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Induction of Methionine Adenosyltransferase in Rat Liver by Corticosteroids* (33079)

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Transmethylation transects nearly every area of metabolism (1). It is an important means of altering the biological activity of many compounds including RNA and DNA (2,3). Methionine, in its active form, *S*-adenosylmethionine, is a major donor of methyl groups in mammalian tissues (4). It may also play a role in the regulation of folic acid-mediated (5,6) and other metabolic pathways (7). Methionine adenosyltransferase (ATP: *L*-methionine *S*-adenosyltransferase, EC 2.5.1.6) is the specific activating enzyme responsible for the formation of *S*-adenosylmethionine from *L*-methionine and ATP

(8). It is also the first enzyme on the catabolic pathway from methionine leading to a variety of products.

Our previous work (9) has shown that methionine adenosyltransferase activity was increased in the livers of both intact and adrenalectomized rats treated with cortisone, while adrenalectomy caused a slight decrease in level of this enzyme. Marked elevation of the enzyme activity also occurred when dietary protein levels were increased (9) and following the induction of alloxan-diabetes.¹ The present study is concerned with the specificity of the response of hepatic methionine adenosyltransferase to different types of steroid hormones and the mechanism un-

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¹ Lien Shou and Foo Pan, unpublished data.

derlying the glucocorticoid-induced increase of this enzyme as studied with the inhibitors of protein and RNA synthesis.²

The available data indicate that only those adrenocortical steroids and synthetic analogs with glucocorticoid activity are effective as inducers of hepatic methionine adenosyltransferase. The results further suggest that the increase in the enzyme activity brought about by glucocorticoids represent an accelerated enzyme synthesis *de novo* probably involving enhanced DNA-directed RNA production. The results obtained from the experiments with estrogens and androgenic-anabolic steroids are reported elsewhere (10).

Experimental procedure. Treatment of animals. General information about the experimental animals has been described (10). Female Sprague-Dawley rats weighing 120–160 gm at start of the experiment were used. They were maintained on a commercial diet (Taiwan Sugar Corp.) and tap water *ad libitum*. Bilateral adrenalectomies were carried out under light ether anesthesia 8–10 days before the animals were sacrificed. Adrenalectomized rats were given 1% NaCl instead of water, except during the treatment with aldosterone or deoxycorticosterone. Steroids and/or antibiotics were administered by intraperitoneal injection in doses indicated in legends to the figures and tables. Steroids were given as a solution or as a finely dispersed suspension in propylene glycol–0.9% NaCl (1:1, v/v), 0.5 ml or less/100 gm of body weight. Other chemicals were administered in 0.9% NaCl. Controls were given the vehicle only.

Analytical methods. Methionine adenosyltransferase activity was assayed essentially according to Cantoni and Durell (11) as described (9,10) in the supernatant fractions of liver homogenates prepared in 10 mM acetic acid containing 5 mM 2-mercaptoethanol and 0.2 mM EDTA and centrifuged at 20,000g for 30 min. Proteins were measured by the biuret procedure (12) using

crystalline bovine serum albumin as standard.

Expression of results. One unit of methionine adenosyltransferase activity is the amount of enzyme catalyzing the formation of 1 μ mole of S-adenosyl-L-methionine in 30 min at 37°C. Units per mg of protein and per liver of a 100-gm rat are referred to as specific and total activity, respectively. The values cited in discussion of results are those of total activity. The same conclusions were obtained when specific activities were compared, except where otherwise noted. The data are given for convenient comparison as percentages of values found in adrenalectomized control animals (0.090 ± 0.0031 unit/mg of protein or 34.5 ± 2.3 units/100-gm rat) and expressed as means \pm standard errors. The results were subjected to statistical evaluation by *t* test and the significant differences between the means are shown when the *p* value was <0.05 .

Materials. Actinomycin D and 9 α -fluoro-16 α -hydroxy-1-dehydrocortisol (triamcynolon) were generously supplied by Merck, Sharp and Dohme and American Cyanamid Co., respectively. Puromycin hydrochloride, cortisol, 1-dehydrocortisol (prednisolone) and aldosterone were purchased from Sigma Chemical Co; corticosterone, deoxycorticosterone, and 11-epicortisol from Mann Research Laboratories; cortisone acetate from Upjohn Co., and cycloheximide from Nutritional Biochemicals Corp. All other chemicals used were obtained commercially and were of reagent grade.

