A Study of Properties of Renal Microbodies of the Rat [1,2]

MARGARET E. BEARD AND JOHN M. ALLEN
Department of Zoology, University of Michigan, Ann Arbor, Michigan [3]

ABSTRACT The *in vitro* response of renal microbodies and lysosomes to a variety of altered physical and chemical conditions was examined. The influence of these conditions upon the integrity of the organelles was determined as that proportion of the total activity of various "marker" enzymes (α-hydroxy acid oxidase, catalase and D-amino acid oxidase for microbodies, and acid phosphatase for lysosomes) which was released from the organelles and recovered in the soluble phase following treatment.

Microbodies and lysosomes were separated by differential centrifugation from 15% homogenates of male, rat kidney prepared in 0.25 M sucrose. After exposure to experimental treatments, the preparation was further separated into sediment and supernatant fractions by centrifugation at 100,000 × g for 30 minutes. The sediment and supernatant fractions were assayed to determine the distribution of the "marker" enzymes.

Eighty to 100% of the activities of α-hydroxy acid oxidase and catalase were found in the supernatant after exposure of the microbody-lysosome fraction to low concentrations of Triton-X 100 or digitonin, or after treatment by sonic vibrations or high speed laminar shearing. Lesser but significant proportions of the total activities of these enzymes were recovered in the soluble phase after exposure of the organelles to distilled water, 0.1 M Sorensen's phosphate buffer or 0.1 M TRIS-HCl buffer prepared in distilled water. Acid phosphatase showed lower levels of release into the supernatant under most of these conditions.

When microbody-lysosome preparations were exposed to sucrose in concentrations of 0.1 M to 0.8 M to phosphate buffer in 0.25 M sucrose or to TRIS-HCl buffer prepared in 0.25 M sucrose, the release of α-hydroxy acid oxidase, catalase and acid phosphatase was insignificant. However, under these conditions D-amino acid oxidase was released into the soluble phase to a significant extent.

These results suggest that α-hydroxy acid oxidase and catalase are soluble constituents of renal microbodies while D-amino acid oxidase is associated firmly with a sedimentable component of the organelle, and that renal microbodies are more fragile than renal lysosomes.

Microbodies are intracellular organelles, distinct morphologically and biochemically from mitochondria and lysosomes. These particles have been identified in liver and kidney of the rat and other vertebrates by means of electron microscopy (deDuve and Baudhuin, '66; Ericsson, '64; Hruban and Reichgl, '67; Maunsbach, '66; Novikoff and Shin, '64; Rhodin, '54; Shnitka, '66), and light microscopy (Allen and Beard, '65; Sobel, personal communication). Microbodies appear as spherical particles 0.2 μ to 1.0 μ in diameter, bounded by a single membrane and usually containing an electron dense core (Baudhuin et al., '65a; Biempica, '66; deDuve and Baudhuin, '66; Ericsson, '64; Maunsbach, '66; Shnitka, '66).

Microbodies have been further characterized in liver and kidney of the rat and in *Tetrahymena pyriformis* by their enzyme content. Hepatic microbodies were found to contain three oxidative enzymes, α-hydroxy acid oxidase, D-amino acid oxidase and urate oxidase which form hydrogen peroxide, and catalase which degrades hydrogen peroxide (Baudhuin et al., '65a; deDuve and Baudhuin, '66; deDuve et al., '60). The enzyme content of rat renal microbodies is similar to that of hepatic microbodies, except that in kidney these organelles lack urate oxidase (Beard, unpublished; Baudhuin et al., '65b). In *T. pyriformis*, isocitrate lyase (Eichel and

[1] This research was supported by a grant from the U.S. Public Health Service, NIH 5 T1 GM 989; and by the Horace H. Rackham School of Graduate Studies, The University of Michigan, Ann Arbor, Michigan 48104.

[2] This study comprised a portion of the dissertation submitted in partial fulfillment of the requirements for the degree Doctor of Philosophy at The University of Michigan.

[3] Dr. Beard's present address is: Department of Pathology, Albert Einstein College of Medicine, Yeshiva University, Bronx, New York.

Rem, '62; Müller and Hogg, '67) and glyoxylate oxidase (Levy and Hunt, '67) seem to be associated in microbodies.

Other properties which are characteristic of microbodies, such as the fragility and permeability of the limiting membrane, sedimentation behavior, frequency of occurrence and the physical nature of the constituent enzymes have been determined only for microbodies derived from rat liver (Baudhuin, '64; deDuve and Baudhuin, '66). In order for the nature of microbodies to be more fully understood, it is necessary that their properties and occurrence be determined in a variety of tissues derived from various taxa. This study defines the physical characteristics of microbodies obtained from kidney of the rat. Emphasis has been placed on elucidating the nature of the association of the constituent enzymes to this organelle.

MATERIALS AND METHODS

The tissue employed in this study was the kidney from 90 to 180-day-old male rats, weighing 180 to 200 gm. Animals were kept in wire cages, two per cage, and were fed a diet of Purina Lab Chow and water *ad libitum*. Animals were killed by cervical dislocation. Tissue was excised immediately and placed on ice.

A 15% (wet weight/volume) tissue homogenate was prepared in 0.25 M sucrose (Mallinckrödt Analytical Reagent). Initial homogenization consisted of three up-down strokes of a smooth glass homogenizer over a loose-fitting Teflon pestle (clearance, 0.01 inch), rotating at 6000 RPM. Throughout the procedures of homogenization and subsequent tissue fractionation, the tissue temperature was maintained near 0°C. Following homogenization, differential centrifugation was carried out according to Allen and Beard ('65).

