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STUDY ON ROLE OF RETICULOENDOTHELIAL SYSTEM IN METABOLIC REGULATION

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ABSTRACT

This report deals with the relationship between liver L-Alanine: 2-oxoglutarate aminotransferaes (EC 2.6.1.2) activity and RES function.

In fasted rats, decrease in Δ^4-3-ketosteroid hydrogenase (EC 1.3.1.3 and 1.3.1.4) and phagocytic activity and increase in alanine aminotransferase activity were noted. On the other hand, increase in Δ^4-3-ketosteroid hydrogenase and phagocytic activity and decrease in alanine aminotransferase activity were observed in RES stimulated ones. Possible mechanism for these changes in alanine aminotransferase activity was discussed in relation to the role of reticuloendothelial system in steroid hormone metabolism.

INTRODUCTION

There have been some reports on the relation between the reticuloendothelial system (RES) and metabolism. Berry *et al.*[1)-5)] studied the activities of tryptophan oxygenase and tyrosine aminotransferase of mouse liver in regard to the function of the reticuloendothelial system in endotoxin tolerance. Nicol *et al.*[6)] reported on the effect of various hormones on the phagocytic activity of RES, and DiCarlo *et al.*[7)8)] on the relation between RES function and hepatic glycogen. These studies, however, did not attempt to elucidate the role of RES in the regulation of metabolism. Hence, no noticeable progress was made in this field until Berliner *et al.*[9)10)11)] reported that RES has an important role in steroid hormone metabolism, particulary that Δ^4-3-ketosteroid hydrogenase, which catalyzes the first step of inactivation of the hormone, which exists in Kupffer cells.

The present study was carried out to elucidate how RES takes part in metabolic regulation, by clarifying the relationship between RES function and liver alanine aminotransferase activity under varied dietary protein levels, fasting and RES stimulation.

MATERLALS AND METHODS

Animals: Male albino rats of Wistar strain weighing 150 to 200 g were

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used, but the animals which were given experimental diet weighed around 120 g before they were fed with it.

Diet: The animals were maintained on a standard diet of Oriental pellets NMF and water *ad libitum*. Furthermore, diets of various protein levels were prepared as shown in Table 1, and were given for 2 weeks before experiment.

TABLE 1. Composition of Experimental Diets

	Casein	Corn starch	Sucrose	Oil*	Salt mixture*	Vitamin mixture*	Choline chloride	C.M.C.
60% Casein	60	10	11.5	10	5	0.85	0.15	2.5
20% Casein	20	50	11.5	10	5	0.85	0.15	2.5
5% Casein	5	65	11.5	10	5	0.85	0.15	2.5

* Tanabe Amino Acids Research Foundation.

RES stimulation: Typhoid paratyphoid vaccine was given by injections for 3 consecutive days into the tail vein in a dose of 10^9 cells per 100 g body weight once a day, and experiments were conducted 24 hours after the last injection. Zymosan suspension in a concentration of 4 mg per ml of sterilized physiological saline was given similarly in a dose of 4 mg per day per 100 g body weight, and experiments were conducted 24 hours after the last injection.

RES phagocytic activity: Carbon clearance studies were performed according to the method described by Halpern *et al.*[12]. After the injection of Pelikan Ink C 11/1431a in a dose of 16 mg per 100 g body weight into the tail vein, blood samples were taken from the retroorbital plexus after 5, 10 and 15 minutes, and phagocytic index K was obtained.

Determination of activities of liver enzymes: Δ^4-3-ketosteroid hydrogenase activity was determined by the method of Tomkins[13] using corticosterone as substrate at pH 7.4. The activity was expressed in the amount (μ moles) of substrate reduced per hour per 100 g body weight.

L-Alanine: 2-oxoglutarate aminotransferase activity was determined by the method of Reitman-Frankel[14]. The activity was expressed in the amount of reaction product (μ moles) per hour per 100 g body weight.

