NON-MITOCHONDRIAL OXIDIZING PARTICLES (MICROBODIES) IN RAT LIVER AND KIDNEY AND IN TETRAHYMENA PYRIFORMIS

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Biochemical investigations carried out over the last ten years have established the existence in rat liver of a special group of cytoplasmic particles containing the enzymes urate oxidase, catalase and D-amino acid oxidase (Beaufay et al., 1964; de Duve, 1965). These particles have been identified morphologically by Baudhuin et al. (1965) as corresponding to the "microbodies" described in liver by Gansler and Rouiller (1956) and Rouiller and Bernhard (1956).

The three enzymes identified so far as components of the microbodies are linked together by hydrogen peroxide which is formed by the two oxidases and destroyed either peroxidatically or catalatically by catalase. It has accordingly been postulated that microbodies are involved in cellular oxidations associated with the formation and utilization of hydrogen peroxide. This hypothesis has guided a search for additional substrates of microbody oxidations and for similar particles in other biological materials.

Rat liver and kidney were fractionated by means of differential and isopycnic centrifugation as described by de Duve et al. (1955) and Beaufay et al. (1964). Similar techniques were used to fractionate T. pyriformis cells (var. 1, mating type II) homogenized according to Hogg and Kornberg (1963). Enzyme assays relied on chemical analyses (Beaufay et al., 1964), on polarographic determinations of oxygen consumption and on measurements of hydrogen peroxide production, using the oxidation of ^{14}C-formate in the presence of an excess of catalase as trapping system (Aebi et al., 1957). The reduction of dichlorophenol-indophenol (Redfearn and Dixon, 1961) was used to measure succinate dehydrogenase in T. pyriformis.

RESULTS

The most important results are summarized in Table I. In all three experiments

Table I. Intracellular distribution of enzymes

Material	Fraction	Protein %	Cytochrome Oxidase %	Cytochrome Oxidase R.S.A.	Urate Oxidase %	Urate Oxidase R.S.A.	Catalase %	Catalase R.S.A.	D-Amino acid Oxidase %	D-Amino acid Oxidase R.S.A.	L-α-OH-acid Oxidase %	L-α-OH-acid Oxidase R.S.A.	L-Amino acid Oxidase %	L-Amino acid Oxidase R.S.A.	Acid Phosphatase %	Acid Phosphatase R.S.A.
Rat liver (8 days after Triton WR-1339)	Nuclear	11.2	4.7	0.42	9.5	0.85	8.4	0.74	18.9	1.7	5.6	0.50			7.8	0.70
	Large granules (M+L)												Not			
	ρ<1.17	2.0	0.9	0.45	0.4	0.20	6.4	3.2	2.3	1.1	4.0	2.0			24.7	12.3
	1.17<ρ<1.20	19.0	84.3	4.4	7.0	0.37	3.3	0.17	5.2	0.27	5.4	0.28	assayed		7.2	0.38
	1.21<ρ<1.25	2.1	0.9	0.43	56.2	27	56.7	27	40.6	19	32.1	15			1.8	0.86
	Other fract.	2.7	4.5	1.7	17.1	6.3	7.4	2.7	8.6	3.2	6.4	2.4			2.7	1.0
	Supernatant (P+S)	63.0	4.7	0.08	9.8	0.16	17.8	0.28	24.4	0.39	46.5	0.74			55.8	0.89
	Recovery	105.0	74.3		83.2		95.3		73.5		90.0				107.2	
Rat kidney	Nuclear	27.8	35.2	1.3			24.4	0.88	30.2	1.1	14.3	0.51	29.1	1.0	31.0	1.1
	Large granules (M+L)				Not											
	1.14<ρ<1.21	14.9	56.5	3.8	measurable		3.9	0.26	5.2	0.35	6.1	0.41	2.6	0.17	12.8	0.86
	1.21<ρ<1.26	2.3	1.8	0.78			35.6	15	31.1	14	22.4	9.7	23.1	10	21.8	9.5
	Other fract.	4.3	0.5	0.12			12.4	2.9	12.5	2.9	13.2	3.1	13.7	3.2	12.5	2.9
	Supernatant (P+S)	50.7	6.0	0.12			23.7	0.47	21.0	0.41	44.0	0.87	31.5	0.61	21.9	0.43
	Recovery	105.7	92.8				98.4		91.5		93.6		95.0		99.4	
T. pyriformis	Large granules		Succinate dehydrogenase													
	ρ<1.19	18.3	11.2	0.61	Not		17.0	0.93	14.6	0.80	5.7	0.31	Not		59.0	3.2
	1.19<ρ<1.22	24.6	79.2	3.2			30.6	1.2	28.6	1.2	24.1	0.98			17.6	0.72
	ρ>1.22	12.6	9.6	0.76	measurable		46.0	3.7	46.3	3.7	56.6	4.5	measurable		11.4	0.91
	Supernatant	44.5	0	0			6.4	0.14	10.5	0.24	13.6	0.31			12.0	0.27
	Recovery	95.7	109.0				96.3		97.0		-				119.0	