Microbodies and lysosomes derived from the "lysosomal" fraction of the differential centrifugation scheme were subjected to mechanical and chemical treatments which were expected to affect the integrity of the membranes surrounding these organelles. The extent to which these particles were affected by such procedures was determined as the release of α-hydroxy acid oxidase, D-amino acid oxidase and catalase from microbodies and of acid phosphatase from lysosomes into the soluble phase. Prior to treatment the "lysosomal" fraction was rinsed in 0.25 M sucrose and sedimented at 100,000 × g for 30 minutes in a Spinco Model L Ultracentrifuge maintained at 4°C, using a Spinco #40 rotor. The pellet resulting from this centrifugation was suspended in either: (1) 0.25 M sucrose to serve as the control preparation; (2) 0.25 M sucrose prior to mechanical treatment; or (3) a medium appropriate at the various chemical treatments. The control preparation was held on ice for the duration of the mechanical and chemical procedures. Following the experimental procedures, samples of all preparation were recentrifuged at 100,000 × g for 30 minutes. The resulting sediments and supernatants, as well as the remaining unsedimented samples, were assayed for enzyme activities. The per cent of solubilization was expressed as the ratio of enzyme activity recovered in the supernatant to the total enzyme activity in the unsedimented material. The sum of the enzyme activity of the sediment and supernatant was equal to the enzyme activity in the unfractionated, but similarly treated material.

Activities of α-hydroxy acid oxidase, D-amino acid oxidase and acid phosphatase were determined as outlined by Allen and Beard ('65). The method used for determining catalase activity was modified from Baudhuin et al., ('64). The reaction mixture contained the following final concentrations of components: 0.02 M imidazole

Fig. 1 Effect of mechanical procedures upon the release of enzymes found in lysosomes and microbodies.

Effect of timed periods of sonic vibrations (top left). Catalase and α-hydroxy acid oxidase were released totally by four minutes of treatment. Lower levels of activity of acid phosphatase and of D-amino acid oxidase were released.

Effect of repeated freezing and thawing (top right). Maximum release of catalase and of α-hydroxy acid oxidase occurred after four cycles of freezing and thawing. Acid phosphatase was released maximally after one cycle. D-Amino acid oxidase resisted release.

Effect of timed periods of blending (bottom). Catalase and α-hydroxy acid oxidase were nearly completely released after 30 seconds of treatment. Lower levels of activity of acid phosphatase and of D-amino acid oxidase were released.

A, catalase; B, α-hydroxy acid oxidase; C, acid phosphatase; D, D-amino acid oxidase.

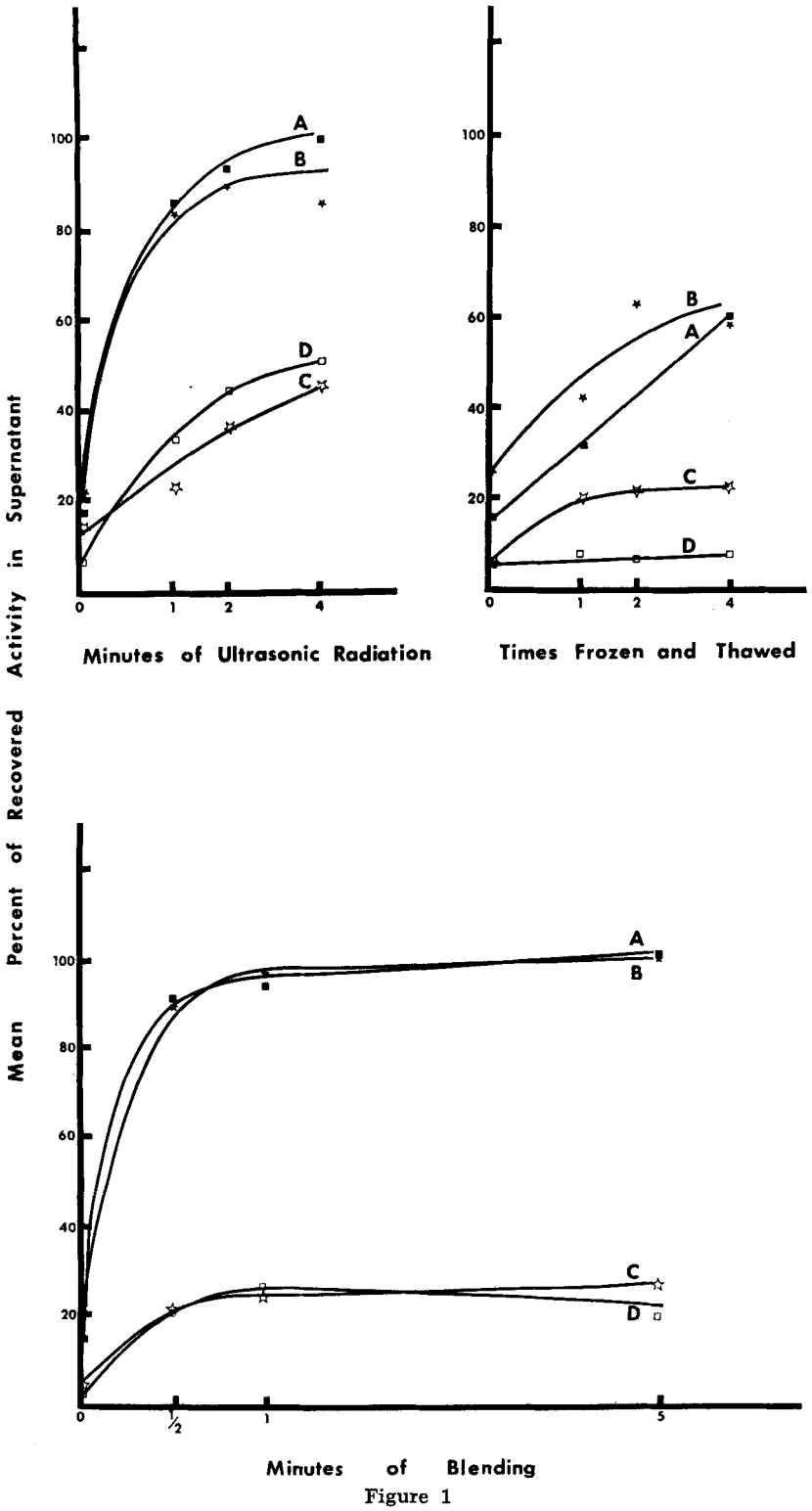
Mean Percent of Recovered Activity in Supernatant
100
80
60
40
20
A
B
D
C
0
1
2
4
Minutes of Ultrasonic Radiation
Times Frozen and Thawed
½
5
Minutes of Blending