Relationship between RES function and alanine aminotransferase activity was explored by the changes in activities of RES phagocytosis, Δ^4-3-ketosteroid hydrogenase, alanine aminotransferase and adrenal weight induced by changes in dietary protein level, fasting and RES stimulation.

RESULTS

(I) Activities of RES phagocytosis, Δ^4-3-ketosteroid hydrogenase and alanine

aminotransferase and adrenal weight without RES stimulation.

Effect of dietary protein level

Rats were divided into three groups, of 60 per cent, 20 per cent and 5 per cent casein diet groups respectively. After they were maintained on the respective diets for two weeks, RES phagocytic activity, Δ^4-3-ketosteroid hydrogenase activity, adrenal weight and alanine aminotransferase activity were measured. As shown in Table 2, no significant relationship was noticed between RES phagocytic activity and dietary protein level. Δ^4-3-ketosteroid hydrogenase activity was found to be highest in the 60 per cent casein diet group, and lowest in the 5 per cent group as shown in Table 3. The adrenal weight showed the same tendency as Δ^4-3-ketosteroid hydrogenase activity, that is, it was greatest in the 60 per cent casein diet group and least in the 5 per cent group. Liver alanine aminotransferase activity, also in the same manner as the Δ^4-3-ketosteroid hydrogenase and the adrenals, showed the highest value in the 60 per cent casein diet group, less high in the 20 per cent group and lowest in the 5 per cent group (Table 3).

TABLE 2. Effect of Dietary Protein Level on Phagocytic Activity in Rats

Diet	No. of rats	Phagocytic activity, K
60% Casein	5	0.0206±0.0030*
20% Casein	5	0.0176±0.0019*
5% Casein	5	0.0157±0.0020*

Values are mean±standard deviation.

* No significant difference was noticed among these three groups.

TABLE 3. Effect of Dietary Protein Level on Δ^4-3-ketosteroid Hydrogenase Activity, Adrenal Weight and Liver Alanine Aminotransferase Activity of Rats

Diet	No. of rats	Δ^4-Hydrogenase activity	Adrenal weight mg/100 g body weight	Alanine amino-transferase activity
60% Casein	5	20.20±2.16*	24.72±1.41**	4674.0± 88.8*
20% Casein	5	15.28±1.80	22.56±1.38	2033.9±153.3
5% Casein	5	12.55±0.88**	19.84±1.19**	1723.5± 95.5*

Values are mean±standard deviation.

* Significantly different from the mean of 20% Casein diet group at a P value of 0.01 or less.

** Significantly different from the mean of the 20% Casein diet group at a P value of 0.05 or less.

Effect of fasting

Rats were fasted for 1 to 4 days, but water was given *at libitum*. It was found that as the duration of fasting was lengthened, RES phagocytic activity became gradually depressed (Fig. 1). Δ^4-3-ketosteroid hydrogenase activity

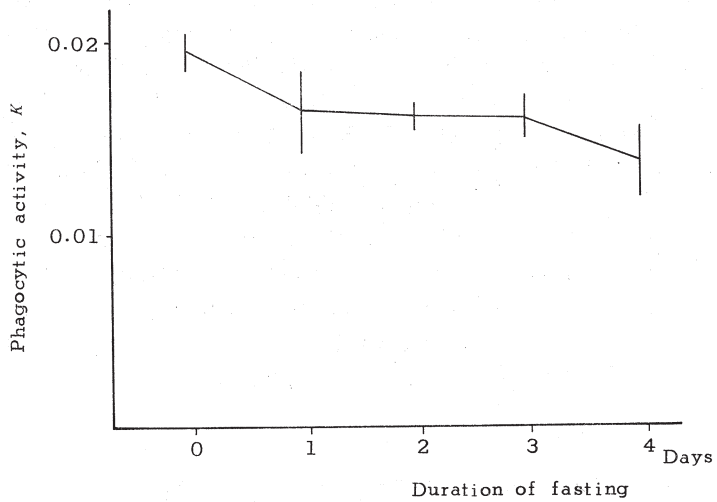

FIG. 1. Effect of fasting on phagocytic activity of rats.
Each point represents the mean of 5 rats and vertical bar standard deviation, respectively.

