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9. I thank Professor J. Z. Young for help and advice and Miss B. Mansell and Mrs. B. C. Wyatt for technical assistance.

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α-Hydroxy Acid Oxidase: Localization in Renal Microbodies

Abstract. *Differential and density equilibrium centrifugation have established the presence of α-hydroxy acid oxidase in microbodies of the kidney of the rat. The enzyme has been demonstrated in cells of the distal convoluted tubule by a microscopic cytochemical method. This enzyme, like certain others in microbodies, produces hydrogen peroxide.*

Microbodies are subcellular particles characterized biochemically by their content of D-amino acid oxidase, urate oxidase, and catalase (*1*). While the metabolic function of microbodies is unknown, it seems significant that two of these enzymes, D-amino acid oxidase and urate oxidase, produce hydrogen peroxide, while the third, catalase, breaks down this product. We have identified a fourth enzyme in microbodies derived from renal tissue of the rat. This enzyme, α-hydroxy acid oxidase, like D-amino acid oxidase and urate oxidase, produces hydrogen peroxide (*2*). Thus the suspicion that microbodies are engaged in the metabolism of hydrogen peroxide is further strengthened (*1*). A detailed account of these studies will be presented elsewhere.

α-Hydroxy acid oxidase was characterized biochemically (*3*) with the supernatant fluid obtained by brief blending of homogenates prepared in 0.1*M* Sörenson's phosphate buffer of *p*H 7.5 followed by centrifugation at 35,000*g* for 30 minutes at 4°C. These preparations, which contained over 90 percent of the enzyme in the soluble phase, were also used in electrophoretic studies. The enzyme oxidized a variety of α-hydroxy acids (Table 1). Neither flavin mononucleotide, flavin adenine dinucleotide, nicotinamide adenine dinucleotide, nor nicotinamide adenine dinucleotide phosphate increased the rate of substrate oxidation. However, inhibition by quinacrin (Table 1) suggests that the enzyme may utilize a flavin cofactor (*4*). The enzyme was inhibited by appropriate α-keto acids (Table 1).

α-Hydroxy acid oxidase could be identified after electrophoresis in acrylamide gels (*5*), since the enzyme was able to transfer electrons from substrate to nitro blue tetrazolium, thereby reducing this material to an insoluble blue pigment. The rate of tetrazole reduction was accelerated by phenazine methosulfate. The enzyme occurred as

Table 1. Properties of α-hydroxy acid oxidase.

Reaction mixture containing:	Relative activity of enzyme in crude supernatant	Relative activity of enzyme by photometric scanning after gel electrophoresis	Relative activity of enzyme by visual estimation of cytochemical reaction*
0.1*M* D, L-α-hydroxy valeric acid	1.00	1.00	++
0.1*M* D, L-α-hydroxy butyric acid	1.30	0.98	++
0.5*M* L-lactic acid	0.99	.46	+
0.5*M* D-lactic acid	.00	.00	—
0.1*M* D, L-α-hydroxy iso-butyric acid	.01	.00	—
0.1*M* D, L-α-hydroxy valeric acid, 0.1*M* D, L-α-keto valeric acid	.25	.34	±
0.1*M* D, L-α-hydroxy valeric acid, 10 m*M* quinacrin	.75	.72	+

* ++, maximal activity; +, modest activity; ±, just-detectable activity; —, no detectable activity. All estimations based upon evaluation of slides incubated for equal lengths of time.

