*GLUCOCORTICOID STIMULATION OF THE BIOSYNTHESIS OF GLUTAMIC-ALANINE TRANSAMINASE**

By Harold L. Segal† and Yee S. Kim

DEPARTMENT OF PHARMACOLOGY, ST. LOUIS UNIVERSITY SCHOOL OF MEDICINE

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Among the mechanisms which have been considered as possible descriptions of the molecular events underlying hormone action, one which has attracted increasing attention among contemporary biochemists is the proposal that control of the biosynthesis of specific enzymes may be an important factor (see, e.g., ref. 1). The plethora of enzyme activities which are found to increase or decrease in response to an alteration in endocrine state in the whole animal[2] has been an insistent factor in favor of this view. More sophisticated studies have come forth in recent years which have given further encouragement to this concept. These have been of three types, of which the following are examples. It has been demonstrated that there is an increased amino acid incorporation into carbamyl phosphate synthetase of tadpole liver after exposure of the animals to thyroxine[3] and into a partially purified preparation of glutamic-tyrosine transaminase of rat liver after exposure of the animals to cortisol.[4] Early hormonal effects have been found on RNA metabolism,[5, 6] particularly of the nuclear fraction.[7, 8] Finally, altered hormonal states have a striking influence on amino acid incorporation into total protein in cell-free systems.[9–15] We are presenting in this paper what we believe to be unequivocal evidence that a change in endocrine state leads to a change in the rate of biosynthesis of a specific enzyme.

Materials and Methods.—The definition of activity units and methods of enzyme assay and antibody titration of rat liver glutamic-alanine transaminase have been presented previously.[16, 17] The procedure for purification of the enzyme to a homogeneous state and some of its physical and chemical properties will be published elsewhere.[18]

Results.—In order to interpret meaningfully the results of measurements of the influence of hormones on amino acid incorporation into a specific protein, it is necessary to know that the period of exposure to the labeled amino acid is short relative to the half-life of the enzyme. Otherwise, the enzyme may have become fully labeled during the experimental period under all conditions. This information is lacking in the studies with carbamyl phosphate synthetase.[3] The half-life of a protein can be measured by isotopic methods if it can be isolated, or more simply by enzyme activity measurements, if a method is available for perturbing its steady-state level in tissues. The latter possibility is readily realized with enzymes, the activity levels of which in tissues are susceptible to hormonal alteration, once it has been established that the activity level is a valid measure of the amount of catalytically active protein present, as in the case at hand.[17]

The prednisolone-mediated transformation from the normal steady-state level to what may be called the high glucocorticoid steady-state level is shown in Figure 1. The high hormone steady-state level is virtually achieved in 5 days, as previously reported by Rosen *et al.*[19] On the eighth day, at the point marked by arrows on the figure, hormone administration was discontinued, and the activity allowed to return

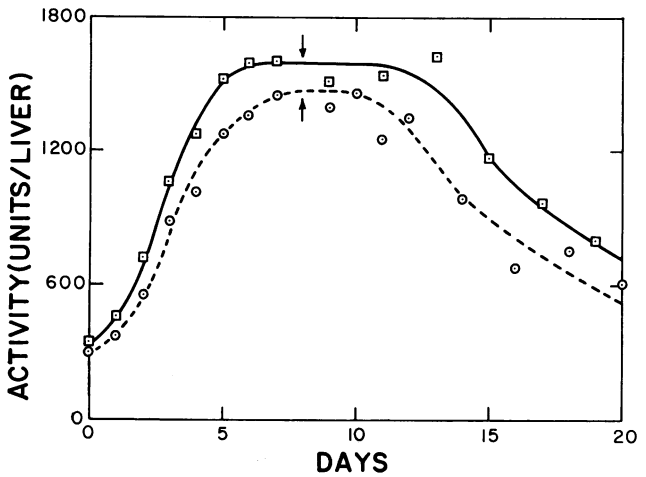

FIG. 1.—Effect of administration of prednisolone and subsequent withdrawal on glutamic-alanine transaminase activity of rat liver. In the upper curve (squares), animals were injected subcutaneously with 4 mg/day of prednisolone acetate, and in the lower curve (circles), 2 mg/day. Each point represents the mean of 3, or occasionally 2, 200–250-gm male rats. Injections were in the morning, and for the first 7 days the animals were sacrificed 2 hr after the last injection. At the points marked with the arrows, the administration of hormone was discontinued.

to the normal steady-state level. Thus, the first part of the curve represents the effects in animals under the influence of a high hormone level, and the second part represents animals under the influence of a normal or lower hormone level. It can be seen from inspection of the curves that the rise in enzyme activity to the steady-state value characteristic of the high hormone level is more rapid than the fall to the normal steady-state level. This is shown quantitatively in the log plots in Figures 2 and 3 (see below).