Figure 1

(Sigma)-HCl buffer, pH 7.2; and 1.5 mM hydrogen peroxide (Baker) prepared by dilution from a 3% solution. Following incubation for exactly two minutes in an ice-water slush, the reaction was stopped by the addition of one-fourth saturated titanium sulfate (Sargent) in 2 N sulfuric acid. The optical density of the titanium peroxy-sulfate was measured at 405 m μ using a Coleman Junior Spectrophotometer.

In certain instances, the variability of the data required the use of the Kendall Sum, a non-parametric test for statistical significance (Siegel, '56). Probability values (P) refer to the significance of the increase in enzyme activity associated with the soluble phase following experimental treatments as compared to the soluble enzyme activity of appropriate control preparations.

RESULTS

Effect of mechanical procedures upon the release of enzymes from microbodies and lysosomes

Effect of timed periods of exposure to sonic oscillations (fig. 1, table 1). Catalase and α-hydroxy acid oxidase were released when the microbody-lysosome fraction was exposed at 4°C to sonic vibrations in an MSE Sonicator with a one centimeter probe. Following four minutes of treatment, 100% of the activity of catalase and 89% of the activity of α-hydroxy acid oxidase were recovered in the soluble phase. Treatment for shorter periods of time still caused the release of more than 80% of the total activities of both enzymes.

The response of D-amino acid oxidase differed from that of catalase and α-hydroxy acid oxidase. After four minutes of treatment 51% of the total activity of this enzyme was found in the supernatant. Treatment for one or two minutes resulted in the release of even smaller proportions of the total enzyme activity.

The response of acid phosphatase was similar to that of D-amino acid oxidase. As the duration of exposure to sonic vibrations increased, the portion of the activity of acid phosphatase recovered in the supernatant increased to a maximum of 46% after four minutes.

Effect of repeated freezing and thawing (fig. 1, table 2). Four cycles of freezing in an ice-alcohol slush followed by thawing at 25°C in water bath caused maximum release of catalase and α-hydroxy acid oxidase. More than 50% of the total activities of both enzymes was recovered in the soluble phase. Fewer cycles of freezing and thawing resulted in correspondingly smaller proportions of these enzyme activities recovered in the supernatant.

Twenty per cent of the total activity of acid phosphatase was released into the supernatant following one cycle of freezing and thawing. Further treatment did not increase the proportion of the enzyme activity released.

D-Amino acid oxidse resisted release into the soluble phase under these conditions; there was no significant increase in enzyme activity recovered in the soluble phase following this treatment over control values.

Effect of timed periods of blending (fig. 1; table 3). Catalase and α-hydroxy acid oxidase were nearly totally released following exposure of a microbody-lysosome preparation at 4°C to 30 seconds of high speed laminar shearing (blending) in a Waring Blender Micro-attachment (Eberbach). More than 90% of the activities of both enzymes was recovered in the soluble phase following treatment. After further blending, 100% of activities of catalase and α-hydroxy acid oxidase were recovered in the supernatant.

D-Amino acid oxidase and acid phosphatase were less susceptible to release by blending. Twenty-one per cent of the total activity of D-amino acid oxidase was found in the supernatant after 30 seconds of treatment. Further exposure to the sharing forces did not cause additional release of this enzyme. The response of acid phosphatase following blending was similar to that of D-amino acid oxidase. Blending for 30 seconds effected release of 21% of the total acid phosphatase activity. With additional treatment only a slightly greater portion of the total enzyme activity was found in the soluble phase.

Effect of detergents upon the release of enzymes from microbodies and lysosomes

Effect of digitonin (fig. 2, table 4). Catalase, α-hydroxy acid oxidase and acid

TABLE 1

Release of acid phosphatase, catalase, α-hydroxy acid oxidase and D-amino acid oxidase by exposure of a lysosomal-microbody preparation to ultrasonic radiation in MSE sonicator

Enzyme	Duration of exposure			
Acid Phosphatase	Untreated	One minute	Two minutes	Four minutes
$\bar{v}$ [1]	14.0	22.8	36.0	45.6
n [2]	3	3	3	3
r [3]	8.4–23.4	22.2–23.6	31.2–40.1	42.9–49.8
Catalase				
$\bar{v}$	17.2	83.9	93.5	99.5
n	3	4	4	4
r	1.1–26.6	67.4–100	87.6–100	97.9–100
α-Hydroxy acid oxidase				
$\bar{v}$	21.8	82.9	89.1	85.1
n	2	2	2	2
r	14.6, 28.9	82.5, 83.3	81.4, 96.7	84.6, 85.7
D-Amino acid oxidase				
$\bar{v}$	6.3	33.6	44.3	50.7
n	3	3	3	3
r	3.3–8.3	30.0–38.2	42.6–45.6	44.4–58.9

[1] $\bar{v}$, mean per cent of the total enzyme activity recovered in the supernatant after treatment and centrifugation.
[2] n, total number of determinations.
[3] r, range of values from which $\bar{v}$ was derived.