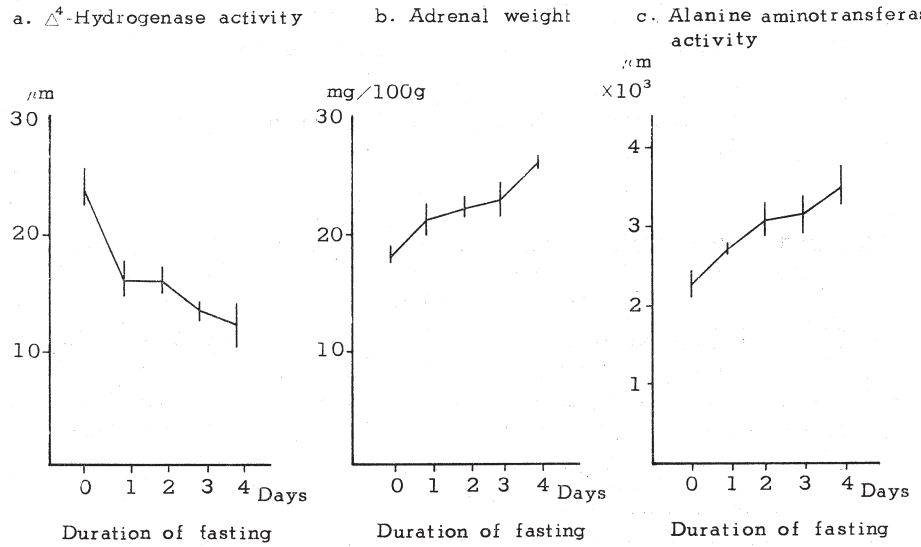

FIG. 2. Changes in Δ^4-3-Ketosteroid hydrogenase activity, adrenal weight and liver alanine aminotransferase activity of fasted rats.
Each point represents the mean of 5 rats and vertical bar standard deviation, respectively.

was noted to be gradually lowered with the lengthening in duration of fasting as in the case of phagocytic activity (Fig. 2 a). While loss of body weight in the fasted rats was prominent, loss of adrenal weight was less marked. Therefore, the ratio of adrenal weight to body weight increased (Fig. 2 b).

Liver alanine aminotransferase activity was noted to rise as the duration of fasting became longer as in the case of adrenal weight (Fig. 2 c).

(II) Activities of RES phagocytosis, Δ^4-3-ketosteroid hydrogenase and alanine aminotransferase and adrenal weight under RES stimulation.

Effect of dietary protein level

Rats were divided into three groups: standard, 60 per cent casein and 5 per cent casein diet group. After they were maintained on these diets for 2 weeks, they were subjected to RES stimulation by injecting typhoid paratyphoid vaccine or zymosan into the tail vein. In each of the above groups, RES phagocytic activity, Δ^4-3-ketosteroid hydrogenase activity, adrenal weight and alanine aminotransferase activity were determined and compared with those of the respective controls. As indicated in Table 4 and 5, in every group

TABLE 4. Effect of RES Stimulation with Typhoid Paratyphoid Vaccine on Phagocytic Activity of Rats fed on Various Diets

Group		No. of rats	Phagocytic activity, K
Standard diet	Untreated	4	0.0195 ±0.0030
	RES stimulated	5	0.0524 ±0.0049*
60% Casein	Untreated	5	0.02057±0.0032
	RES stimulated	4	0.05048±0.0037*
5% Casein	Untreated	5	0.0157 ±0.0020
	RES stimulated	4	0.0506 ±0.0052*

Values are mean±standard deviation.

