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CHANGES IN *N*-ACETYL-β-GLUCOSAMINIDASE ACTIVITY IN HEPATIC DAMAGE

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SUMMARY

In order to elucidate the mechanism of the elevation of the serum *N*-acetyl-β-glucosaminidase activity found in hepatic damage, the intracellular distribution pattern of the enzyme in the liver was investigated.

1. In acute hepatic injury, a significant decrease of the enzyme activity was seen in both the lysosomal and supernatant fractions of the liver while in the serum activity increased simultaneously.

2. In chronic hepatic injury, the enzyme activity in the serum and liver supernatant fraction was found to be increased. This effect was more marked with advance of the damage. Changes in the enzyme activity both in the serum and in the supernatant fraction of the liver were seen to be nearly inversely proportional to the change in enzyme activity in the particle fraction of the liver.

3. In the process of convalescing from chronic hepatic injury, the enzyme activity was markedly increased in both the particle and supernatant fractions of the liver.

4. It was found that the release of the enzyme from the particle fraction of the liver occurs under various conditions; this is discussed in relation to the changes in hepatic acid mucoploysaccharides.

INTRODUCTION

N-Acetyl-β-glucosaminidase (EC 3.2.1.30) is widely distributed in mammalian tissues and its distribution and localization have been described by several workers[1–4]. This enzyme is mainly associated with the lysosomes and occurs largely in the latent form in fresh particulate preparations and treatments which cause a release of acid phosphatase liberated *N*-acetyl-β-glucosaminidase[2]. Liver was the tissue used almost exclusively in the studies of the intracellular distribution of the enzyme and its changes under various conditions[2,4–6]. Little has been known, however, about the changes in the intracellular distribution pattern of the enzyme occurring in hepatic damage.

We previously reported that the serum *N*-acetyl-β-glucosaminidase activity in patients with various liver diseases was elevated above the normal range, and that the

elevation of the enzyme activity in serum was most prominent in patients with active chronic hepatitis[3]. In the present study, in order to elucidate the mechanism of the elevation of the serum enzyme activity in hepatic damage, the interrelation between the serum enzyme activity and the intracellular distribution pattern of the enzyme in the liver was investigated under various conditions. In addition, a study was made of the effects of labilizing and stabilizing agents on enzyme release in healthy and damaged livers.

MATERIALS AND METHODS

Male albino rats of the Sprague–Dawley strain, weighing approx. 150 g at the beginning of the experiment, were used. 4 to 8 rats of each group were fed *ad libitum* on a standard laboratory diet. Chronic hepatic damage was induced by carbon tetrachloride inhalation twice a week for periods of 1, 2, 3, 4 and 5 months. For acute hepatic damage, animals were given 1.25 ml of carbon tetrachloride per kg of body weight through a stomach tube and sacrificed after 24 h. In the experiment on ischemia in rat liver, the left liver lobe was ligated according to the method described by DE DUVE AND BEAUFAY[4]. Rats with hypervitaminosis A were given orally a daily dose of 20 000 I.U. of vitamin A acetate per 100 g of body weight for 7 to 10 days. Hydrocortisone was injected intraperitoneally in a daily dose of 5 mg per 100 g of body weight for 5 days. Adrenalectomy was performed bilaterally by retroperitoneal approach and the rats were sacrificed after 24 and 48 h. The animals were bled under anesthesia, and the blood was collected and allowed to clot in a refrigerator. The livers were perfused *in situ* by the portal vein with ice-cold 0.25 M sucrose solution and removed. Before perfusion, a small piece of the liver was taken from the right lobe for histological study. Tissue fractionation was performed as follows. The liver homogenates (10%, w/v) were prepared according to a strictly standardized grinding technique in an ice-cold 0.25 M sucrose solution in a Potter–Elvehjem glass homogenizer for 2 min at about 1000 rev./min. For the study of enzyme release from lysosomes the liver was homogenized in 0.25 M sucrose containing 0.05% of Triton X-100. Fractions of subcellular particles were prepared from the sucrose homogenates of rat liver according to the method described by DE DUVE *et al.*[5]. Estimation of *N*-acetyl-β-glucosaminidase activity was performed by a modified method of WALKER, WOOLEN AND PUGH[7]. The assay system for *N*-acetyl-β-glucosaminidase activity contained 0.2 ml of enzyme solution and 1.8 ml of 3.6 mM *p*-nitrophenyl-*N*-acetyl-β-glucosaminide in 0.5 M acetate buffer (pH 4.3) in a final volume of 2 ml. After incubation for 30 min at 37°, 4 ml of 0.2 M borate buffer (pH 9.8) was added, the solution was centrifuged, and the liberated *p*-nitrophenol was measured at 420 mμ. Unless otherwise stated the results presented throughout this report are expressed as specific activity (μg of *p*-nitrophenol/mg of protein per 30 min for the liver, and mg of *p*-nitrophenol/100 ml of serum per 30 min for the serum). Protein was determined colorimetrically by the method of LOWRY *et al.*[8] with crystalline bovine serum albumin as a standard.