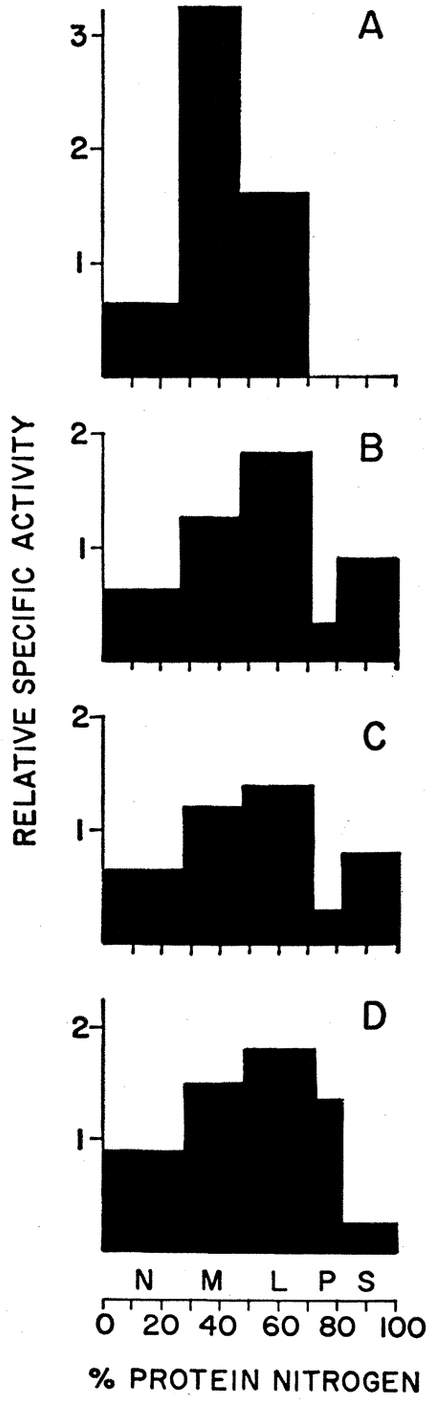

Fig. 1. Distribution of succinic dehydrogenase (*A*), α-hydroxy acid oxidase (*B*), D-amino acid oxidase (*C*), and acid phosphatase (*D*) after differential centrifugation. *N*, nuclear fraction; *M*, mitochondrial fraction; *L*, lysosomal fraction; *P*, microsomal fraction; *S*, soluble. Relative specific activity equals the percent of total enzyme activity divided by percent of total protein nitrogen (Biuret).

a single major electrophoretic form accompanied by a minor form. Photometric scanning of the developed gels (*5*) showed the rate of tetrazole reduction to be linear with time of incubation and enzyme concentration. The properties of the major form of the enzyme determined by photometric scanning were nearly identical to the properties of α-hydroxy acid oxidase determined by assay of crude supernatant material (Table 1). This close correspondence indicates that the same enzyme was measured in both analytical systems.

The sedimentation of particles containing α-hydroxy acid oxidase was studied by differential centrifugation (*6*) and density equilibrium centrifugation (*6*). As marker enzymes we chose succinic dehydrogenase (*6*), for mitochondria, acid phosphatase (*6*) for lysosomes, and D-amino acid oxidase (*6*) for microbodies. In these experiments homogenates were prepared in 0.25*M* sucrose by gentle grinding in smooth-bore glass homogenizers fitted with loose Teflon pestles. α-Hydroxy acid oxidase was easily released by rough mechanical treatment. In differential centrifugation studies (Fig. 1) α-hydroxy acid oxidase, D-amino acid oxidase, and acid phosphatase behaved similarly and showed highest relative specific activities in the "lysosomal" fraction. Succinic dehydrogenase, to the contrary, was concentrated in the "mitochondrial" fraction. This analysis indicates, therefore, that particles containing α-hydroxy acid oxidase resemble lysosomes and microbodies rather than mitochondria in their sedimentation behavior.