TABLE 2

Release of acid phosphatase, catalase, α-hydroxy acid oxidase and D-amino acid oxidase by exposure of a lysosomal-microbody preparation in 0.25 M sucrose to freezing in an ice-alcohol slush followed by thawing at room temperature

Enzyme	Number of times frozen and thawed			
Acid Phosphatase	Untreated	Two times	Four times	Eight times
$\bar{v}$ [1]	4.9	19.7	21.0	22.3
n [2]	3	3	3	3
r [3]	3.0–7	16.6–21.7	20.4–21.8	19.1–24.9
Catalase				
$\bar{v}$	14.6	28.5	23.0	64.5
n	2	2	2	2
r	12.5, 16.6	25.5, 31.5	18.0, 28.0	50.0, 79.0
α-Hydroxy acid oxidase				
$\bar{v}$	24.9	42.4	52.5	48.8
n	2	2	2	2
r	20.4, 19.4	39.0, 45.7	45.0, 60.0	43.0, 54.7
D-Amino acid oxidase				
$\bar{v}$	6.0	7.1	6.3	6.9
n	3	3	3	3
r	2.9–7.7	5.4–9.3	0–8.5	4.2–9.0

[1,2,3] See table 1.

phosphatase were released from microbody-lysosome-containing material which was exposed to increasing concentrations of digitonin. Maximal release of these enzymes occurred in the presence of 0.5% digitonin. Nearly 100% of the total activity of catalase, more than 80% of the activity of α-hydroxy acid oxidase, and

TABLE 3

Release of acid phosphatase, catalase, α-hydroxy acid oxidase and D-amino acid oxidase by exposure to blending

Enzyme	Minutes of treatment in the waring blender			
Acid Phosphatase	Untreated	One-half minute	One minute	Five minutes
$\bar{v}$ [1]	3.6	20.8	25	26.8
n [2]	2	1	2	1
r [3]	2.0, 4.3		23.1, 27	
Catalase				
$\bar{v}$	7.4	90.7	94.3	100
n	2	1	2	1
r	5.9, 8.8		88.6, 100	
α-Hydroxy acid oxidase				
$\bar{v}$	22.4	90.0	97.0	100
n	2	1	2	1
r	18.8, 26.0		94.1, 100	
D-Amino acid oxidase				
$\bar{v}$	2.5	20.8	26.0	19.5
n	2	1	2	1
r	2.3, 2.7		16.6, 35.5	

[1,2,3] See table 1.

50% of the activity of acid phosphatase were recovered in the soluble phase under these conditions. Lower concentrations of the detergent caused the release of small proportions of the enzyme activities.

The distribution of D-amino acid oxidase activity between the soluble and sedimentable fractions was unaffected by exposure of microbodies to this detergent. There was no significant release of the activity of this enzyme into the soluble phase as compared to the untreated control.

Effect of Triton-X 100 (fig. 2, table 5). Catalase, α-hydroxy acid oxidase and acid phosphatase were released into the soluble phase when the microbody-lysosome fraction was exposed to low concentrations of Triton-X 100. One-hundred per cent of the activity of catalase was recovered in the soluble phase in the presence of 0.05% Triton-X 100. Maximal release of both α-hydroxy acid oxidase (94%) and acid phosphatase (82%) occurred in the presence of 0.1% Triton-X 100. Concentrations of this detergent greater than 0.2% inhibited the activities of acid phosphatase and α-hydroxy acid oxidase. The activity of D-amino acid oxidase was not significantly released in the presence of any concentration of Triton-X 100.

Effect of solutions of various composition upon release of enzymes from microbodies and lysosomes

Effect of solutions of varying osmotic concentrations (table 6). A significant portion of the total activity of catalase was recovered in the soluble phase only when the microbody-lysosome preparation had been resuspended in distilled water (24.8%) or 1.25 M sucrose (21.6%). α-Hydroxy acid oxidase was significantly released by exposure of the lysosome-microbody fraction to distilled water (32.5%) and to solutions containing 0.8 M sucrose (19.5%) or 1.25 M sucrose (27.6%). Acid

Fig. 2 Effect of detergents upon the release of enzymes from lysosomes and microbodies.

Effect of increasing concentrations of digitonin (top). Catalase, α-hydroxy acid oxidase and acid phosphatase were released by exposure to digitonin. D-amino acid oxidase was unaffected by the detergent.

Effect of increasing concentrations of Triton-X 100 (bottom). Catalase, α-hydroxy acid oxidase and acid phosphatase were effectively released by exposure to low concentrations of Triton-X 100. Concentrations of Triton-X 100 greater than 0.2% inhibited activity of acid phosphatase and α-hydroxy acid oxidase. D-amino acid oxidase was not significantly released in the presence of this detergent.

A, catalase; B, α-hydroxy acid oxidase; C, acid phosphatase; D, D-amino acid oxidase.