RESULTS

Distribution pattern of N-acetyl-β-glucosaminidase activity in rat liver and serum

In acute hepatic damage, as shown in Table I, the total *N*-acetyl-β-glucosaminidase activity of the liver was significantly lowered.

TABLE I

INTRACELLULAR DISTRIBUTION OF *N*-ACETYL -*β*-GLUCOSAMINIDASE ACTIVITY IN RAT LIVER AND SERUM

4 to 8 rats of each group were examined and enzyme activity was expressed as mean value ± S.D.

	Liver						*Serum****
	*Total activity**	*Super-natant***	*Micro-some***	*Lysosome***	*Mitochon-dria***	*Nucleus cell debris***	
Healthy	11.2 ± 0.6	5.2 ± 0.8	30.7 ± 6.5	306.4 ± 60.3	156.7 ± 15.6	20.5 ± 6.4	19.1 ± 1.4
Acute injury§	7.3 ± 0.9	0.6 ± 0.3	69.4 ± 7.1	87.4 ± 18.7	187.4 ± 19.3	19.1 ± 8.2	25.7 ± 2.7
Chronic injury§§	17.1 ± 1.2	6.8 ± 1.0	48.1 ± 6.3	118.7 ± 24.0	177.1 ± 17.5	20.2 ± 8.1	20.2 ± 1.7

* Total activity: the enzyme activity of total liver homogenate expressed as mg of *p*-nitrophenol liberated/g of wet liver per 30 min.

** Specific activity: *μ*g of *p*-nitrophenol/mg of protein per 30 min.

*** Serum activity: mg of *p*-nitrophenol liberated/100 ml of serum per 30 min.

§ Acute hepatic injury was caused by giving carbon tetrachloride through a stomach tube; rats were sacrificed after 24 h.

§§ Chronic hepatic injury was caused by carbon tetrachloride inhalation twice a week for 1 month.

With respect to the intracellular distribution pattern, the specific activities were markedly reduced in the supernatant and lysosomal fractions and slightly enhanced in the microsomal fraction; the serum activity was elevated. In chronic hepatic damage caused by carbon tetrachloride inhalation for 1 month, the serum enzyme activity showed no significant change. The total activity and distribution pattern of the enzyme, however, were entirely different from those of healthy rats.

The total activity and the specific activity of the supernatant and of the microsomal fractions were slightly increased and the lysosomal specific activity was significantly decreased.

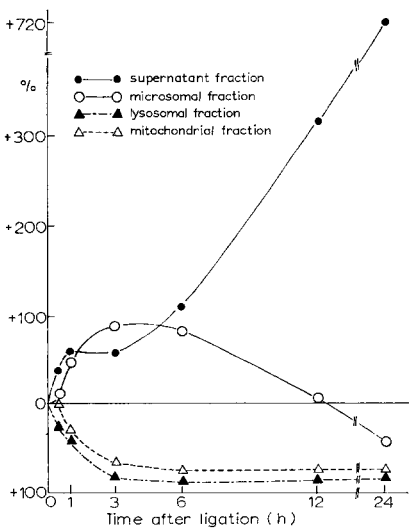

Fig. 1. Change in the intracellular distribution of *N*-acetyl-*β*-glucosaminidase in ligated liver lobe of healthy rat. Enzyme activity in each fraction is percent of the activity in the unligated part of the same liver.