Results and Discussion. Effects of various corticosteroid hormones on hepatic methionine adenosyltransferase activity. In an attempt to obtain information on the specificity of the response, effects of various steroid hormones on the hepatic levels of methionine adenosyltransferase have been comparatively studied in adrenalectomized rats. Besides gonadal hormones (10), five adrenocorticoids including cortisone acetate, cortisol, corticosterone, deoxycorticosterone, and aldosterone and three synthetic analogues, namely triamcinolone, prednisolone, and 11-epicortisol, were examined. The results presented in Table I show that, among the

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TABLE I. Effects of Adrenal Corticosteroids and Synthetic Analogues on Hepatic Methionine Adenosyltransferase Activity.^a

No. of rats	Treatment	Enzyme activity (% of control)	
		Specific act.	Total act.
11	Control	100 ± 4.9	100 ± 4.6
9	Cortisol	158 ± 13.0	179 ± 16.9
10	Cortisone acetate	151 ± 2.1	190 ± 6.5
7	Corticosterone	121 ± 7.9	121 ± 10.8
4	Aldosterone	98 ± 9.4 ^b	104 ± 10.8 ^b
4	Deoxycorticosterone	87 ± 3.3	92 ± 3.7 ^b
5	11-Epicortisol	79 ± 3.5	74 ± 2.5
4	Prednisolone	147 ± 13.7	177 ± 14.0
7	Triamcinolone	182 ± 9.0	230 ± 12.2

^a Adrenalectomized female rats were given intraperitoneally a single dose (6 mg/100 gm) of various steroids or vehicle (controls) at zero time and sacrificed 24 hours later, except the aldosterone-treated animals. The latter were given 75 µg of aldosterone/100 gm daily for 4 days and sacrificed 24 hours after the last injection.

^b Except those marked as *b* footnote, all other data are statistically significantly different as compared with the values of adrenalectomized control rats ($p < 0.05$).

steroids studied, all the compounds known to have potent glucocorticoid activity increased the activity of methionine adenosyltransferase in the livers of treated rats. The increase found with triamcinolone was more marked than those obtained with cortisol and other natural glucocorticoids. This accords with the higher gluconeogenic potency of the fluorinated steroids (13). On the other hand, two mineralocorticoids, aldosterone and deoxycorticosterone, failed to raise the hepatic level of the enzyme under our experimental conditions. In fact, the rats treated with deoxycorticosterone had slightly lower enzyme activity than the controls, when calculated on the basis of units per mg of protein. Further correlation concerning the specificity of this response was obtained by testing a stereoisomer of cortisol, 11-epicortisol, which was biologically inactive with respect to the glycogen deposition and anti-inflammatory assays (14). It was also found to be inactive in inducing the transferase in liver. Actually

it lowered the enzyme activity to 74% of the control value.

The response of methionine adenosyltransferase to the treatment with glucocorticoids, but not to the treatment with structurally related but inactive steroids indicated a specific association of the response with gluconeogenic activity. This relationship might be explained on a teleological basis, since gluconeogenesis requires the catabolism of amino acids, and *S*-adenosylmethionine formation is the first step in this process with methionine.

Dose-response study with triamcinolone. Since triamcinolone was found to be the most potent inducer of liver methionine adenosyltransferase, a dose-response study was carried out at one time interval (24 hours) with this steroid. Figure 1 shows that a dose of 0.25–0.5 mg/100 gm was sufficient to induce a statistically significant increase in this enzyme. An optimum induction was observed with a dose of 6 mg/100 gm which resulted in an 246% increase in enzyme activity.

Since enzyme increases were lower in the

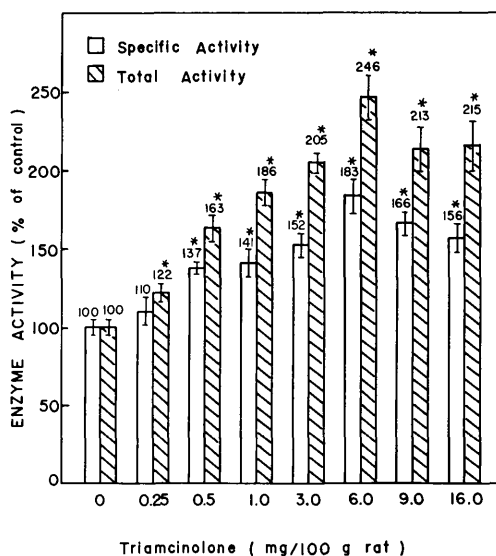


FIG. 1. Effect of varying doses of triamcinolone on liver methionine adenosyltransferase. Groups of 5 or more adrenalectomized female rats were given triamcinolone in doses varying from 0.25 to 16.0 mg/100 gm and sacrificed after 24 hours. (*), Statistically significant difference as compared with the values of control rats ($p < 0.05$).