For the calculation of half-lives, it was assumed that the rate of change of enzyme level was the algebraic sum of a zero-order rate of synthesis and a first-order rate of breakdown (Table 1). It is not assumed that the rate constants for either synthesis

TABLE 1

ENZYME LEVEL AS A FUNCTION OF TIME

	Normal	Prednisolone
	$\frac{d(E)}{dt} = k_1 - k_2(E)$	$\frac{d(E)}{dt} = k_1' - k_2'(E)$
At steady state:	$k_1 = k_2(E_0)$	$k_1' = k_2'(E_0')$
At any time t:	$\ln [(E) - (E_0)] = \ln [(E_0') - (E_0)] - k_2 t$	$\ln [(E_0') - (E)] = \ln [E_0') - (E_0)] - k_2' t$

The unprimed terms are the values in normal animals and the primed terms the corresponding value in the prednisolone-treated animals.

or breakdown are the same in the normal and the high hormone animals. A plot of $\ln [(E) - (E_0)]$ versus t as the activity is returning to the normal steady-state level allows the determination of k_2, the rate constant for the degradation reaction under normal hormone conditions (Fig. 2); and a plot of $\ln [(E_0') - (E)]$ versus t as the activity is rising to the steady-state value of the high hormone level allows the cal-

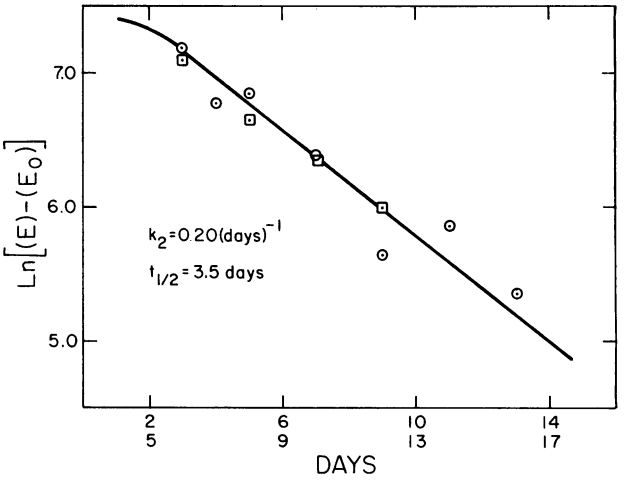

FIG. 2.—Logarithmic plot of the decline of enzyme activity after withdrawal of hormone. Symbols as in Fig. 1. The abscissa is days after withdrawal of hormone—the upper set of numbers for the experiment with 2 mg/day of prednisolone and the lower set for the experiment with 4 mg/day. If allowance is made for the greater lag period in the latter experiment, the data from the two experiments lie on the same line.

culation of k_2', the rate constant for the degradation reaction under conditions of high hormone (Fig. 3 and Table 1). The rate constant for degradation of the enzyme is about 3 times as great when the animal is under the influence of a high hormone level compared with the normal, and the half-life is therefore $^1/_3$ as great. These data appear to indicate that the administration of the hormone stimulated the rate of breakdown of the enzyme some threefold. It has been reported that glucocorticoid administration also increased the rate of turnover of serum albumin in the rabbit and man.[20] The half-lives thus obtained for glutamic-alanine transaminase may be compared with values for other rat liver proteins of about 3 hr for glutamic-tyrosine transaminase,[4, 21] 3 hr for tryptophan pyrrolase,[22] 1 day for catalase,[23] 5 days for arginase,[24] and 3 days for total liver protein.[24] Significantly in muscle, where levels of enzyme activities are notably refractory to environmental influences, the half-life of aldolase was 20 days in the rat.[25]

From the rate constants for degradation and the steady-state levels of the enzyme, it is possible to calculate the rates of synthesis of the enzyme in the normal and prednisolone-treated animals (Table 2).