Percentage values are given in terms of the sum of the activities recovered in all fractions; the percentage ratio of this sum to the activity of the homogenate defines the recovery. R.S.A. is the relative specific activity (R.S.A. of the homogenate = 1.0)

described, a large granule fraction was first isolated by differential centrifugation and then further subfractionated by density equilibration in a gradient of sucrose (containing 5% dextran for the experiments on liver and kidney). Highly significant similarities in enzyme distribution patterns were observed between the three materials studied and the data are best examined in a comparative fashion.

Catalase. In liver, this enzyme serves as a good index of intact microbodies since it is easily lost from these particles when they are injured. In the experiment shown in Table I, it was purified almost 30-fold with a more than 50% yield in the fraction of density 1.216 to 1.25. This fraction has a low content in mitochondria (cytochrome oxidase); in normal animals, it would be heavily contaminated with lysosomes, but these were caused to band in the upper region of the gradient by previously injecting the animal with Triton WR-1339 which selectively decreases their density (Wattiaux et al., 1963).

As already shown by Wattiaux (personal communication), the particulate catalase of kidney behaves very much like the liver enzyme and can be similarly separated almost quantitatively from the mitochondria by isopycnic centrifugation. In this case, the dense fractions also contain a high proportion of lysosomes, but these are certainly not the bearers of catalase, as has been shown by Wattiaux et al. (1964).

In T. pyriformis catalase is also associated with particles showing a higher average equilibrium density in a sucrose gradient than the succinate dehydrogenase-bearing mitochondria. The lysosomes, which were identified by means of several other acid hydrolases in addition to acid phosphatase, tended to accumulate in the regions of lower density, as they do when separated from the liver of animals injected with Triton WR-1339. However, there was a high degree of overlapping between the three groups of particles and the separations were much less clearcut.

D-amino acid oxidase. This enzyme was measured with D-alanine as substrate. The results of Table I confirm its association with the liver microbodies and show that it is similarly associated with the catalase-containing particles in rat kidney, as well as in Tetrahymena. To our knowledge, this enzyme has not been demonstrated before in the latter organism.

Urate oxidase. This enzyme is firmly attached to an insoluble component of the hepatic microbodies, presumably their crystalloid core (Baudhuin et al., 1965). It could not be demonstrated in rat kidney nor in Tetrahymena. It is interesting that the particles described in mouse kidney tubule cells by Rhodin (1954) under the name "microbody" differ from the hepatic microbodies by the absence of a crystalloid core.

