ACTA CHEMICA SCANDINAVICA 7 (1953) 1170—1172

On the Intracellular Distribution of Catalase and Alcohol Dehydrogenase in Horse, Guinea Pig and Rat Liver Tissues

A. NYBERG, J. SCHUBERTH and L. ÄNGGÅRD

Medical Nobel Institute, Biochemical Department, Stockholm, Sweden

These investigations have been made preliminary to isotopic studies on the velocity of amino acid incorporation in catalase and alcohol dehydrogenase (ADH) in liver.

The intracellular distribution of catalase in rat liver has been studied by H. von Euler and L. Heller [1]. They used the differential centrifugation method developed by Hogeboom, Schneider and Pallade [2] but did not isolate the submicroscopical particles. Their final supernatant thus contained the ground substance of the cytoplasm as well as the submicroscopical particles. They found that the catalase activity per mg nitrogen was almost equally distributed between the mitochondria and the final supernatant. Our investigation concerns the same question in some other species.

No studies of this kind have been reported on ADH.

EXPERIMENTAL

Methods: In our preparations we have followed the method described by Hogeboom, Schneider and Pallade [2]. Fresh liver was forced through a masher in order to remove as much as possible of the connective tissue. 5 – 20 g of the mash was used in each preparation. In the following the figures given for the volumes of sucrose solution added before the homogenisation and added for the washings are calculated per g liver mash. The mash was ground for 10 minutes in a Potter-Elvehjem homogenizer together with 8 ml of 0.25 *M* sucrose solution. The big particles consisting chiefly of nuclei were separated by centrifugation for 10 min. at 700 g(I). The sediment (nuclei) was washed once with 4 ml of 0.25 *M* solution and centrifuged under the same conditions. The two supernatants were combined and centrifuged for 10 min. at 20 600 g(II) to isolate the mitochondria. They were washed twice with 4 ml of 0.25 *M* sucrose solution and then centrifuged at the same speed. The submicroscopical particles were isolated from the combined supernatants by centrifugation for 60 min. at 70 000 g(III). This fraction was washed once with 4 ml of 0.25 *M* sucrose solution and centrifuged for 60 min. at 140 000 g(IV). All operations were performed at 0 – 4° C. The sediments were suspended in 0.01 *M* phosphate buffer (pH 7) in order to cause lysis of the cell components.

(I) 2 000 r.p.m. with rotor No. 269 of the International refrigerated centrifuge PR-1.
(II) 16 300 r.p.m. with multispeed attachment No. 296 of the International refrigerated centrifuge PR-1.
(III) 27 690 r.p.m. with the preparative K rotor of the Spinco centrifuge model E.
(IV) 39 460 r.p.m. with the preparative K rotor of the Spinco centrifuge model E.

Table 1. Horse. 5 g liver tissue.

Fraction	Nitrogen	Catalase			ADH		
	mg	mg	%	Catalase / Nitrogen	mg	%	ADH / Nitrogen
Homogenate	106	32.5	100	0.307	25.7	100	0.242
Nuclei	21.7	0.4	1.2	0.018	1.6	6.2	0.074
Mitochondria	17.7	1.2	3.7	0.068	0.3	1.2	0.017
Microsomes	14.4	0.1	0.3	0.007	2.1	8.2	0.146
Supernatant	49.2	27.2	83.7	0.553	20.1	78.3	0.408
Sum	103	28.9	88.9		24.1	93.9	

The catalase activities were calculated into mg by using Kat.f. = 60 000.
The alcohol dehydrogenase activities were calculated into mg by considering 1 mg alcohol dehydrogenase to give $\Delta\Sigma = 40$ in three minutes.

Table 2. Guinea pig.

Fraction	Nitrogen mg	Catalase		
		mg	%	Catalase / Nitrogen
Homogenate	—	233.0	100	—
Nuclei	463	16.2	6.9	0.035
Mitochondria	215	8.6	3.7	0.040
Microsomes Supernatant	203	170.5	73.0	0.861
Sum	881	195.3	83.9	—

Table 3. Rat. 8.0 g liver tissue.

Fraction	Nitrogen	Catalase			ADH		
	mg	mg	%	Catalase / Nitrogen	mg	%	ADH / Nitrogen
Homogenate	220	5.4	100	0.025	2.1	100	0.010
Nuclei	62	1.0	18.6	0.016	0.4	19.0	0.006
Mitochondria	44	3.3	61.0	0.075	0.3	14.3	0.007
Microsomes	26	0.2	3.7	0.008	0.0	0.0	0.000
Supernatant	84	1.9	35.2	0.023	2.0	95.2	0.024
Sum	216	6.4	118.5		2.7	128.5	

The catalase activity was measured according to the rapid titration method described by Bonnichsen, Chance and Theorell [3]. The ADH activity was determined according to the spectrophotometrical method described by Theorell and Bonnichsen [4]. The nitrogen determinations were made with the micro Kjeldahl method.

Materials: Horse, guinea pig and rat liver tissue has been investigated. The microsomes were not isolated from guinea pig liver.

RESULTS

Six series of experiments were made, two in each of the species. The results from one of the series for each kind of animal are given in Tables 1—3. The other series essentially agreed with those given in the tables.

In guinea pig liver most of the catalase, about 70 %, is found in the fraction containing microsomes and ground substance and only minor quantities are present in the other fractions. This fraction (microsomes + ground substance) shows an extremely high catalase/nitrogen ratio.

The final supernatant of the horse liver preparations contains about 80 % of the catalase and has a very high catalase/nitrogen ratio compared with those of the other fractions.

The distribution of catalase in rat liver shows a different pattern. In essential agreement with von Euler and Heller [2] we found about 50 % of the catalase activity in the mitochondrial fraction, 30 % in the final supernatant and the rest in the other two fractions. The catalase/nitrogen ratio of the mitochondria is about 4 times as big as those of the other fractions.

Guinea pig liver did not give measurable quantities of ADH. In horse liver most of the ADH is located in the ground substance. The final supernatant shows a high ADH/nitrogen ratio.

Rat liver contains very small quantities of ADH, so the accuracy of the activity determinations was low. Most of the ADH activity was found in the supernatant.

SUMMARY

The intracellular distribution of catalase and alcohol dehydrogenase in horse, guinea pig and rat liver tissues has been investigated by using the differential centrifugation method.

REFERENCES

1. Euler, H. v., and Heller, L. *Z. Krebsforsch.* **56** (1949) 393.
2. Hogeboom, G. H., Schneider, W. C., and Pallade, G. E. *J. Biol. Chem.* **172** (1948) 619.
3. Bonnichsen, R. K., Chance, B., and Theorell, H. *Acta Chem. Scand.* **1** (1947) 685.
4. Theorell, H., and Bonnichsen, R. K. *Acta Chem. Scand.* **5** (1951) 1105.

Received June 2, 1953.