TABLE 2

CALCULATED RATES OF GLUTAMIC-ALANINE TRANSAMINASE SYNTHESIS IN NORMAL AND PREDNISOLONE-TREATED RAT LIVERS

Status	(E_0) (units/gm liver)	k_2 (days)$^{-1}$	Calculated k_1 (units/gm liver) × (days)$^{-1}$	Calculated k_1 (mg/gm liver) × (days)$^{-1}$
N	31.4	0.20	6.3	0.021
P	108	0.60	65.4	0.22

N refers to normal animals and P refers to animals injected with 2 mg of prednisolone acetate per day. (E_0) is the steady-state level of enzyme. k_2 is the experimental value for the rate constants of enzyme degradation (Figs. 2 and 3). $k_1 = k_2(E_0)$ (see Table 1). The conversion of units of enzyme to mg of enzyme is based on a specific activity of 300 for virtually homogeneous preparations of the enzyme (ref. 18).

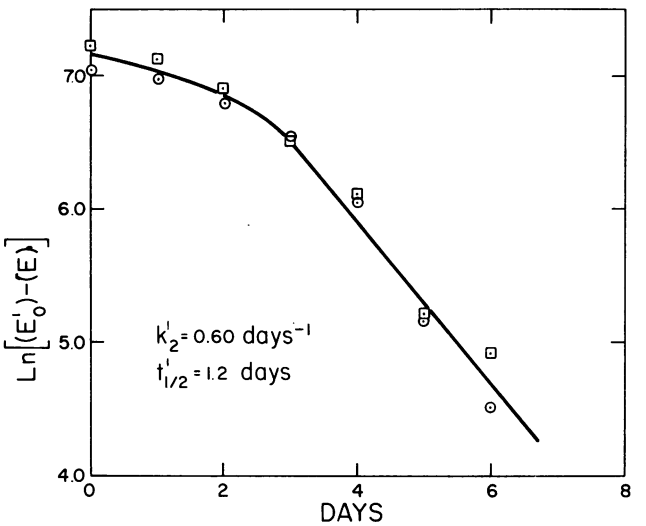

FIG. 3.—Logarithmic plot of increase in enzyme activity consequent to administration of hormone. Symbols as in Fig. 1.

The conclusion from these experiments is that the increase in glutamic-alanine transaminase level after glucocorticoid administration is not an effect of a decreased rate of breakdown; in fact the rate of breakdown is increased in the hormone-treated animals, and must therefore be a result of an increased rate of synthesis. Direct evidence to support this conclusion was obtained by measurements of the relative extent of incorporation of C^{14}-labeled leucine into the enzyme in normal and prednisolone-treated rats. The results are presented in Table 3.

As can be seen, there was a markedly greater incorporation of isotope into the enzyme from glucocorticoid-treated animals, by a factor of about fivefold. Since the half-life of the enzyme is of the order of days, these incorporation data during a 1-hr period can be taken as approximations of the relative rates of synthesis of the enzyme in the two cases, unless the specific activity of leucine in the precursor pools is drastically different. In order to estimate a possible effect of the hormone on the leucine pool specific activity, counts incorporated into the total soluble protein of the liver were also measured. As shown in the table, there was only a minor effect of

TABLE 3

RADIOACTIVE LEUCINE INCORPORATION IN LIVER GLUTAMIC-ALANINE TRANSAMINASE IN NORMAL AND PREDNISOLONE-TREATED RATS

Status	Enzyme activity (units)	Cpm in enzyme*	Cpm in soluble protein	Soluble protein (mg)
N (5)	31.4 ± 1.1	21.0 ± 2.6	14,200 ± 1,400	8.3 ± 0.3
P (7)	108 ± 5.9	99 ± 6.4	18,500 ± 1,900	8.5 ± 0.1
P/N	3.44	4.71	1.3	1.0

* Although there was some variation from animal to animal in the counts incorporated into the enzyme, the ratio of this value to the counts incorporated into total soluble protein (as a measure of relative pool specific activity) was strikingly constant, especially in the normal animals. This indicates that the rate of synthesis of the enzyme from one animal to another is quite uniform.