* Significantly different from the mean of the respective untreated group at a P value of 0.01 or less.

TABLE 5. Effect of RES Stimulation with Zymosan on Phagocytic Activity of Rats fed on Various Diets

Group		No. of rats	Phagocytic activity, K
Standard diet	Untreated	4	0.0195 ±0.0030
	RES stimulated	5	0.0526 ±0.0037*
60% Casein	Untreated	5	0.02057±0.0032
	RES stimulated	5	0.0484 ±0.0024*
5% Casein	Untreated	5	0.0157 ±0.0020
	RES stimulated	5	0.0581 ±0.0076*

Values are mean±standard deviation.

* Significantly different from the mean of the respective untreated group at a P value of 0.01 or less.

TABLE 6. Effect of RES Stimulation with Typhoid Paratyphoid Vaccine on Δ^4-3-ketosteroid Hydrogenase Activity, Adrenal Weight and Liver Alanine Aminotransferase Activity of Rats fed on Various Diets

Group		No. of rats	Δ^4-Hydrogenase activity	Adrenal weight mg/100 g body weight	Alanine amino-transferase activity
Standard diet	Untreated	5	24.14±1.00	18.24±1.18	3014.2±342.3
	RES stimulated	5	27.93±1.83*	23.86±1.06*	1984.0±126.9*
60% Casein	Untreated	5	19.10±0.60	19.82±1.19	5698.4±517.2
	RES stimulated	5	24.30±1.24*	25.28±0.84*	3882.2±157.2*
5% Casein	Untreated	5	9.68±0.50	18.00±0.87	1054.8±132.1
	RES stimulated	5	12.04±1.02*	24.79±1.17*	1185.3±162.8

Values are mean±standard deviation.
* Significantly different from the mean of the respective untreated group at a P value of 0.01 or less.

TABLE 7. Effects of RES Stimulation with Zymosan on Δ^4-3-ketosteroid Hydrogenase Activity, Adrenal Weight and Liver Alanine Aminotransferase Activity of Rats fed on Various Diets

Group		No. of rats	Δ^4-Hydrogenase activity	Adrenal weight mg/100 g body weight	Alanine amino-transferase activity
Standard diet	Untreated	5	32.38±1.89	20.23±0.73	3256.8±334.5
	RES stimulated	5	41.76±2.31*	24.42±1.40*	2116.5±102.1*
60% Casein	Untreated	5	20.20±2.16	24.72±1.41	4674.0± 88.8
	RES stimulated	5	28.99±2.50*	29.45±1.57*	4030.5±259.8*
5% Casein	Untreated	5	12.55±0.88	19.84±1.19	1723.5± 95.5
	RES stimulated	5	14.36±0.75**	28.23±1.10*	1669.6±104.3

Values are mean±stndard deviation.
* Significantly different from the respective untreated group at a P value of 0.01 or less.
** Significantly different from the mean of the respective untreated group at a P value of 0.05 or less.

RES phagocytic activity was found to be markedly elevated by RES stimulation with typhoid paratyphoid vaccine or zymosan, and difference in dietary protein level had no influence on the process of stimulation of RES phagocytic activity. RES stimulation with typhoid paratyphoid vaccine or zymosan caused a rise in Δ^4-3-ketosteroid hydrogenase activity in all the three group. RES stimulation with typhoid paratyphoid vaccine or zymosan also caused an increase in adrenal weight in all the three groups. On the other hand, typhoid paratyphoid vaccine or zymosan injection clearly produced a fall in liver alanine aminotransferase activity in both standard and 60 per cent casein diet group, but no change in the 5 per cent casein diet group (Table 6 and 7).

Effect of fasting

Rats were fasted for 3 days, except that they were given water *ad libitum*.