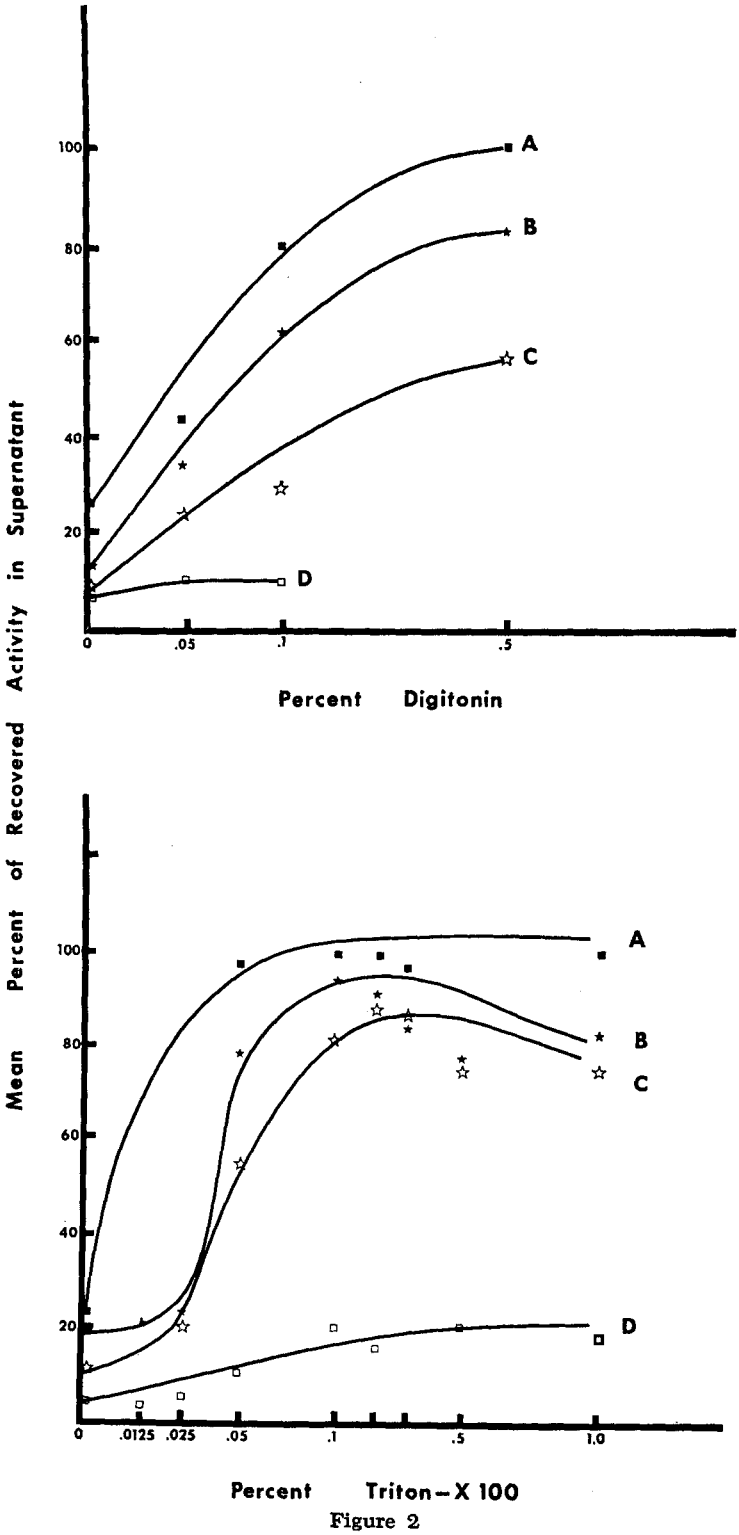
Mean Percent of Recovered Activity in Supernatant
100
80
60
40
20
0
.05
.1
.5
A
B
C
D
Percent Digitonin
100
80
60
40
20
0
.0125
.025
.05
.1
.5
1.0
A
B
C
D
Percent Triton–X 100

Figure 2

TABLE 4

Release of acid phosphatase, catalase, α-hydroxy acid oxidase and D-amino acid oxidase by exposure of a microbody-lysosomal preparation to digitonin

Enzyme	Concentration of digitonin			
Acid Phosphatase	Untreated	0.05%	0.1%	0.5%
$\bar{v}$ [1]	8.4	23.5	29.2	56.8
n [2]	2	2	2	2
r [3]	3.4, 13.5	21.9, 25.1	29.5, 30.4	30.8, 75.1
Catalase				
$\bar{v}$	25.9	43.1	80.1	100
n	2	2	2	2
r	37.0, 14.9	39.0, 47.1	67.1, 93.0	100
α-Hydroxy acid oxidase				
$\bar{v}$	12.9	33.9	61.8	83.3
n	2	2	2	2
r	12.5, 13.3	25.0, 42.8	54.5, 69.2	66.6, 100
D-Amino acid oxidase				
$\bar{v}$	7.2	10.0	9.2	
n	1	1	1	
r				

[1,2,3] See table 1.

TABLE 5

Release of acid phosphatase, α-hydroxy acid oxidase, catalase, and D-amino acid oxidase by exposure of a lysosomal-microbody preparation to Triton-X 100 in 0.25 M sucrose

Enzyme	Concentration of Triton-X 100								
Acid Phosphatase	None	0.0125%	0.025%	0.05%	0.1%	0.2%	0.4%	0.5%	1.0%
$\bar{v}$ [1]	11.9	9.9	20.4	55.1	82.2	88.0	87.4	74.6	74.6
n [2]	5	1	1	5	2	1	1	3	3
r [3]	8.2–21.7			34.3–64.8	79.8, 84.6			69.5–78.3	72.1–76.6
Catalase									
$\bar{v}$				97.8	100	100	97.1		100
n				2	2	1	1		2
r				95.6, 100	100				100
α-Hydroxy acid oxidase									
$\bar{v}$	20.0	21.2	23.8	79.0	94.4	91.8	84.6	78.5	82.6
n	4	1	1	4	2	1	1	2	2
r	5.3–29.7			39.3–100	88.8–100			85.8, 71.1	81.2–84.0
D-Amino acid oxidase									
$\bar{v}$	4.7	3.8	5.8	10.3	20.2		16.6	20.5	18.1
n	5	1	1	5	2		1	3	3
r	1.9–7.6			3.7–18.2	21.2, 19.2			19.7–21.9	15.7–19.7

[1,2,3] See table 1.

phosphatase was released into the soluble phase in the presence of distilled water (19.4%) and 1.25 M sucrose (43.6%). Little activity of this enzyme was recovered in the supernatant after exposure of the particulate material to 0.4 M or 0.8 M sucrose.