5.8–13.8 × 10^6 cpm of uniformly labeled C^{14}-L-leucine (specific activity, *ca.* 1 mc/mg) was injected, and the livers were removed 1 hr later, homogenized in 0.25 *M* sucrose, and centrifuged at 100,000 × *g* for 1 hr. The enzyme was partially purified by heat and acid treatment and ammonium sulfate precipitation (ref. 16), then precipitated with antibody (ref. 17). Cpm were corrected for self-absorption and normalized to 5 × 10^6 cpm injected. Numbers in parentheses under "Status" refer to the number of animals in the group. Prednisolone treatment was 2 mg/day for 5 days. P/N is the ratio of the measured values in the hormone-treated relative to the untreated animals. Values are means for 1 gm of liver with standard errors. Protein was determined by the Lowry method (ref. 26).

the hormone on this parameter, of the order expected from the data of Noall *et al.*[27] on the effect of glucocorticoids on α-aminobutyrate transport. Thus, the specific activity of the leucine pool does not seem to be a significantly variable factor in the comparison of the incorporation rates of normal and hormone-treated animals.

Although the peak of *enzyme level* is not achieved until about the fifth day of prednisolone administration (Fig. 1), the increasing *rate of enzyme synthesis* would be expected to reach a maximum much sooner, then remain constant as long as the high hormone level is maintained. Correspondingly, after withdrawal of the hormone the *rate of enzyme synthesis* would presumably diminish to the normal level, followed by a later return of the *enzyme level* to the normal value. The curves in Figures 1, 2, and 3 are consistent with this prediction. In each case there is a lag of the order of a day or more before the logarithmic relationship is established, indicating that a new and constant rate of synthesis and degradation has been achieved. To test this conclusion directly, measurements were made of the relative rates of enzyme synthesis by the isotopic method described in Table 3 after 1 day and 3 days of hormone administration, and 2 days after withdrawal of the hormone. The results are presented in Table 4. Again, the rate of amino acid incorporation in the hormone-treated animals was 4–6 times that in the normals. In harmony with the prediction from theory, the rate of synthesis of the enzyme has reached the new steady-state value considerably earlier than has the enzyme level. This is particularly noticeable in the 1-day-treated animals where enzyme level is only slightly above normal while the rate of synthesis is already maximal. Two days after hormone withdrawal following a 5-day treatment, the rate of synthesis is still high, consistent with the long lag period preceding the achievement of the normal rates of enzyme synthesis and breakdown (Fig. 3). This may be a reflection of the persistence of high hormone levels in the animal.

TABLE 4

RADIOACTIVE LEUCINE INCORPORATION INTO GLUTAMIC-ALANINE TRANSAMINASE AT VARIOUS TIMES AFTER PREDNISOLONE TREATMENT

Days of hormone treatment	Enzyme activity (units)	Cpm in enzyme*	Cpm in soluble protein
0 (6)	35.0 ± 2.1	45.0 ± 9.8	18,700 ± 2,800
1 (3)	59.0 ± 8.5	298 ± 51	23,600 ± 1,500
3 (3)	117 ± 4.1	203 ± 13.3	23,700 ± 1,300
5† (6)	150 ± 12.1	189 ± 21.5	20,500 ± 3,100

* The same constancy in the ratio of counts incorporated into enzyme to counts incorporated into total soluble protein in individual animals was observed here that is referred to in the footnote in Table 3. In these experiments, performed several months after those in Table 3, the counts incorporated into the enzyme were in all cases about double the corresponding values in Table 3. The reason for this difference is not known.

† This group was injected with radioactive leucine and sacrificed 2 days after discontinuance of hormone treatment.

Livers were removed 1 hr after administration of C^{14}-leucine, homogenized in 0.25 *M* sucrose, and centrifuged at 100,000 × *g* for 1 hr. The enzyme was partially purified and precipitated with antibody. Cpm were corrected for self-absorption and normalized to 5 × 10^6 cpm injected. Values are means for 1 gm of liver with standard errors. Numbers in parentheses under "Days of hormone treatment" are the numbers of animals in each group. There were no significant differences in the total soluble protein content in the several groups.

