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Studies on Glutamic Oxalacetic Transaminase in the Rat. III.[1] Hormonal Control of Isoenzyme Levels in Liver by the Pituitary-Adrenal System[2]

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Glutamic oxalacetic transaminase (GOT) is one of main transaminase in liver. This enzyme has not been induced so active by glucocorticoids as other transaminases. However, the presence of isoenzymes and their characteristics were investigated and the same hormonal sensitivity to glucocorticoids was observed in the enzyme localized in the soluble fraction (s-GOT). In our present paper, the regulation by adrenal hormones was proven to be exist not only in s-GOT but also in the mitochondrial enzyme (m-GOT) which is major component in rat liver. Furthermore, it is suggested that catecholamines would play a role on defining of isoenzyme level in liver and leakage of enzyme from tissue.

The study of the hormonal regulation of this enzyme is not complete although it has been studied by several groups of workers.[4-6] The investigation of the separation of isoenzymes for the estimation of enzyme activity was further developed for this purpose. In this communication the possibility of regulation by adrenal hormones was studied utilizing glucocorticoids, catecholamines or adrenocorticotropic hormone (ACTH) with our developed assay program[7] for isoenzyme activities in liver homogenate. Discussion is directed not only to the regulation of s-GOT activity but also to m-GOT activity, which has received little study.

Methods

Animals——Male rats of the Wister strain were used in this experiment, all within 100—150 g of body weight. Adrenalectomized rats were used a week after operation, and given saline instead of water. Hypophysectomized rats were also given saline and treated with drugs 24 hours after operation.

Methods of Drug Administration——Cortisone or corticosterone was injected subcutaneously once a day by suspension in sesame oil in most of experiments. A commercial preparation of ACTH[8] was injected subcutaneously. Catecholamines or dehydroxyphenylalanine (DOPA) were dissolved with diluted acid and neutralized before use. Administration of these drugs was repeated four times every hour by intraperitoneal injection to avoid the drastic action of the drugs.

Enzyme Materials and Assay Procedure for GOT Activity——As materials, liver homogenate and serum were prepared and their GOT activities determined according to a previously described procedure.[7]

The enzyme activities in the liver homogenate were assayed by the cellulose acetate method and electrophoretic procedure for the isoenzyme ratio, and their isoenzyme activities calculated by simultaneous equations.

1) Part II: Y. Ogawa, Y. Kometani, and Y. Baba, *Chem. Pharm. Bull.* (Tokyo), **16**, 1942 (1968).
2) This work was presented at the 87th Annual Meeting of Pharmaceutical Society of Japan, Kyoto, April 1966.
3) Location: *Fukushima-ku, Osaka.*
4) K. Kato, *Vitamins* (Kyoto), **28**, 531 (1963).
5) F. Rosen and C. A. Nichol, *Vitamins and Hormones*, **21**, 136 (1963).
6) C. A. Nichol and F. Rosen, *Advan. Enzyne Regulation*, **1**, 341 (1963).
7) Y. Ogawa, Y. Kometani, and Y. Baba, *Chem. Pharm. Bull.* (Tokyo), 16, 1937 (1968).
8) Cortrophine-Z.

Results

Effects of Adrenalectomy

Within a week after operation, only a slight decrease was observed in the total activities in liver, but a further dramatic decrease could no longer be recognized because this decrease was based on only that of s–GOT as shown in Table I.

Table I. Effects of Adrenalectomy on GOT Activities in Rat Liver

	Days after operation	Treatment	No. of rats	s–GOT (units/g liver)	m–GOT (units/g liver)
I	7	control	4	14600±900	19200±1100
		adrenalectomized	7	11400±800[a]	18900±1100
II	9	control	6	10800±500	14600±400
		adrenalectomized	6	8500±700[a]	14000±800
III	7	control	6	12600±500	11900±500
		adrenalectomized	6	9800±400[b]	10400±700

M±SE statistically significant a) $P<0.05$ b) $P<0.01$

Effects of Glucocorticoids

As shown by other workers,[9] it seems reasonable to assume that cortisone would induce the activity of s–GOT in the liver. This was also shown in our investigation as seen in Fig. 1. However, it was also proven as seen in Table II, that cortisone would affect m–GOT activity depending on the vehicle employed. The effects of cortisone could also be observed with corticosterone, which is well known as the main glucocorticoid in rats, as shown in Table III.

Table II. Effects of Vehicle on Action of Cortisone on Liver GOT Activities

	Treatment	No. of rats	s–GOT (units/g liver)	m–GOT (units/g liver)
I	suspended into water			
	control	4	12000±1500	13300±500
	cortisone 25 mg/kg/day×4	4	17200±1500[a]	13100±700
II	suspended into sesame oil			
	control	6	9800±900	15300±500
	cortisone 25 mg/kg/day×4	6	18200±200[b]	13300±600*

M±SE statistically significant a) $P<0.05$ b) $P<0.01$

Table III. Effects of Cortisone or Corticosterone on Liver GOT Activities in Adrenalectomized Rat

Treatment	No. of rats	s–GOT (units/g liver)	m–GOT (units/g liver)
Control (sesame oil)	4	11700±400	16400±1400
Cortisone 25 mg/kg/day×4	4	17700±1400[a]	12200±800[b]
Corticosterone 25 mg/kg/day×4	4	17200±900[a]	12400±600[b]

M±SE statistically significant a) $P<0.01$ b) $P<0.01$

9) N. Katsunuma and T. Katsunuma, *J. Clin. Sci.* (Tokyo), **1**, 803 (1965).