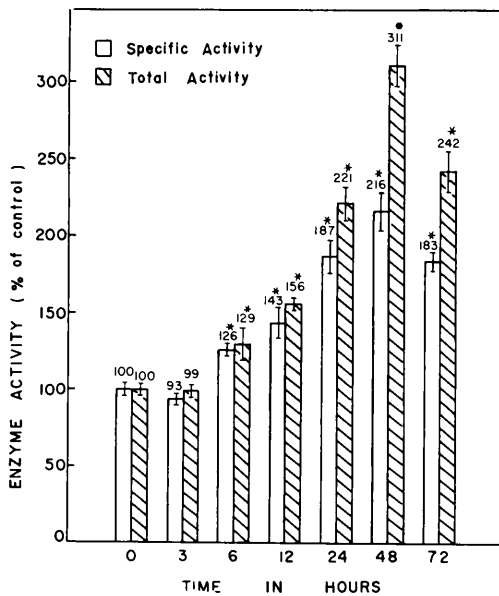


FIG. 2. Time course of the induction of hepatic methionine adenosyltransferase in adrenalectomized female rats by a single dose of triamcinolone, 6 mg/100 gm body weight. Each group had 4 or more rats. (*), Statistically significant difference as compared with the values of control rats ($p < 0.05$).

group treated with 9 or 16 mg of triamcinolone/100 gm than in the group receiving 6 mg/100 gm, toxic effects of the steroid might be operating at the higher dose level. Similar interpretations were proposed for analogous findings on the inducing effects of high doses of corticosteroid hormones on the activities of hepatic tyrosine aminotransferase (15), glucose-6-phosphatase and fructose-1,6-diphosphatase (16).

Time course and magnitude of response of methionine adenosyltransferase to triamcinolone. The rate of increase in methionine adenosyltransferase activity in the livers of adrenalectomized rats injected intraperitoneally with a single dose (6 mg/100 gm) of triamcinolone is shown in Fig. 2. A significant rise (129%) in the enzyme activity was detectable within 6 hours and the enzyme reached peak level (311%) after 48 hours.

Figure 3 shows the time course and extent of response of the transferase to the administration of repeated doses of triamcinolone. A maximal induction (403%) of the enzyme

was achieved after 4 daily doses of 6 mg/100 gm.

The time course of triamcinolone-induced methionine adenosyltransferase increase revealed that this enzyme elevation occurs gradually which is more compatible with a synthetic mechanism than with activation (cf. the experiments with antibiotics discussed below). The rather long time required for a maximal induction of methionine adenosyltransferase by glucocorticoid also suggests a relatively long half-life for this enzyme. The apparent small response of arginase and the slow response of alanine aminotransferase, as compared to the rapid and large response of tryptophan oxygenase and tyrosine amino transferase, have been shown to be a reflection of the slower turnover of these enzymes (17).

Mechanism of glucocorticoid action on methionine adenosyltransferase activity. Glucocorticoids could elevate methionine adenosyltransferase level by directly stimulating enzyme activity, or by affecting the synthesis or breakdown of the enzyme protein. To

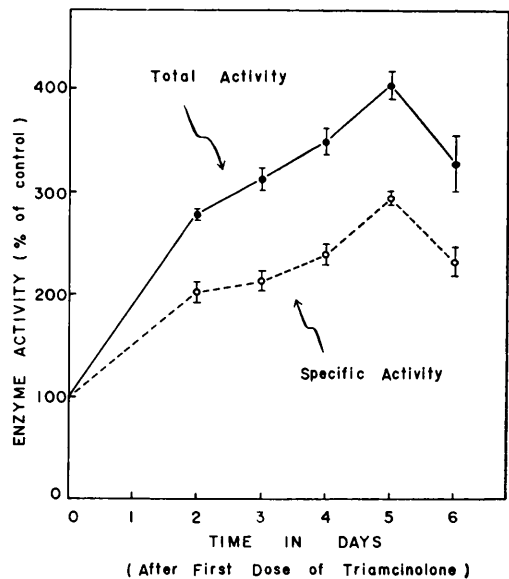


FIG. 3. Sequential changes in hepatic methionine adenosyltransferase activity induced by daily injection of triamcinolone. Groups of 4 or more adrenalectomized female rats were given triamcinolone, 6 mg/100 gm daily for 1-5 days, and sacrificed 48 hours after the last injection.

TABLE II. Effects of Puromycin and Actinomycin D on the Methionine Adenosyltransferase Induction by Triamcinolone.^a

Expt. no.	Treatment	No. of rats	Enzyme activity (% of control)	
			Specific act.	Total act.
1	Control	5	100 ± 4.8	100 ± 5.8
	Puromycin (P)	4	102 ± 5.9	94 ± 5.3
	Triamcinolone (T)	8	149 ± 6.5 ^b	152 ± 6.7 ^b
	T + P	8	115 ± 7.3 ^c	117 ± 4.7 ^c
	Inhibition of induction by puromycin (%) ^d		(69)	(67)
2	Control	5	100 ± 1.5	100 ± 2.6
	Actinomycin D (A)	5	93 ± 7.7	85 ± 5.9 ^b
	Triamcinolone (T)	6	143 ± 10.7 ^b	156 ± 4.5 ^b
	T + A	7	116 ± 8.1 ^c	122 ± 4.6 ^{cc}
	Inhibition of induction by actinomycin D (%) ^d		(63)	(61)