The response of D-amino acid oxidase to high concentrations of sucrose was difficult to determine since sucrose interfered

TABLE 6

Release of acid phosphatase, catalase, α-hydroxy acid oxidase and D-amino acid oxidase after treatment of a lysosomal-microbody preparation with solutions of varying osmotic strength

Enzyme	Suspending medium				
	Distilled water	0.25 M Sucrose	0.4 M Sucrose	0.8 M Sucrose	1.25 M Sucrose
Acid Phosphatase					
$\bar{v}$ [1]	19.4	5.4	3.5	14.1	43.6
n [2]	6	17	2	3	2
r [3]	15.6–24.2	1.0–26.0	1.2, 5.8	6.6–19.2	41.2–46.0
Catalase					
$\bar{v}$	24.8	5.4	3.8	6.9	21.6
n	7	20	2	5	4
r	14.7–40.6	0–15.2	0, 7.6	3.1–12.4	15.6–31.4
α-Hydroxy acid oxidase					
$\bar{v}$	32.5	10.8	13.9	19.5	27.6
n	6	18	2	5	4
r	15.2–60.7	0–38.0	12.5, 15.4	5.0–36.7	17.0–42.8
D-Amino acid oxidase					
$\bar{v}$	3.8	3.1	9.7	0 (Dialyzed)	6.2
n	6	19	2	3	3
r	0–14.1	0–10.3	8.9, 10.4	0	4.9
$\bar{v}$				15.6 (Undialyzed)	21.0 (Undialyzed)
n				2	1
r				13.0, 18.1	

[1,2,3] See table 1.

with the enzyme assay employed. For this reason most preparations were dialyzed for 12 hours against 0.1 M Sorensen's phosphate buffer prior to enzyme assay. No significant release of the activity of D-amino acid oxidase was observed after exposure of the microbody-lysosome fraction to either distilled water or to sucrose in concentrations greater than 0.25 M.

Effect of ionic solutions prepared in 0.25 M sucrose (table 7). Fractions containing microbodies and lysosomes were resuspended in 0.25 M sucrose to which one of the following compounds had been added in the concentrations indicated: 0.1 M Sorensen's phosphate buffer; 0.1 M Tris (hydroxymethyl) amino methane (TRIS): HCl buffer; 0.9% sodium chloride; 0.001 M ethylenediamine tetra-acetic acid (EDTA). All solutions were brought to pH 7.5. α-Hydroxy acid oxidase and catalase were significantly released in the presence of Sorensen's phosphate buffer ($p < 0.01$) and TRIS-HCl buffer ($p < 0.01$). Sixteen per cent of the total activity of α-hydroxy acid oxidase and 21.9% of the activity of catalase were recovered in the soluble phase after exposure to the former solution; 24.2% of the activity of α-hydroxy acid oxidase and 27.5% of the activity of catalase following exposure to the latter.

The response of acid phosphatase following exposure of lysosomes to these solutions differed from the response of microbody enzymes. None of the media significantly increased the proportion of the total activity of this enzyme recovered in the soluble phase ($p > 0.05$).

In the presence of all added chemicals, with the exception of EDTA, the portion of the total activity of D-amino acid oxidase (12% to 13%) recovered in the soluble phase was increased significantly ($p < 0.01$).

Effect of ionic solutions prepared in distilled water (table 8). Aqueous solutions of 0.1 M Sorensen's phosphate buffer, 0.1 M TRIS-HCl buffer, 0.9% sodium chloride and 0.001 M EDTA at pH 7.5 caused no significant increase in the activities of catalase, α-hydroxy acid oxidase or acid phosphatase recovered in the super-

TABLE 7

Release of acid phosphatase from lysosomes and of catalase, α-hydroxy acid oxidase and D-amino acid oxidase from microbodies in the presence of various ions dissolved in 0.25 M sucrose

Enzyme	Suspending medium				
Acid Phosphatase	0.25 M Sucrose	0.1 M Phosphate buffer pH 7.5 (Na, K salts)	0.1 M Tris-HCl buffer pH 7.5	0.9% NaCl	0.001 EDTA
$\bar{v}$ [1]	5.4	3.7	4.7	3.2	3.8
n [2]	17	3	2	2	1
r [3]	1.0–26.0	2.4–4.9	4.6, 4.9	2.8, 3.6	
Catalase					
$\bar{v}$	5.4	21.9	27.5	10.6	13.7
n	20	3	2	2	1
r	0–15.2	16.5–29.1	25.0–30.0	12.5, 8.7	
P [4]		(0.01)	(0.01)		
α-Hydroxy acid oxidase					
$\bar{v}$	10.8	16.4	24.2		12.5
n	18	3	2		1
r	0–38.0	12.0–19.0	23.3, 25.0		
D-Amino acid oxidase					
$\bar{v}$	3.1	13.0	12.0	12.9	8.0
n	19	3	2	2	1
r	0–10.3	11.8–14.4	11.5–12.4	11.9–13.9	
P		(0.01)	(0.01)	(0.01)	(0.05)

[1] $\bar{v}$, mean per cent of the total enzyme activity recovered in the supernatant after treatment and centrifugation.
[2] n, total number of determinations.
[3] r, range of values from which $\bar{v}$ was derived.
[4] P, level of significance as determined by the Kendall Sum.

natant when compared to the effect which distilled water lacking ions had upon the microbody-lysosome material ($p > 0.05$). Indeed, the activity of acid phosphatase found in the soluble phase actually decreased upon the addition of these compounds to the suspending medium.

On the other hand, D-amino acid oxidase was released into the soluble phase to a significant extent in the presence of aqueous solutions containing all adjuvants, with the exception of EDTA, ($p < 0.01$). Twelve per cent to 25% of the total activity of this enzyme was recovered in the supernatant fraction following exposure of microbodies to the various solutions.