TABLE 8. Effect of RES Stimulation with Typhoid Paratyphoid Vaccine on Phagocytic Activity of Fasted Rats

Group		No. of rats	Phagocytic activity, K
Standard diet Untreated		4	0.0200±0.0025
Fasting	Untreated	4	0.0167±0.0020
	RES stimulated	5	0.0290±0.0020*

Values are mean±standard deviation.
* Significantly different from the mean of the untreated group at a P value of 0.01 or less.

TABLE 9. Effect of RES Stimulation with Typhoid Paratyphoid Vaccine on Δ^4-3-ketosteroid Hydrogenase Activity, Adrenal Weight and Liver Alanine Aminotransferase Activity of Fasted Rats

Group		No. of rats	Δ^4-Hydrogenase activity	Adrenal weight mg/100 g body weight	Alanine amino-transferase activity
Standard diet Untreated		5	29.21±1.86	18.48±0.38	2367.3±204.5
Fasting	Untreated	5	14.95±1.03	22.52±1.73	3184.1±302.0
	RES stimulated	5	23.07±1.62*	28.03±1.99*	1848.9±151.7*

Values are mean±standard deviation.
* Significantly different from the mean of the untreated group at a P value of 0.01 or less.

At the same time they were injected into the tail vein with typhoid paratyphoid vaccine for 3 days in the RES stimulated group. As shown in Table 8, even in the fasted group, phagocytic activity was evidently increased by RES stimulation, but, compared with the group which was given food, the rate of increase in the fasted group was less marked. As described above, Δ^4-3-ketosteroid hydrogenase activity was shown to be markedly decreased by fasting. But it was significantly increased by RES stimulation even in the fasted group when compared with the untreated, fasted group. While adrenal weight relative to body weight was increased by fasting, the degree of increase was greater by RES stimulation. Liver alanine aminotransferase activity was observed to be increased in the fasted rats, but decreased in RES stimulated, fasted ones (Table 9).

DISCUSSION

The reticuloendothelial system has been well studied in terms of morphology, phagocytic activity and body defense, but there have been few studies on the role of RES in metabolism. The first step of inactivation of adrenal cortical hormone is the reduction of the C_{4-5} double bond of ring A which is catalyzed by Δ^4-3-ketosteroid hydrogenase, producing a dihydro compound. In the second

step, reduction of C_3 of ring A catalyzed by 3 α-hydroxysteroid dehydrogenase produces a tetrahydro compound. This compound is easily conjugated with glucuronate or sulfate, and is secreted in the urine or faeces as conjugates of glucuronic acid or sulfuric acid. The reaction involving catalysis by Δ^4-3-ketosteroid hydrogenase, which is the first step in the metabolism of the adrenal cortical hormone in the liver, is irreversible[15], and also rate-limiting[16] in the metabolic system leading to conjugation with glucuronic acid or sulfuric acid. It was reported by Berliner *et al.*[9)10)11)] that this process is carried out in the hepatic RE cells. Alanine aminotransferase activity undergoes marked changes dependent on dietary protein level. Since activity of the enzyme is markedly elevated by the adminstration of ACTH or adrenal cortical hormone [17)18)19)] and lowered by adrenalectomy[20], its activity is considered to be controlled by adrenal cortical function or glucocorticosteroid level in the plasma. It is also considered that the enzyme is closely related to protein catabolism and acceleration of gluconeogenesis which is a physiological action of glucocorticosteroid[17)18)19)]. In the present study, an investigation was made on the interrelationship between RES phagocytic activity which represents RES function, Δ^4-3-ketosteroid hydrogenase activity which participates in inactivation of adrenal cortical hormones, adrenal weight and alanine aminotransferase activity which is closely related to adrenal cortical hormones. When the dietary protein level varied, no particular change was noticed in RES phagocytic activity. In contrast, activities of Δ^4-3-ketosteroid hydrogenase and alanine aminotransferase and adrenal weight were found to be increased in rats fed high protein diet, but decreased in those fed low protein diet. In this case, since the change in the level of plasma glucocorticosteroid was not significant, as reported previously[21], these changes in alanine aminotransferase activity are considered to be caused chiefly by dietary protein level. So far no definite relation was noted between RES and alanine aminotransferase activity.