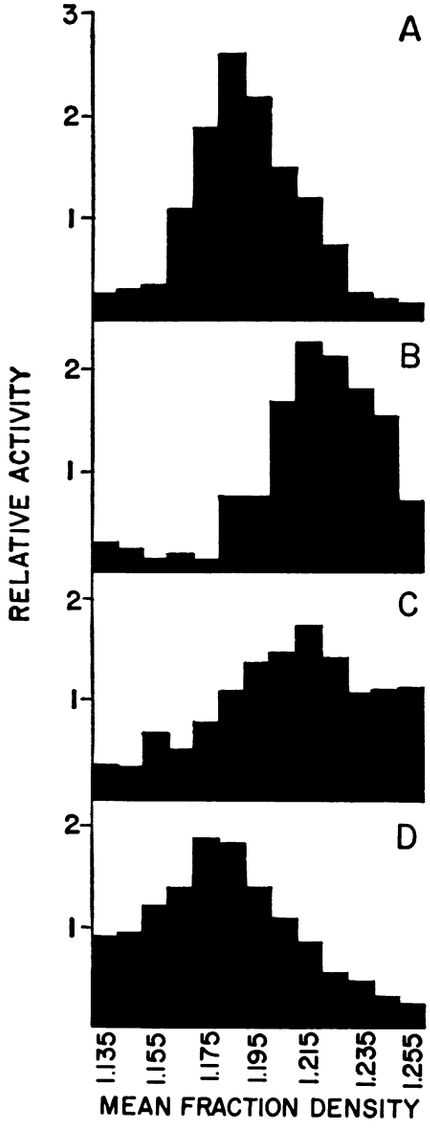

Fig. 2. Distribution of succinic dehydrogenase (*A*), α-hydroxy acid oxidase (*B*), D-amino acid oxidase (*C*), and acid phosphatase (*D*) after density equilibrium centrifugation in a sucrose-water gradient of density 1.12 to 1.26. Relative activity equals enzyme activity in fraction divided by mean recovered enzyme activity per fraction.

Density equilibrium centrifugation was carried out with a variety of linear sucrose-water gradients (*1*). In these studies, material from the "lysosome" fraction obtained by differential centrifugation (*6*) was layered on top of the gradient. In sucrose-water gradients extending from a density of 1.12 to 1.26, α-hydroxy acid oxidase and D-amino acid oxidase showed similar density distributions (Fig. 2). This distribution differed from that shown by succinic dehydrogenase and acid phosphatase (Fig. 2). In this gradient, the median density of particles containing α-hydroxy acid oxidase was 1.219 and that of particles containing D-amino acid oxidase was 1.210. These values are contrasted to median densities of 1.190 for particles containing succinic dehydrogenase and 1.181 for particles containing acid phosphatase. Particles containing α-hydroxy acid oxidase, therefore, resemble microbodies rather than mitochondria or lysosomes in their density equilibrium distribution.

The enzyme derived from density gradient fractions having high α-hydroxy acid oxidase activity had substrate specificities and other properties identical to the enzyme assayed in crude supernatant material. Furthermore, after electrophoresis of these fractions, the sites of enzymatic activity in the gels were identical to those derived from crude supernatant material. Thus, the α-hydroxy acid oxidase present in microbodies appears to be the same as that observed by assay of crude supernatant material and further identified by electrophoresis.

The ability of α-hydroxy acid oxidase to transfer electrons to nitro blue tetrazolium enabled us to design a specific microscopic cytochemical reaction for demonstration of the enzyme (*7*). Sections reacted for the localization of α-hydroxy acid oxidase showed a distribution of reduced nitro blue tetrazolium restricted primarily to cells of the distal convoluted tubule of the kidney (Fig. 3). The dye deposits were spherical, and were most frequently found in the basal portions of the cell; they ranged from 0.5 to 1.0 μ in diameter. This size range compares favorably with that of hepatic microbodies obtained by indirect measurement (0.54 μ) (*8*) or by direct measurement on electron micrographs (0.4 μ to 1.0 μ) (*8*). The rate of tetrazole reduction in sections was dependent upon the substrate molecule provided (Table 1) and closely matched the be-