Intracellular distribution of N-acetyl-β-glucosaminidase in ligated lobe of liver

Fig. 1 shows the pattern of change in *N*-acetyl-β-glucosaminidase activity in each subcellular fraction after liver lobe ligature in a healthy rat, in which values of the activity of the ligated lobe were expressed as percent of the values in the unligated part of the same liver. No difference was seen in the total activity between the unligated liver lobe after operation and the non-operated liver. The change in the intracellular distribution pattern was already evident 30 min after the ligation. The activity in the supernatant fraction was found to increase slowly just after ligation, and to increase rapidly thereafter, reaching a level more than 7-fold the normal level after 24 h. The activity of the microsomal fraction, on the other hand, increased slightly after 3 h and decreased progressively thereafter. In contrast, a significant decrease in the activity of mitochondrial and lysosomal fractions was seen throughout the experiment.

Rat livers with chronic hepatic damage were ligated in the same way as the healthy livers, and the rat was killed 3 h after the ligation. Table II shows the activity

TABLE II

N-ACETYL-β-GLUCOSAMINIDASE ACTIVITY IN SUPERNATANT AND PARTICLE FRACTIONS WITH AND WITHOUT LIGATION OF LIVER LOBE OF HEALTHY RATS AND RATS WITH DAMAGED LIVERS

		*Per cent value**		*S/P ratio***
		Supernatant (%)	*Particle (%)*	
Healthy	Control	2.6 (0.22 ± 0.03)***	97.4 (8.16 ± 0.95)***	7.3
	Non-ligated lobe	2.6 (0.19 ± 0.02)	97.4 (7.10 ± 0.16)	7.3
	Ligated lobe§	6.5 (0.39 ± 0.06)	93.5 (5.58 ± 0.98)	11.5
Chronic injury§§	Control	4.3 (0.35 ± 0.06)	95.7 (7.75 ± 0.85)	22.2
	Non-ligated lobe	4.0 (0.43 ± 0.07)	96.0 (10.3 ± 0.96)	22.2
	Ligated lobe§	11.8 (1.04 ± 0.09)	88.2 (7.8 ± 0.98)	30.3

* Per cent value: total activity of supernatant/total activity of whole homogenate × 100.
** S/P ratio: specific activity of supernatant fraction/specific activity of particle fraction × 100.
*** Values in parentheses show total activity ± S.D.
§ 3 h after ligation.
§§ Chronic hepatic injury was caused by carbon tetrachloride inhalation twice a week for 1 month.

of supernatant and particle fractions of the liver with chronic damage in comparison with the values obtained for healthy liver. The S/P ratio was expressed as percent of the activity in the supernatant fraction to the activity in the particle fraction. The rate of increase in the activity in the supernatant fraction in the ligated lobe of damaged liver was about the same as that of healthy rat liver. The S/P ratio in the ligated lobe of the damaged liver, however, was higher than that of normal liver. This result seems to suggest a significant increase in the enzyme release into the supernatant fraction from the particle fraction of the liver with chronic damage.

Release of N-acetyl-β-glucosaminidase with different treatments

In order to study the difference in enzyme liberation in healthy and damaged livers, a freezing and thawing treatment was used. As shown in Table III, with this

TABLE III

EFFECT OF THE TREATMENT OF REPEATED FREEZING AND THAWING OR TRITON X-100 ON THE CHANGE IN *N*-ACETYL-β-GLUCOSAMINIDASE ACTIVITY[1,*] IN SUPERNATANT AND PARTICLE FRACTIONS IN RAT LIVER WITH AND WITHOUT HEPATIC DAMAGE