L-α-hydroxy-acid oxidase and L-amino acid oxidase. An oxidase acting on a variety of L-amino and L-α-hydroxy-acids has been characterized by Blanchard et al. (1944, 1946) in rat kidney; the authors briefly mention the presence of a similar enzyme in rat liver. Eichel and Rem (1962) and Eichel (1964) have described a particle-bound L-α-hydroxy-acid oxidase in T. pyriformis. The results of Table I indicate that these enzymes (as measured by pyruvate formation from L-lactate or by the coupled oxidation of formate with L-lactate or L-leucine as substrate) accompany catalase and D-amino acid oxidase in all three materials. An excess of lactate oxidase activity over the other enzymes was found in the supernatant fraction from liver and kidney, but this may have been due to a second oxidative pathway involving lactate dehydrogenase and diaphorase.

Relative activities observed on various substrates are shown in Table II. It will be noted that the activity of the liver enzyme on α-hydroxy-acids decreases with increasing chain length; the reverse is true, at least up to α-hydroxy-butyrate with the enzymes of kidney and of Tetrahymena. All three enzymes (or groups of enzymes) differ in their relative activities on L-lactate and L-leucine. The ratio of L-lactate oxidase to D-amino acid oxidase activity also varies greatly in the three materials.

Other oxidases. Not all the oxidases of liver capable of supporting the coupled oxidation of formate "in vitro" are located in the microbodies. In agreement with the findings of Aebi et al. (1962), positive results were also obtained with substrate of the mitochondrial monoamine oxidase and of the soluble xanthine oxidase; several other substrates, including NADH and NADPH, were active with extracts or mitochondrial supernatants, but not with purified microbodies. No hydrogen peroxide production could be detected with succinate or α-glycerophosphate as substrate, even in liver

Table II. Relative oxidation rates of various substrates by microbody oxidases (Measured by the coupled oxidation of formate in the presence of 40 mM substrate, unless stated otherwise)

	Liver	Kidney	Tetrahymena pyriformis*
Glycolate, 5 mM	173	4.6	21
40 mM	75	11	20 (30)
L-Lactate	100	100	100 (100)
DL-α-OH-Butyrate	60	146	174 (180)
DL-Glycerate	27	59	65 (50)
L-Leucine	3	10	0.7
L-Alanine	0	0	0
D-Alanine	330	912	14
Urate 1.25 mM	750	0	0

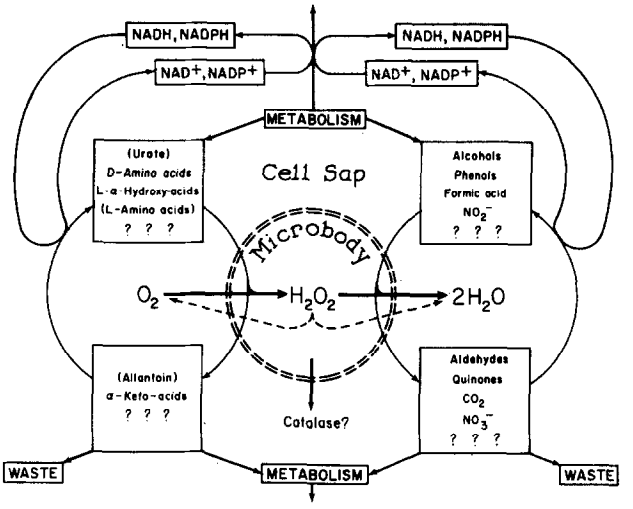
* Values between parentheses are from Eichel (1964).

extracts from animals treated with triodothyronine which enhances considerably the α-glycerophosphate oxidase activity (Lee et al., 1959). Subfractionation of the large granules showed the latter enzyme to belong to the true mitochondria where it must be functionally linked with the electron transport chain.

However, the microbodies may contain additional as yet unidentified oxidases. The purified liver particles were found to exhibit active respiration and hydrogen peroxide production in the presence of small amounts of a boiled high-speed supernatant from rat liver. It remains to be seen whether the substances responsible for this activity are substrates of known or unknown oxidases.