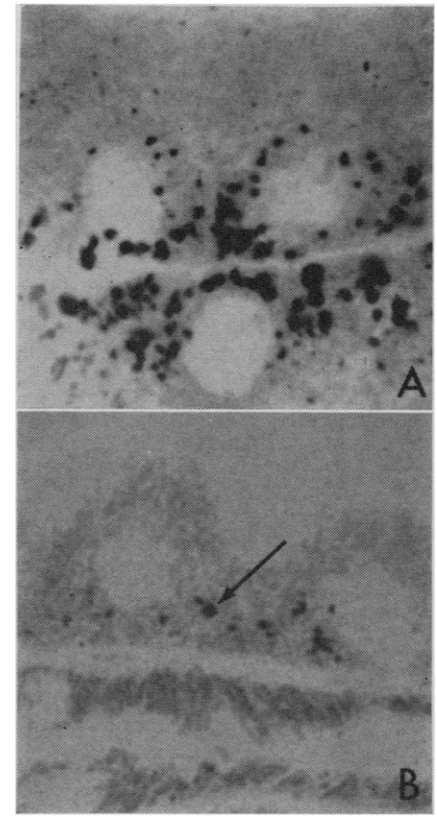

Fig. 3. (*A*) Cytochemical localization of α-hydroxy acid oxidase in cells of the distal convoluted tubule of the kidney of the rat. (× 1500) (*B*) Combined cytochemical localization of succinic dehydrogenase and α-hydroxy acid oxidase (arrow) in cells of the kidney of the rat. Elongate mitochondria are clearly visible in the cells of the proximal convoluted tubule (bottom). (× 1500)

havior of the enzyme separated in acrylamide gels or present in crude supernatant material. Tetrazole reduction did not occur in sections immersed in reaction mixtures containing all components except substrate. Inclusion of quinacrin or an α-keto acid in cytochemical reaction mixtures decreased tetrazole reduction (Table 1). Substitution of succinic acid for α-hydroxy acid in cytochemical reaction mixtures resulted in typical mitochondrial staining (Fig. 3). We feel that the cytochemical reaction system used to produce these results yields a valid image of the cellular distribution of α-hydroxy acid oxidase and, hence, of those renal microbodies which contain the enzyme.

The functional significance of microbodies is obscure. The identification of yet another hydrogen peroxide–producing enzyme in microbodies strongly implicates peroxide metabolism as a significant functional feature of these structures. The disparate catalytic functions of D-amino acid oxidase, urate oxidase, and α-hydroxy acid oxidase appear to preclude any unified metabolic role other than an as yet undefined association with catalatic oxidation. Perhaps microbodies will be found to contain diverse catalysts which have in common only the production of hydrogen peroxide. In this case, microbodies would serve to degrade hydrogen peroxide by catalatic oxidation and would be a device to protect cell components from indiscriminate oxidative attack by this molecule. The high concentration of catalase in hepatic microbodies (*8*), and presumably in those of other cells, would be admirably adapted to this role in cellular decontamination.

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3. α-Hydroxy acid oxidase in supernatant preparations was assayed by 2,6-dichlorophenolindophenol reduction according to J. Robinson *et al.* (*4*).
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6. Fractions in differential centrifugation experiments were obtained by the following schedule: nuclear fraction, 270*g* for 10 minutes followed by two washes at 270*g* for 10 minutes; mitochondrial fraction, 3020*g* for 10 minutes followed by one wash at 2600*g* for 10 minutes; lysosomal fraction, 22,000*g* for 20 minutes; microsomal fraction, $10^6 g$ for 1 hour. Density equilibrium centrifugation was done according to H. Beaufay *et al.* (*1*), with the Spinco SW 39 rotor operated at 39,000 rev/min for 2.25 hours exclusive of acceleration and deceleration times. Gradients were prepared and fractions were removed by the procedure of R. Martin and B. Ames, *J. Biol. Chem.* **236**, 1372 (1961). Enzymatic activity in fractions was determined as follows: α-hyroxy acid oxidase and D-amino acid oxidase by determination of α-keto acid formation, by the method of J. Robinson *et al.* (*4*) in the presence of 0.1*M* D, L-α-hydroxy valeric acid or 0.05*M* D-alanine; succinic dehydrogenase by 2,6-dichlorophenolindophenol coupled to phenazine methosulfate reduction as suggested by T. Singer and E. Kearney, in *Methods of Biochemical Analysis*, vol 4, D. Glick, Ed. (Interscience, New York, 1957), p. 307; acid phosphatase by determination of α-naphthol liberated from α-naphthyl acid phosphate according to J. Allen and J. Gockerman, *Ann. N.Y. Acad. Sci.* **121**, 616 (1964).
7. The reaction system used for cytochemical localization in sections was identical to that used for visualization of α-hydroxy acid oxidase after electrophoresis (*5*). All cytochemical results mentioned in this report were obtained with 2- or 4-μ cryostat sections of rat kidney fixed according to D. Walker and A. Seligman, *J. Cell Biol.* **16**, 455 (1963).
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9. Supported by grants from PHS, AM 5731 C-2, and from the University of Michigan Cancer Research Institute, American Cancer Society, Michigan Division Account.

