—The tight control over hepatic tyrosine transaminase synthesis exercised by hormones of the pituitary-adrenal system is demonstrated by the striking differences in response to stressing agents observed in animals with different components of the system removed. Synthesis is increased in stressed intact animals, repressed by stress in adrenalectomized animals, and is unaffected by stress in animals lacking both pituitary and adrenals. The steady-state level in the normal animal thus reflects a delicate balance between opposing endocrine influences, one promoting enzyme synthesis and the other inhibiting it. Increased enzyme synthesis is known to be the result of glucocorticoid action, as these steroids are effective inducers under all conditions tested, including the isolated, perfused liver[10, 21] and hepatoma cells in culture.[22] Identification of the presumed hormone promoting enzyme repression has not yet been made, but the data presented here indicate that it is of pituitary origin, or depends upon the pituitary for its elaboration. Both hormone actions are inhibited by actinomycin, and thus both appear to act on transcriptional processes.

Induction of tyrosine transaminase by hydrocortisone is associated with increased synthesis of all three of the major kinds of RNA; as discussed elsewhere,[7, 23] this general response in RNA synthesis has been difficult to reconcile with the specificity of enzyme induction. The highly selective action of repressors of enzyme synthesis could provide the solution to this problem, if it were possible to establish that enzyme synthesis is normally subject to repressor restraint and that hydrocortisone releases this restraint. The available evidence suggests that these conditions obtain. Rosen, Raina, Milholland, and Nichol[24] have shown that administration of very low doses of actinomycin over a period of days increases the tyrosine transaminase level severalfold in adrenalectomized rats. This indicates that the capacity for enzyme synthesis is considerably greater than that usually operative, even in the absence of glucocorticoids; thus, enzyme synthesis is normally partially repressed. Induction by actinomycin can be understood in terms of its inhibition of repressor formation, as demonstrated above and earlier by Garren *et al.*[11] But the actinomycin induction is slow and limited, in contrast to the increase in enzyme synthesis caused by hydrocortisone. The rapid, extensive induction that follows hydrocortisone treatment could be due to a combination of two effects, one being inhibition of formation of the selective repressor, and the other a generally increased capacity for protein synthesis due to increased synthesis of the various types of RNA. That glucocorticoids do inhibit repressor formation is indicated by previous observations[2] of the response of tyrosine transaminase in adrenalectomized rats given both hydrocortisone and stressing agents. The stress-initiated repression of enzyme synthesis is not apparent in these animals, suggesting that it is blocked in the presence of hydrocortisone.

Whereas the response to stress resulting in repressor formation appears to involve transcriptional processes, the data indicate that repressor action is at the translational level. Translational control of specific protein syntheses in higher forms has been implicated in several recent reports (see, for example, refs. 25–27), and experiments providing indications of potential mechanisms of repressor action at the ribosomal level have been described.[28, 29] From the present data and the considerations discussed above, it appears that hormones participate in this type of regulation by controlling the rates of repressor formation.

Summary.—Administration of stressing agents (tyrosine, Celite) to adrenalectomized rats initiates a highly selective repression of the synthesis of hepatic tyrosine-α-ketoglutarate transaminase. The enzyme level falls with a $t^1/_2$ of about 2.5 hr. Immunochemical measurement of the rate of enzyme synthesis indicates that it is reduced essentially to zero in stressed, adrenalectomized rats, whereas labeling of total liver soluble proteins is unaffected. Actinomycin does not itself influence the enzyme level, but it blocks the stress-initiated repression of enzyme synthesis, indicating that repression acts at the translational level, whereas initiation of repression involves transcriptional processes. The stressing agents are ineffective in hypophysectomized rats, implicating an hormonal factor of pituitary origin in the initiation of repression.

Note added in proof: Since submission of this article, we have provisionally identified growth hormone (somatotropin) as the pituitary factor active in eliciting transaminase repression.

We are pleased to acknowledge the kind assistance of Dr. Darold Holten, who carried out some

of the immunochemical analyses described, and Dr. Carl Wust, who aided in the preparation of the antiserum used in these analyses.

* Research jointly sponsored by the National Cancer Institute, the National Institutes of Health, and by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

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[3] Some controversial terms and their definitions as used here: induction, selective stimulation of enzyme synthesis; repression, selective inhibition of enzyme synthesis; transcription, readout of genetic information to form an RNA template; translation, readout of the RNA-coded information in the process of enzyme synthesis.

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