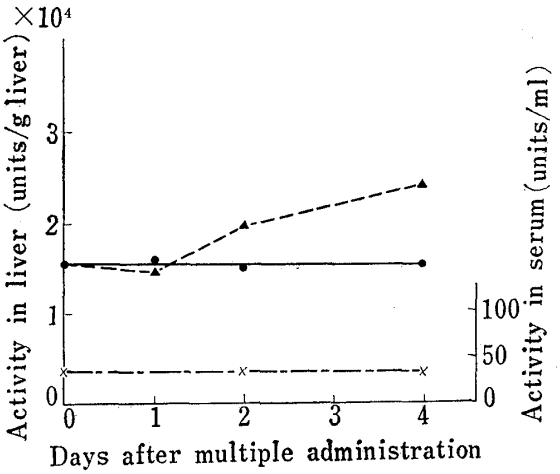

Fig. 1. Effects of Cortisone on GOT Activity in Serum and Liver

Mean values of four rats are plotted.
▲------▲ s-GOT ●——● m-GOT
×------× GOT in serum

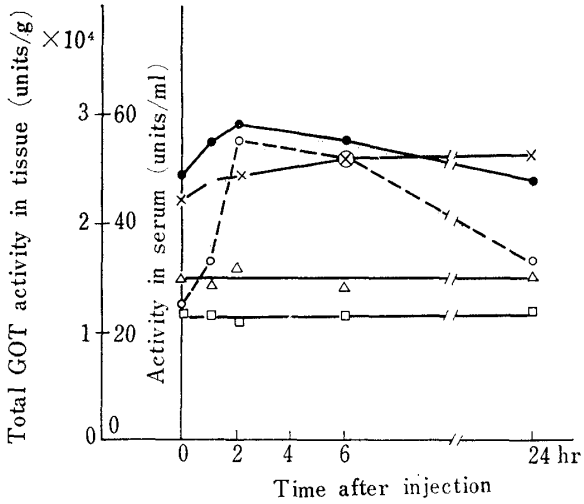

Fig. 2. Effects of l-Epinephrine on GOT Activity in Tissues or Serum

l-Epinephrine was injected 0.5 mg/kg intraperitoneally.
×——× heart ●——● liver ○------○ serum
△——△ kidney □——□ brain

Effects of ACTH

The effects of ACTH are summarized in Table IV. Administration of low doses of ACTH gave no observable effects in intact rats. Effects were noted, however, in hypophysectomized

Table IV. Effects of ACTH on GOT Activities in Rat

Treatment	No. of rats	Liver GOT (units/g liver) s-GOT	Liver GOT (units/g liver) m-GOT	Serum GOT (units/ml)
I low dose (10 IU/kg/day × 3)				
a) intact				
control	6	12500±1500	15000±500	26.7±2.3
ACTH	6	14400±900	15100±600	28.2±1.4
b) hypophysectomized				
control	5	10500±600	15800±400	35.2±3.0
ACTH	5	18400±2900[a]	13900±1100[b]	39.2±4.8
II high dose 1 (20 IU/kg 3 times/day × 1)				
a) intact				
control	6	12200±1100	17100±500	22.8±2.4
ACTH	6	14000±1000	12800±700[b]	30.2±1.9
b) hypophysectomized				
control	5	14200±1300	17000±300	35.6±2.6
ACTH	5	18800±1500	10600±900[b]	30.7±1.3
c) adrenalectomized				
control	5	11100±800	17200±1100	32.2±3.7
ACTH	5	10800±900	18500±900	30.3±2.2
III high dose 2 (20 IU/kg 3 times/day × 3)				
a) intact				
control	6	14000±500	16000±700	26.2±1.1
ACTH	6	23500±2000[b]	12300±700[b]	54.2±15.6
b) hypophysectomized				
control	5	10500±600	15800±400	35.2±3.0
ACTH	5	25700±1200[b]	10700±800[b]	42.4±8.3
c) adrenalectomized				
control	5	11200±900	15400±1000	26.6±2.2
ACTH	5	8300±1200	16400±1000	25.3±1.4

M±SE statistically significant a) $P<0.05$ b) $P<0.01$

Table V. Effects of Epinephrine on Liver GOT Activity in the Rat

Treatment	No. of rats	Total GOT activity	
		units/g liver	units/mg protein
Control	4	22300±600	104±3
Epinephrine 0.5 mg/kg 4 times 4 hr after	4	27600±1400[a)]	124±5[a)]
Epinephrine 0.5 mg/kg 24 times 24 hr after	4	20200±600[a)]	108±7

M±SE statistically significant a) $P<0.05$

Table VI. Effects of Epinephrine or Related Agents on GOT Isoenzymes in Rat Liver

Agents	No. of rats	s-GOT (units/g liver)		m-GOT (units/g liver)	
		4 hr after	24 hr after	4 hr after	24 hr after
None	4	12600±700		17600±700	
Epinephrine	4	14500±400	12500±1000	20500±500[a)]	16200±1200
Norepinephrine	4	15200±800	12200±700	20500±1300	16500±800
DOPA	4	14700±600	10700±300[a)]	18400±400	16900±500

M±SE statistically significant a) $P<0.05$
All of agents were injected 4 times every an hour, 0.5 mg/kg subcutaneously.