DISCUSSION

The properties of microbodies from rat's liver have been extensively studied (see deDuve and Baudhuin, '66). From these investigations it was concluded that catalase is a soluble component of hepatic microbodies (Beaufay et al., '64) since under certain conditions all or part of the activity of this enzyme is found in the soluble phase. Although catalase at first appeared to demonstrate latency, and thus to resemble lysosomal acid phosphatase, this response was found to be a "pseudo-latency" in which enzyme activity could be measured only after its total release from its particulate association was effected. Such a response was attributed to the very high concentration of catalase within microbodies and the limited rate of diffusion of the substrate, hydrogen peroxide, into the organelle (deDuve and Baudhuin, '66). The property of "pseudo-latency," however, allowed studies to be made of the effects of various procedures upon the integrity of microbodies with concomitant release of the soluble catalase (deDuve, '65; deDuve and Baudhuin, '66).

D-Amino acid oxidase is considered a soluble enzyme of hepatic microbodies, being released into the soluble phase by blending and by detergent treatment, al-

TABLE 8

Effect of ions suspended in distilled water upon the release of acid phosphatase, catalase, α-hydroxy acid oxidase and D-amino acid oxidase from a microbody-lysosomal preparation

Enzyme	Suspending medium				
	Distilled water	0.1 M Phosphate buffer pH 7.5 (Na, K salts)	0.1 M Tris-HCl buffer pH 7.5	0.9% NaCl	0.001 EDTA
Acid Phosphatase					
$\bar{v}$ [1]	19.4	5.9	11.2	7.6	23.1
n [2]	6	5	6	2	2
r [3]	15.6–24.2	4.1–10.1	7.5–19.0	7.7, 7.6	21.8, 24.5
Catalase					
$\bar{v}$	24.8	36.9	40.1	38.7	25.0
n	7	5	6	2	2
r	14.7–40.6	23.6–45.2	18.5–50.2	40.0, 37.4	20.1, 30.0
P [4]			(0.05)		
α-Hydroxy acid oxidase					
v	32.5	47.1	41.3		36.2
n	6	5	6		2
r	15.2–60.7	41.1–6.6	23.5–57.0		29.0, 43.5
D-Amino acid oxidase					
$\bar{v}$	3.8	22.2	12.4	24.3	5.8
n	6	4	6	2	2
r	0–14.1	15.9–27.0	2.9–31.5	27, 21.5	5.1, 6.6
P		(0.01)	(0.05)	(0.01)	

[1,2,3,4] See table 7.

though being unaffected by repeated cycles of freezing and thawing. Further it is not characterized by a latency-like phenomenon (Beaufay et al., '64).

Urate oxidase of hepatic microbodies, on the other hand, was not recovered in the soluble phase after such treatments. It seems firmly associated with the sedimentable, electron dense core (Baudhuin et al., '65; Shnitka, '66; Tsukada et al., '66).

Hepatic microbodies do not behave as osmometers; they are freely permeable to sucrose and to molecules of similar size, as is indicated by their density independent behavior upon density equilibrium centrifugation (deDuve '65). Further, hepatic microbodies, when suspended in distilled water, show no loss of the soluble enzymes into the suspending medium (Baudhuin et al., '65; deDuve, '65).

The data presented here indicate that renal microbodies differ from hepatic microbodies in their response to altered environmental conditions *in vitro*. This difference in behavior may reflect a difference in the structural organization between hepatic and renal microbodies.

The catalase activity of renal microbodies demonstrates the latency-like phenomenon described for liver catalase. It is necessary to treat microbody-containing material with Triton-X 100, digitonin or mechanical procedures in order to measure the total activity of this enzyme. Catalase of renal microbodies appears to be a soluble component of this organelle, restrained within the microbody only by the presence of an intact, limiting membrane, since the enzyme was recovered nearly completely in the supernatant after the above treatments.

α-Hydroxy acid oxidase displayed no "pseudolatency," but like catalase was completely or nearly completely released into the soluble phase following exposure of microbody-containing material to Triton-X 100, digitonin, sonic oscillations, repeated cycles of freezing and thawing or high speed laminar shearing. It is concluded that α-hydroxy acid oxidase is also a soluble constituent of renal microbodies.

The behavior of D-amino acid oxidase of renal microbodies does not correspond to that described previously for hepatic

D-amino acid oxidase (Beaufay et al., '64). With the exception of the response of this enzyme to sonic vibrations, treatment of microbody-lysosome fractions by mechanical procedures or with detergents released an insignificant portion of the total activity of D-amino acid oxidase from a particulate association. The recovery of much of the activity of this enzyme in the supernatant following treatment with sonic oscillations may signify release of the enzyme from a "structure-bound" condition or may indicate fragmentation of the structural or membraneous components of microbodies into particles which failed to sediment during subsequent centrifugation. It is possible that D-amino acid oxidase is an integral structural component of renal microbodies as urate oxidase is of the nucleoid of hepatic microbodies (Baudhuin et al., '65). Alternatively, this enzyme may be more loosely bound to a sedimentable component by ionic or electrostatic forces. Only when lysosome-microbody fractions were resuspended in a solvent to which various ionizable salts had been added was a significant portion of the activity of D-amino acid oxidase recovered in the soluble phase.

It has been noted that solutions of varying osmotic strength, (distilled water and aqueous solutions of increasing sucrose concentration), did not release the soluble enzymes from hepatic microbodies (deDuve, '65). This is not the case for renal microbodies. Microbody-containing preparations which were exposed to distilled water or to an aqueous solution containing 1.25 M sucrose lost a greater portion of the activities of the soluble enzymes, catalase and α-hydroxy acid oxidase, into the supernatant than control preparations suspended in isosmotic sucrose. This suggests that renal microbodies are not as freely permeable to sucrose as are hepatic microbodies and that they behave as partial osmometers.