	Healthy			*Acute injury***			*Chronic injury****		
	Super-natant	*Particle*	*S/P ratio*§	*Super-natant*	*Particle*	*S/P ratio*§	*Super-natant*	*Particle*	*S/P ratio*§
Control	5.2 ± 0.8	68.7 ± 0.6	7.3	0.6 ± 0.3	61.4 ± 6.0	1.0	6.8 ± 1.0	30.5 ± 3.8	22.2
Freezing and thawing (2 times)	8.7 ± 1.2	83.1 ± 8.3	10.5	24.7 ± 4.9	49.3 ± 5.2	50.0	25.2 ± 3.2	27.3 ± 4.0	92.0
Triton X-100 treated§§ (0.05%)	20.5 ± 4.6	73.4 ± 3.8	28.0	23.4 ± 4.3	58.3 ± 6.0	40.0	24.5 ± 5.7	58.6 ± 5.2	41.8

* Values show specific activities (μg of *p*-nitrophenol liberated/mg of protein per 30 min).
** Acute hepatic injury was caused by giving carbon tetrachloride through a stomach tube; rats were sacrificed after 24 h.
*** Chronic hepatic injury was caused by carbon tetrachloride inhalation twice a week for 1 month.
§ S/P ratio: specific activity of supernatant fraction/specific activity of particle fraction × 100.
§§ Liver was homogenized in 0.25 M sucrose containing 0.05% of Triton X-100.

treatment a significant increase was found in the activity of the supernatant fraction and the S/P ratio in liver with acute and chronic damage, while the increase was not as marked in healthy liver. On the other hand, no significant difference in the activity

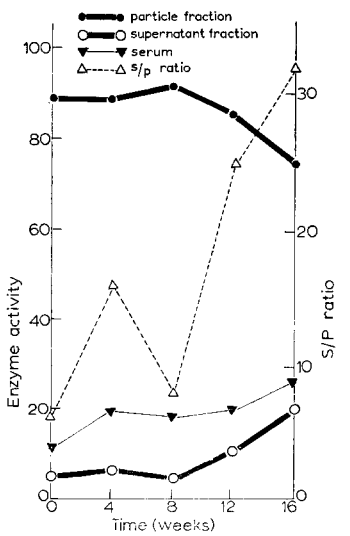

Fig. 2. *N*-acetyl-β-glucosaminidase activity of liver and serum in rats with chronic hepatic damage due to carbon tetrachloride inhalation. The ordinate on the left shows the enzyme activities of the serum and of the particle and supernatant fractions. On the right it shows the S/P ratio. Values of particle and supernatant fractions are given as specific activity of the enzyme and value of serum enzyme activity is given as mg of *p*-nitrophenol liberated/dl per 30 min. The S/P ratio is calculated as follows: specific activity of supernatant fraction/specific activity of particle fraction × 100. The abscissa shows duration of serial carbon tetrachloride inhalation.

of the supernatant fraction was seen between the groups after treatment with 0.05% Triton X-100 in 0.25 M sucrose solution. With the Triton treatment, however, the S/P ratio was increased in acute hepatic damage.

Change in N-acetyl-β-glucosaminidase activity in chronic hepatic damage

The change in *N*-acetyl-β-glucosaminidase activity in the serum and in the supernatant and particle fractions of the liver in chronic hepatic damage is shown in Fig. 2. The enzyme activity of the serum and the supernatant fraction was found to be increased with progression of hepatic damage, while that of the particle fraction was decreased. The S/P ratio was seen to be remarkably elevated in the advanced stage of hepatic damage. The changes in the activity of the serum and the supernatant fraction were shown to be nearly inverse to the change in activity of the particle fraction.

Change in N-acetyl-β-glucosaminidase activity during convalescence from chronic hepatic damage

The effect of interruption of hepatic injury on the enzyme activity of the serum and of the supernatant and particle fractions is shown in Table IV. In this experiment,

TABLE IV

N-ACETYL-β-GLUCOSAMINIDASE ACTIVITY IN PARTICLE AND SUPERNATANT FRACTIONS AND IN THE SERUM OF RATS CONVALESCING FROM LIVER DAMAGE

	Liver			*Serum*
	Supernatant	*Particle*	*S/P ratio**	
Control**	10.8 ± 1.7	86.3 ± 8.5	12.5	20.3 ± 3.7
2 weeks after***	37.2 ± 2.8	112.9 ± 8.2	33.0	22.2 ± 4.5
4 weeks after***	22.7 ± 4.5	70.6 ± 10.9	32.0	20.8 ± 3.6

* S/P ratio: specific activity of supernatant fraction/specific activity of particle fraction × 100.