^a Adrenalectomized female rats were treated as follows: puromycin hydrochloride (pH adjusted to 6.0), 1.25 mg/rat at 0, 2, 4, 6, 8, and 10 hours (total dose, 7.5 mg/rat); triamcinolone, 6 mg/100 gm at 0.5 hour; actinomycin D, 50 µg/100 gm, about 15 min before the injection of triamcinolone. Controls and other rats were given the vehicles instead of the steroid and/or antibiotic at the same time. All rats were sacrificed at 12.5 hours.

^b Statistically significant difference as compared with the values of adrenalectomized controls ($p < 0.05$).

^c Statistically significant difference as compared with the values of triamcinolone but not antibiotic-treated rats ($p < 0.05$).

^d The enzyme levels in control animals were subtracted in calculating this value.

examine the possibility of direct effect, supernatant fluids from the livers of control and cortisol- or triamcinolone-treated animals were mixed and then assayed for methionine adenosyltransferase activity. Such admixing failed to produce any appreciable activation or inhibition of enzyme activity and resulted only in the expected summation. In addition, enzyme extracts obtained from normal or adrenalectomized rats were assayed for methionine adenosyltransferase activity with or without the addition *in vitro* of cortisol or triamcinolone. The steroids, added as a solution in 25 µl of propylene glycol to a final concentration of 10^{-5} M, as well as the solvent used, had no effect on the enzyme activity.

To determine whether the glucocorticoid-induced rise in the transferase activity resulted from increased synthesis of the enzyme protein, the effect of triamcinolone was examined in animals treated with compounds known to inhibit RNA or protein synthesis.

The data presented in Table II show that actinomycin D, 50 µg/100 gm, given shortly before triamcinolone, or puromycin, 1.25 mg/rat every 2 hours starting 30 min before the administration of triamcinolone, inhibited the induction by 61–69% within 12 hours. Puromycin given alone produced no significant alteration in the enzyme activity under experimental conditions. Actinomycin D lowered the enzyme activity by 15% when calculated as total activity. Table III shows the effect of another protein synthesis inhibitor, cycloheximide (18), on the cortisone-induced increase in liver methionine adenosyltransferase activity. The results were similar to those obtained with puromycin. These data are consistent with the interpretation that the changes in methionine adenosyltransferase activity brought about by glucocorticoids are due to increases in the rate of synthesis of the enzyme protein. Immunological-isotopic analyses (19, 20) would provide more direct evidence for this conclu-

TABLE III. Effect of Cycloheximide on the Methionine Adenosyltransferase Induction by Cortisone Acetate.*

No. of rats	Treatment	Enzyme activity (% of control)	
		Specific act.	Total act.
8	Control	100 ± 2.3	100 ± 4.4
5	Cycloheximide	78 ± 10.0 ^b	74 ± 7.3 ^b
8	Cortisone acetate	123 ± 5.9 ^b	125 ± 5.6 ^b
7	Cortisone acetate + cycloheximide	93 ± 5.7 ^c	103 ± 7.2 ^c
Inhibition of induction by cycloheximide (%) ^d		(100)	(88)

* Adrenalectomized female rats were given intraperitoneally cortisone acetate (10 mg/100 gm) and/or cycloheximide (50 µg/100 gm) and sacrificed 12 hours later. Both compounds were in 0.9% NaCl. Controls were given the vehicle.

^{b,c,d} See footnotes of Table II.

sion. However, this enzyme is not available in the highly purified form required for the preparation of the antitransferase antiserum since it was found to be very unstable (21, 22).

Summary. The induction of hepatic methionine adenosyltransferase by adrenal cortical hormones has been studied in adrenalectomized rats. Experiments with cortisol, cortisone, corticosterone, prednisolone, and triamcinolone revealed that triamcinolone was the most potent inducer of methionine adenosyltransferase activity. On the contrary, two mineral corticoids, aldosterone and deoxycorticosterone, and a biologically inactive isomer of cortisol, 11-epicortisol, were found to be ineffective in raising the hepatic level of this enzyme, indicating the specificity of the action of glucocorticoids in this respect. Actinomycin, puromycin, and cycloheximide, inhibitors of RNA or protein synthesis, largely blocked the glucocorticoid-induced increase in the enzyme, observed 12 hours after hormone administration. The results suggest that the changes in the hepatic levels of methionine adenosyltransferase brought about by glucocorticoids represent enzyme synthesis *de novo* probably involving enhanced DNA-directed RNA production.

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