Renal microbodies differ from hepatic microbodies and hepatic lysosomes in still another way. The limiting membrane of the renal microbody seems to be more fragile, for nearly 100% of the activities of α-hydroxy acid oxidase and catalase were released from this organelle in the presence of lower concentrations of digitonin and Triton-X 100 than were required to release soluble enzymes from lysosomes and microbodies of liver.

Further, the response of renal microbodies to the various altered environmental conditions studied here differs from that of renal lysosomes. The lysosomal membrane remained intact when lysosome-microbody-containing material was exposed to low concentrations of ionized salts. A smaller portion of the total acid phosphatase activity was recovered in the supernatant under these conditions than following exposure of lysosomes to distilled water lacking adjuvants. In contrast, the presence of salts in the suspending medium caused release of a greater portion of the soluble enzymes from microbodies. Both organelles were affected by exposure to hyperosmotic sucrose solutions. Lysosomes, however, were more sensitive to these media than were microbodies, as indicated by the release of a larger proportion of acid phosphatase than of catalase or α-hydroxy acid oxidase into the soluble phase. Further renal microbodies seem to be more permeable to sucrose than renal lysosomes as demonstrated by their greater density dependence during density gradient centrifugation (see Allen and Beard, '65).

LITERATURE CITED

Afzelius, B. A. 1965 The occurrence and structure of microbodies. A comparative study. J. Cell Biol., *26:* 835–843.

Allen, J. M., M. E. Beard and S. Kleinbergs 1965 The localization of α-hydroxy acid oxidase in renal microbodies. J. Exp. Zool., *160:* 329–344.

Allen, J. M., and J. Gockerman 1964 Electrophoretic separation of multiple forms of particle-associated acid phosphatase. Annals N.Y. Acad. Sci., *121:* 615–633.

Baudhuin, P., H. Beaufay and C. deDuve 1965a Combined biochemical and morphological study of particulate fractions from rat-liver. Analysis of preparations enriched in lysosomes or in particles containing urate oxidase, D-amino acid oxidase and catalase. J. Cell Biol., *26:* 219–243.

Baudhuin, P., M. Müller, B. Poole and C. deDuve 1965b Non-mitochondrial oxidizing particles (microbodies) in rat liver and kidney, and in *Tetrahymena pyriformis*. Biochem. Biophys. Res. Comm., *20:* 53–59.

Beaufay, H., P. Jacques, P. Baudhuin, O. Z. Sellinger, J. Berthet and C. deDuve 1964 Tissue fractionation studies 18. Resolution of mitochondrial fractions from rat liver into three distinct populations of sytoplasmic particles by means of density-equilibration in various gradients. Biochem. J., *92:* 184–205.

Biempica, L. 1966 Human hepatic microbodies with crystalloid cores. J. Cell Biol., 29: 383–386.

Chantrenne, H. 1955 Effets d'un inhibiteur de la catallase sur la formation induite de cet enzyme chez la levure. Biochim. Biophys. Acta, 16: 410–417.

deDuve, C. 1965 The separation and characterization of subcellular particles. In: Harvey Lectures, Series 59, Academic Press, New York, 49–87.

deDuve, C., and P. Baudhuin 1966 Peroxisomes (microbodies and related particles). Phys. Rev., 46: 323–357.

deDuve, C., H. Beaufay, P. Jacques, Y. Rahman-Li, O. Z. Sellinger, R. Wattiaux and S. deConnick 1960 Intracellular localization of catalase and of some oxidases in rat liver. Biochim. Biophys. Acta, 40: 186–187.

Eichel, H. J., and L. T. Rem 1962 Respiratory enzyme studies in *Tetrahymena pyriformis*. V. Some properties of an L-lactate oxidase. J. Biol. Chem., 237: 940–945.

Domench, C. E. 1967 α-Hydroxy Acid Oxidases in subcellular fractions from rat kidney. Biochem. Biophys. Res. Comm., 28: 209–213.

Ericsson, J. L. E. 1964 Absorption and decomposition of homologous hemaglobin in renal proximal tubular cells. Acta Path. Microb. Scand. Suppl., 168.

Hruban, Z., and M. Reichigl 1967 Comparative ultrastructure of microbodies. Abstract, Fed. Proc., 26: 1428.

Levy, M. R., and A. E. Hunt 1967 L-α-hydroxy acid oxidase activity in Tetrahymena. Change with physiological state. J. Cell Biol., 34: 911–914.

Maumsbach, A. B. 1966 The ultrastructure of renal proximal tubule cells as related to protein absorption. Almquist and Wiksells Boktryckeri Ab, Uppsala, Sweden.

Müller, M., and J. F. Hogg 1967 Occurrence of protozoal isocitrate lyase and malate synthase in the peroxisome. Fed. Proc., 26: Abstract 158.

Novikoff, A. B., and W. Shin 1964 The endoplasmic reticulum in the Golgi zone and its relation to microbodies, Golgi apparatus and autophagic vacuoles in rat liver cells. J. Micro., 3: 187–206.

Rhodin, J. 1954 Correlation of ultrastructural organization and function in normal and experimentally changed proximal convoluted tubule cells of mouse kidney. Aktiebolaget Godvil, Stockholm.

Shnitka, T. K. 1966 Comparative ultrastructure of hepatic microbodies in some mammals and birds in relation to species differences in uricase activity. J. Ultrastruct. Res., 16: 598–625.

Siegel, S. 1956 Non-parametric Statistics for the Behavioral Sciences. McGraw-Hill Book Co., New York.

Tsukada, H., Y. Mochizuke and S. Fujiwara 1966 The nucleoids of rat liver cell microbodies. Fine structure and enzymes. J. Cell Biol., 27: 449–460.