12 July 1965

Algal Cultures: Ability To Reduce Turbulent Friction in Flow

Abstract. *Liquid cultures of several freshwater and marine algae required less pressure to flow through a pipe at a given rate than the pure liquid medium before algal growth. This increased ease of flow can be attributed to long-chain polysaccharides produced in the medium during growth. Measurements of friction were used to estimate the molecular weight of an algal polysaccharide and to show the effect of bacterial action on the polymer.*

In tanks for towing model ships, the occasional drastic decrease in the normal drag associated with a given model has been of great concern (*1*). Because solutions of many natural and synthetic high-polymers are effective in damping turbulence and reducing friction (*2*), dissolved algal polysaccharides have been proposed as the cause of the variations in drag in test tanks. It has been shown (*2*) that solutions of carrageenin, derived from *Chondrus crispus*, have lower friction in turbulent flow than does pure water.

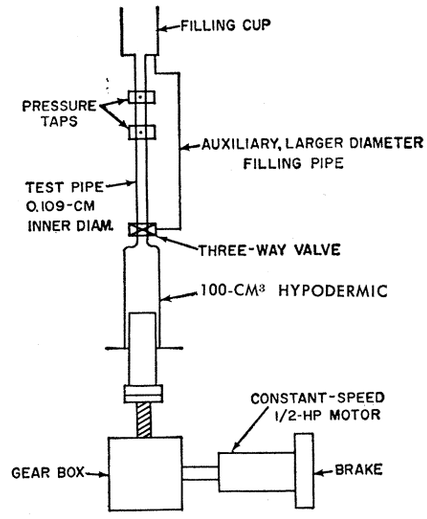

Fig. 1. Diagram of apparatus for measuring pressure losses by turbulent friction, caused by flow of algal cultures through a test pipe.

In order to show that algal growth can influence turbulent friction drag, we have examined liquid cultures of several freshwater and marine algae in an apparatus that measures the loss of pressure by friction in a short length of small-bore pipe. The instrument is designed to give a constant rate of flow of a sample of test solution (initially contained in a 100-cm^3 hypodermic syringe) through a measuring pipe, regardless of the magnitude of friction loss in the pipe; a fully turbulent flow is obtained, with the Reynolds number in the pipe approximately 14,000. The test consists in measuring pressures, during a run, at the two points marked "pressure taps" in Fig. 1. Strain-gage pressure transducers together with an oscillograph are used

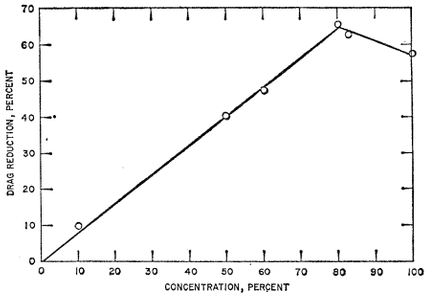

Fig. 2. Effect of dilution of culture of *Anabaena flos-aquae* with distilled water on observed reduction of friction.