** The activity of the control is the value of rats injured by 3 months of carbon tetrachloride inhalation.

*** 2 and 4 weeks after interruption of carbon tetrachloride inhalation.

rats injured by inhaling carbon tetrachloride for 3 months were used as controls. 2 weeks after interruption of carbon tetrachloride inhalation, the activity was significantly increased in both the particle and supernatant fractions of the liver, while no change was seen in the serum activity. After 4 weeks, the activity of the particle fraction was decreased, reaching the control level, while that of the supernatant remained at a slightly higher level. The S/P ratio of rat liver in the convalescent stage both 2 and 4 weeks after the interruption was higher than that of the control group.

Effect of hypervitaminosis A on the release of N-acetyl-β-glucosaminidase

A significant increase in the enzyme activity of the supernatant and particle fractions of the liver and of serum was found both in healthy rats and rats with chronic

TABLE V

EFFECT OF HYPERVITAMINOSIS A ON *N*-ACETYL-β-GLUCOSAMINIDASE ACTIVITY IN THE SUPERNATANT AND PARTICLE FRACTIONS OF THE LIVER AND IN THE SERUM OF HEALTHY RATS AND RATS WITH CHRONIC HEPATIC DAMAGE

		Liver			*Serum*
		Supernatant	*Particle*	*S/P ratio**	
Healthy	Control	3.1 ± 0.5	70.2 ± 3.4	4.4	13.6 ± 1.3
	Hypervitaminosis A**	5.0 ± 0.5	106.6 ± 3.1	4.6	18.3 ± 2.5
Chronic injury***	Control	3.7 ± 0.3	82.1 ± 3.1	4.5	20.8 ± 1.9
	Hypervitaminosis A**	5.6 ± 0.4	103.4 ± 10.7	5.4	25.3 ± 5.3

* S/P ratio: specific activity of supernatant fraction/specific activity of particle fraction × 100.

** Vitamin A was given orally to rats in a daily dose of 20 000 I.U. per 100 g of body weight for 7 days.

*** Chronic hepatic damage was induced by serial carbon tetrachloride inhalation twice a week for 2 months.

hepatic damage due to administration of excess of vitamin A. Practically no difference was found in the S/P ratios of control and hypervitaminosis A in healthy livers. On the contrary, in chronic hepatic damage, an increase was seen in this ratio in hypervitaminosis A as shown in Table V.

This result seems to indicate that the effect of hypervitaminosis A on enzyme release in the damaged liver is more marked than in healthy liver.

Effect of glucocorticoid on release of N-acetyl-β-glucosaminidase

The results presented in Table VI show that the enzyme activity in the particle fractions of both healthy liver and liver with chronic damage was slightly reduced by administration of hydrocortisone. No significant influence of hydrocortisone was

TABLE VI

EFFECT OF GLUCOCORTICOID ON *N*-ACETYL-β-GLUCOSAMINIDASE ACTIVITY IN THE SUPERNATANT AND PARTICLE FRACTIONS OF THE LIVER AND IN SERUM OF HEALTHY RATS AND RATS WITH CHRONIC HEPATIC DAMAGE

		Liver			*Serum*
		Supernatant	*Particle*	*S/P ratio**	
Healthy	Control	4.8 ± 1.9	71.4 ± 10.6	6.7	14.2 ± 3.1
	Hydrocortisone**	4.5 ± 0.3	67.7 ± 2.8	6.6	11.9 ± 1.1
Chronic injury***	Control	11.2 ± 2.0	83.4 ± 3.7	13.4	23.8 ± 0.6
	Hydrocortisone**	11.5 ± 1.4	75.4 ± 13.9	15.3	23.1 ± 2.4

* S/P ratio: specific activity of supernatant fraction/specific activity of particle fraction × 100.

** Hydrocortisone was injected intraperitoneally for 5 days in a daily dose of 5 mg per 100 g of body weight. The rats were sacrificed 24 h after the last injection.