DISCUSSION

The results described provide further support for the hypothesis that microbodies are centers of hydrogen peroxide metabolism. The following diagram combining the information recorded in this paper with the known properties of catalase gives some idea of the possible functions of the microbodies.



METABOLIC FUNCTIONS OF MICROBODIES

It is probably significant that many of the substrates and products of microbody reactions can themselves either arise as products or act as substrates for reactions known to take place in the soluble fraction of the cell (de Duve et al., 1962). It may accordingly be postulated that microbodies carry out non-phosphorylative oxidations associated with metabolic functions of the cell sap. In addition to catalysing the specific destruction of some metabolites, they could, as illustrated in the above diagram, play a significant role in the disposal of electrons released by the soluble dehydrogenases. In the cells where they are present and where the existence of an efficient electron shuttle system has not been brought to light, microbodies rather than mitochondria could be responsible for this important function.

While catalase has a broad tissue distribution, the oxidases identified in rat microbodies appear to be characteristic of kidney and liver. Measurements of H_2O_2 production with L-lactate as substrate on preparations of rat brain, lung, spleen, heart and testes did not yield significant reaction rates. It will be the task of the future to find out to what extent microbody-like particles exist in the different cell types of higher animals. The finding that similar particles are present in a protozoan is of great interest and suggests that they have a long evolutionary history and presumably perform some vital function.

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REFERENCES

Aebi, H., Frei, E., Knab, R. and Siegenthaler, P. (1957). Helv. Physiol. Pharmacol. Acta, 15, 150.

Aebi, H., Quitt, J. and Hassan, A. (1962). Helv. Physiol. Pharmacol. Acta, 20, 148.

Baudhuin, P., Beaufay, H. and de Duve, C. (1965). J. Cell Biol. (in press).

Beaufay, H., Jacques, P., Baudhuin, P., Sellinger, O.Z., Berthet, J. and de Duve, C. (1964). Biochem. J., 92, 184.

Blanchard, M., Green, D.E., Nocito, V. and Ratner, S. (1944). J. Biol. Chem. 155, 421.

Blanchard, M., Green, D.E., Nocito-Carroll, V. and Ratner, S. (1946). J. Biol. Chem., 163, 137.

de Duve, C. (1965). Harvey Lectures, Series 59, 49.

de Duve, C., Pressman, B.C., Gianetto, R., Wattiaux, R. and Appelmans, F. (1955). Biochem. J., 60, 604.

de Duve, C., Wattiaux, R. and Baudhuin, P. (1962). Adv. Enzymol., 24, 291.

Eichel, H.J. (1964). Abstracts of the Sixth International Congress of Biochemistry. New York, 305.

Eichel, H.J. and Rem, L.T. (1962). J. Biol. Chem., 237, 940.

Gänsler, H. and Rouiller, C. (1956). Schweiz. Z. Allgem. Pathol. Bacteriol., 19, 217.

Hogg, J.F. and Kornberg, H.L. (1963). Biochem. J., 86, 462.

Lee, Y.P., Takemori, A.E. and Lardy, H.A. (1959). J. Biol. Chem., 234, 3051.

Redfearn, E.R. and Dixon, J.M. (1961). Biochem. J., 81, 19P.

Rhodin, J. (1954). "Correlation of Ultrastructural Organization and Function in Normal and Experimentally Changed Proximal Convoluted Tubule Cells of the Mouse Kidney". Aktiebolaget Godvil, Stockholm.

Rouiller, C. and Bernhard, W. (1956). J. Cell Biol., 2 Suppl., 355.

Wattiaux, R., Wibo, M. and Baudhuin, P. (1963). Ciba Foundation Symposium on Lysosomes (Little, Brown, Boston, Mass.), 176.

Wattiaux, R., Rutgeerts, M.J. and Wattiaux-De Coninck, S. (1964). Abstracts of the Sixth International Congress of Biochemistry, New York, 669.