Discussion.—Three general modes of action have been visualized in considering the molecular events underlying hormone effects, viz., interaction with transport barriers, direct modification of enzyme activity, and control of the synthesis and/or degradation of specific enzymes. The first has been established in connection with the effect of insulin on glucose transport[28] and the second as a factor in the actions of glucagon and epinephrine.[29] Other examples could be cited; however, in these we have referred to, there seems to be a clear relationship between the observed events

on the molecular level and the metabolic effects of the hormone. We believe the results reported here establish the hormonal control of the biosynthesis of a specific enzyme. In this case, however, there does not appear to be a connection between the observed biochemical effect and at least the early metabolic response to glucocorticoid administration. Increased incorporation of alanine and other precursors into glycogen can be shown within 2 hr after prednisolone administration,[30] far too early to be ascribed to changes in glutamic-alanine transaminase activity. On the other hand, the possibility that changes in the activity of this enzyme are responsible for later metabolic responses to glucocorticoids remains a possibility. In any event it seems safe to predict that other metabolic control functions of hormones will be found to be directly related to modifications of the rate of synthesis or breakdown of specific enzymes.

The experiments reported here have not touched on the question of the specific site in the machinery of protein synthesis where hormonal control is exerted. One might speculate, on the basis of the lag preceding the achievement of the maximum rate of enzyme synthesis consequent to hormone administration, that the hormone is acting to stimulate the production of an essential component of the system (messenger RNA) rather than to activate preexisting components. It is hoped that studies on the biosynthesis of the enzyme in cell-free systems will provide further information in this regard.

Summary.—Measurements of C^{14}-leucine incorporation into liver glutamic-alanine transaminase in normal and prednisolone-treated rats has provided a direct demonstration of a severalfold greater rate of biosynthesis of the enzyme in the hormone-treated animals. The elevated rate of synthesis precedes and leads to the increase in the steady-state tissue level of the enzyme consequent to hormone administration. The half-life of the enzyme has been determined and found to be 3.5 days in normal animals and 1.2 days in prednisolone-treated animals.

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*AN INFLUENCE OF INSULIN ON THE SYNTHESIS OF A RAPIDLY LABELED RNA BY ISOLATED RAT DIAPHRAGM**

BY IRA G. WOOL AND ALAN J. MUNRO

DEPARTMENT OF PHYSIOLOGY, UNIVERSITY OF CHICAGO, AND DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CAMBRIDGE, ENGLAND

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Insulin, when added *in vitro* to isolated rat diaphragm, will enhance the incorporation of labeled amino acids[1–4] and of amino acid precursors[5] into protein; neither the precise mechanism nor the exact site at which the hormone acts to bring about this effect is known. The influence of insulin on protein biosynthesis in muscle does seem, however, to be separate and distinct from the action of the hormone in facilitating the entry of glucose into the cell.[3, 4] Moreover, there are observations[5–8] that, at least by indirection, support the hypothesis that insulin stimulates protein biosynthesis through an effect on an intracellular process.

To obtain information as to the identity of the intracellular process, the effect of insulin on nucleic acid synthesis was investigated. Insulin was found to enhance RNA synthesis in muscle;[9, 10] this was reflected in an increase in incorporation of C^{14} from several substrates (adenine, orotic acid, and glucose) into RNA and by an actual net synthesis of RNA. Most remarkably, the latter occurred in the absence of any added substrate.

It is a commonplace that considerable importance attaches to the isolation of the primary biochemical locus at which insulin influences metabolic processes, for that information is vital to an analysis of the mechanism of the hormone's action. Especially pertinent is the identification of the exact molecular species of RNA whose synthesis is increased by insulin. The stimulation of the production of a specific RNA, more so if that RNA were limiting for protein synthesis, as may be the case with messenger RNA,[11] would add weight to the speculation that RNA synthesis is the intracellular site of action of insulin in stimulating protein synthesis.

The recognition of the problem and its implication has led to an attempt to identify the fraction of the cellular RNA whose synthesis is stimulated by insulin. It is the purpose of this paper to report the results of that investigation.