*** Chronic hepatic damage was induced by serial carbon tetrachloride inhalation twice a week for 3 months.

found on the S/P ratio of healthy liver. In chronic hepatic damage, however, a slight increase was seen in the S/P ratio with hydrocortisone and no change was found in the activity of the supernatant. These results might suggest that glucocorticoid is not effective in inhibiting enzyme release from the particle fraction of damaged liver.

Effect of adrenalectomy on the release of N-acetyl-β-glucosaminidase

As shown in Table VII, the enzyme activity in the supernatant fraction of healthy liver increased markedly at 24 h and then returned to a nearly normal level 48 h after adrenalectomy. A slight increase of the enzyme activity in the supernatant fraction was found in chronic hepatic damage.

TABLE VII

EFFECT OF ADRENALECTOMY ON THE RELEASE OF *N*-ACETYL-β-GLUCOSAMINIDASE

			Liver			*Serum*
			Supernatant	*Particle*	*S/P ratio**	
Healthy	Control		4.8 ± 1.9	71.4 ± 10.6	6.7	14.2 ± 3.1
	Adrenalectomy**	24 h	16.3 ± 5.1	76.4 ± 24.3	21.3	20.9 ± 5.8
		48 h	9.2 ± 0.6	109.8 ± 28.4	8.4	15.0 ± 1.7
Chronic injury***	Control		23.5 ± 6.2	76.1 ± 22.4	30.9	27.9 ± 6.1
	Adrenalectomy**	24 h	30.0 ± 3.6	120.0 ± 11.0	25.0	26.9 ± 6.4
		48 h	21.7 ± 9.0	112.9 ± 10.9	19.2	20.7 ± 5.8

* S/P ratio: specific activity of supernatant fraction/specific activity of particle fraction × 100.

** Adrenalectomy was performed bilaterally by the retroperitoneal approach and rats were sacrificed after 24 and 48 h.

*** Chronic hepatic damage was induced by serial carbon tetrachloride inhalation twice a week for 4 months.

The enzyme activity of the particle fraction was increased both in healthy liver and in chronic hepatic damage, more markedly in the latter. The change in the S/P ratio due to adrenalectomy was found to be more marked in healthy liver than in damaged liver. The results seem to suggest that the enzyme release from the particle fraction is more pronounced in healthy liver than in damaged liver after adrenalectomy.

DISCUSSION

N-Acetyl-β-glucosaminidase is considered to be involved in the catabolism of acid mucopolysaccharides. Recently ARONSON AND DAVIDSON[9] have shown that hyaluronidase, an endomucopolysaccharidase, is present in the lysosomes of rat liver. The possibility exists, therefore, that the combined action of these enzymes in lysosomes might have biological implications for the degradation of acid mucopolysaccharides. The release of lysosomal hydrolases in ischaemic rat liver was described by DE DUVE AND BEAUFAY[4]. HUTTERER[10] showed that hepatic lysosomes degraded highly polymerized mucopolysaccharides, and the activity of hepatic mucopolysaccharidase was related to the turn-over rate of hepatic acid mucopolysaccharides and to fiber formation

in hepatic fibrosis. The abnormality of acid mucopolysaccharides in the liver and the elevation of *N*-acetyl-β-glucosaminidase activity in the serum in chronic hepatic damage were reported by our laboratory[3,11]. The pathologic role of *N*-acetyl-β-glucosaminidase as well as other lysosomal enzymes is not yet known. In acute hepatic damage, the increase both in the serum and in the supernatant fraction of the lysosomal enzymes seemed to be an indication of cell disruption. In chronic hepatic damage, abnormal intracellular distribution of the enzyme was found. This change became more pronounced in progressive stages of hepatic damage, which may be attributed to release of the lysosomal enzymes. Results suggesting different modes of enzyme release under various conditions were also seen *in vitro* in liver with chronic injury after treatment with Triton X-100 or freezing and thawing. The same result was obtained *in vivo* after administration of excess vitamin A, which is known to be a labilizing agent of lysosomes. It is assumed that the lysosomal alteration is related to the change in hepatic acid mucopolysaccharides.

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