Elevation of alanine aminotransferase activity under fasting condition could be attributed to the raised level of plasma glucocorticosteroid[22)23)] as the result of reduced rate of inactivation of the hormone due to decreased Δ^4-3-ketosteroid hydrogenase activity, as well as relative hypertrophy of adrenals. Thus it is believed, in this case, that there is a close relation between RES function and liver alanine aminotransferase activity.

Administration of RES stimulating agent caused RES phagocytic activity to rise markedly irrespective of dietary protein level, but less marked in the fasted rats[24)25)]. Δ^4-3-ketosteroid hydrogenase activity was also raised by RES stimulation. In contrast, alanine aminotransferase activity fell uniformly by RES stimulation in all groups except the low dietary protein groups. This fact suggests that the level of steroid hormone in plasma was lowered by RES stimulation. Sawyer *et al.*[26)] reported that the rate of disappearance of intravenously injected corticosterone was markedly accelerated in rats subjected

to RES stimulation with zymosan. Since RES stimulation with typhoid paratyphoid vaccine or zymosan increased the adrenal weight, it is conceivable that both production and inactivation of steroid hormone are increased. As to the level of plasma steroid hormone under RES stimulation, no report has yet been avaliable. From the fact that alanine aminotransferase activity is reduced by RES stimulation, it may be considered, however, that inactivation of steroid hormone in the reticuloendothelial cells of the liver is more accelerated than the production of the hormone in the adrenal gland. Since the ratio of adrenal weight to Δ^4-3-ketosteroid hydrogenase activity in the RES stimulated groups did not differ significantly from that in the control group, the acceleration in uptake of steroid hormone into the stimulated liver RE cells could be considered to be an important factor. But this remains to be elucidated. While RES stimulation enhanced both phagocytic and Δ^4-3-ketosteroid hydrogenase activity, little change in liver alanine aminotransferase activity was noted in rats fed low protein diet. Because the activity of this enzyme in rats fed low protein diet is lower than that of adrenalectomized ones[27], it may be presumed that it was not influenced by the lowered plasma level of adrenal cortical hormone.

In the present study, the role of liver RES in metabolic regulation was explored by the changes in activity of liver alanine aminotransferase as an index of metabolism. As a result, it might be concluded that hepatic RE cells participate in the regulation of metabolism through the metabolism of adrenal cortical hormone under such conditions as RER stimulation or fasting. There exists a complex mechanism functioning in metabolic regulation or adaptation of an organism under various conditions, and what role RES plays remains to be studid in detail.

SUMMARY

In order to elucidate the role of RES in metabolic regulation, RES phagocytic activity, Δ^4-3-ketosteroid hydrogenase activity, adrenal weight and alanine aminotransferase activity of rats were determined under various dietary protein levels, by fasting or RES stimulation, and the mutual interrelationship was examined.

i) In rats fed high protein diet, Δ^4-3-ketosteroid hydrogenase and alanine aminotransferase activity and adrenal weight were increased, but the results were exactly reversed in those fed low protein diet. A definite relationship was not noted between RES phagocytic activity and dietary protein level.

ii) In fasted rats, depression of RES phagocytic activity and Δ^4-3-ketosteroid hydrogenase activity was noted on one hand, and rise in adrenal weight and alanine aminotransferase activity noted on the other.

iii) Under RES stimulation, rise in phagocytic activity, Δ^4-3-ketosteroid

hydrogenase activity and adrenal weight and decrease in alanine aminotransferase activity were noted.

iv) Rise in alanine aminotransferase activity in the liver of fasted rats and its depression in RES stimulated ones, suggest that RES function is related to body metabolism through the metabolism of adrenal cortical hormone.

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