Table VII. Effects of Epinephrine or Related Agents on Serum GOT Activity in the Rat

Agents	No. of rats	Serum GOT (units/ml)	
		4 hr after	24 hr after
Control	4	28.8±1.7	
Epinephrine	4	86.1±23.6	39.3±1.4
Norepinephrine	4	45.3±6.1	28.6±1.2
DOPA	4	26.3±0.8	26.8±1.4

M±SE All of agents were injected 4 times every an hour 0.5 mg/kg subcutaneously.

Table VIII. Interaction between Cortisone and Epinephrine on Liver GOT Activities in the Adrenalectomized Rat

	Treatment	No. of rats	s-GOT (units/g liver)	m-GOT (units/g liver)
I	control (no addition)	6	10600±800	13700±600
	cortisone 25 mg/kg/day×4	5	18400±1300[a)]	13100±700
	cortisone 25 mg/kg/day×4 epinephrine 0.5 mg/kg 4 times 24 hr after	4	14900±1000[a)]	9900±1000[a)]
II	control (no addition)	4	9700±800	14100±1200
	cortisone 25 mg/kg/day×4	4	19800±1600[a)]	13800±700
	cortisone 25 mg/kg/day×4 epinephrine 0.5 mg/kg 4 times/day×4	6	17100±500[a)]	11200±400[b)]

M±SE statistically significant a) $P<0.01$ b) $P<0.05$

rats. On the other hand, nothing could be observed even at high doses of ACTH in adrenalectomized rats. The effects of ACTH were not only an increase of s–GOT but a decrease of m–GOT. This decrease of m–GOT could not be found from estimation of specific activity in isolated mitochondria.

Effects of Catecholamines

Epinephrine resulted in an increase of serum GOT activity and a slight increase in liver GOT activity in the early state after administration as seen in Fig. 2. This slight increase could be considered to be significant statistically as shown in Table V, and was also observed with norepinephrine or DOPA as seen in Table VI. The effects on serum GOT were different with these agents, respectively, as shown in Table VII. Although there was an increase in the early state, a slight decrease could also be observed at 24 hr after administration, and this effect was enhanced in the adrenalectomized rat pretreated with cortisone as shown in Table VIII.

Discussion

Although the hormonal induction of liver enzymes has been studied with cortisone or hydrocortisone by many workers,[5] little attention has been paid to GOT which has not shown as dramatic an effect as other enzymes.[10–12] However in this investigation the hormonal effects on liver GOT activity could be demonstrated on each of the isoenzymes, which might cancel out in a determination of total activity. This undoubtedly is reason why the hormonal effects with cortisone or hydrocortisone were not so remarkable in respect to total activity as reported by several workers.[6,13]

While the effect of glucocorticoids on s–GOT has already been found by recent investigations on isoenzymes,[9] that of m–GOT was not well known owing to difficulty of quantitative estimation of the enzyme activity. Therefore, it appeared of interest that there was a decrease of m–GOT by administration of ACTH, cortisone suspended into sesame oil or epinephrine to cortisone–treated rats. As nothing could be observed with ACTH in adrenalectomized rats, the effects of ACTH on intact or hypophysectomized rats is considered to be induced by the adrenal hormone.

Consequently, it seems reasonable to assume that this effect would not be shown only by glucocorticoids released by ACTH, if the action of glucocorticoids was defined by the increase of s–GOT activity. In this assumption, we could consider the role of catecholamines which was found to be slightly decrease in both isoenzymes at 24 hr after administration, and showed a more remarkable decrease in rats treated with cortisone.

This data would suggest the fact that a regulation system by the pituitary–adrenal hormones was possible at the isoenzyme level in liver GOT, and necessitates further investigations on the metabolic control by hormones.

The effects on serum GOT activity was shown in this communication and a characteristic increase was found with epinephrine. Future investigations will involve the study of the mechanism of leakage of tissue GOT into the serum with regard to adrenal injury.

10) F. Cavosto, A. Pileri and A. Brusca, *Biochem. Biophys. Acta*, **24**, 250 (1957).
11) W.E. Knox, *Proc. Intern. Symp. Enzyme Chem.*, **1957**, 414.
12) W.E. Knox, *Brit. J. Expt. Path.*, **32**, 462 (1951).
13) J.R. Puchol and A. Carballido, *Med. Exptl.*, **1**, 305 (1959).