Allantoinase: Association with Amphibian Hepatic Peroxisomes

Abstract. *Differential and density-gradient centrifugation studies have established an association between allantoinase and peroxisomes from the liver of the frog* Rana pipiens. *The presence of allantoinase in the peroxisome indicates a uricolytic function for this organelle in the liver of amphibians.*

Peroxisomes have been identified by ultrastructural criteria in the hepatic and renal tissue of numerous vertebrates (*1*), in plants (*2*), and in yeast (*3*). The organelles from several of these groups have been characterized biochemically by their content of D-amino-acid oxidase (E.C.1.4.3.3), urate oxidase (E.C.1.7.3.3), α-hydroxyacid oxidase (E.C.1.1.3.1), and catalase (E.C.1.11.1.6) (*4, 5*). The three former enzymes are involved in the production of hydrogen peroxide and the latter in its catabolism.

Urate oxidase has been used as a marker enzyme for the organelles. Urate oxidase catalyzes the oxidation of uric acid to allantoin and occupies a position in the chain of enzymes involved in uricolysis. With the ascent of the phylogenetic scale, the deletion of steps in the enzymatic degradation of uric acid is evidenced by the successive loss of urease (E.C.3.5.1.5), allantoicase (E.C.3.5.3.4), allantoinase (E.C.3.5.2.5), and urate oxidase (*6*). We have demonstrated the presence of a second enzyme of the sequence, allantoinase, in peroxisomes derived from the hepatic tissue of the frog. The enzyme allantoinase hydrolyzes allantoin to allantoic acid, which in amphibians is ultimately cleaved to glyoxylic acid and urea.

The sedimentation of particles containing allantoinase was studied by differential and density-gradient centrifugation (*7*). As marker enzymes we chose succinic dehydrogenase (E.C. 1.3.99.1) for mitochondria (*7*), acid phosphatase (E.C.3.1.3.2) for lysosomes (*7*), glucose-6-phosphatase (E.C. 3.1.3.9) for microsomes (*7*), and catalase, D-amino-acid oxidase, α-hydroxyacid oxidase, and urate oxidase for peroxisomes (*7*).

Homogenates (5 percent) of liver tissue were prepared in 0.25*M* sucrose containing 0.001*M* $MgCl_2$ and buffered with 0.01*M* tris(hydroxymethyl)-aminomethane–hydrochloride (*p*H 7.5). Gentle grinding in smooth-bore glass homogenizers that were fitted with loose Teflon pestles was required because catalase, acid phosphatase, and allantoinase can be released by mechanical disruption.

In differential centrifugation studies (Fig. 1) allantoinase, α-hydroxyacid oxidase, D-amino-acid oxidase, urate oxidase, and acid phosphatase behaved similarly and were recovered in the lysosome fraction, with variable amounts appearing in the soluble fraction. Succinic dehydrogenase and glucose-6-phosphatase, in contrast, were recovered in the mitochondrial and microsome fractions, respectively. This analysis indicates that, in sedimentation behavior, allantoinase resembles enzymes associated with lysosomes or peroxisomes rather than enzymes associated with mitochondria or microsomes.

Density-gradient centrifugation was carried out in linear sucrose-water gradients (*7*). Material from the lysosome fraction of differential centrifugations was utilized as the sample. In sucrose-water gradients having a density 1.122 to 1.338, the distributions of allantoinase, urate oxidase, D-amino-acid oxidase, α-hydroxyacid oxidase, and catalase were similar (Fig. 2). These distributions differed from those shown by succinic dehydrogenase and acid phosphatase (Fig. 2). The median density of particles containing allantoinase, catalase, and D-amino-acid

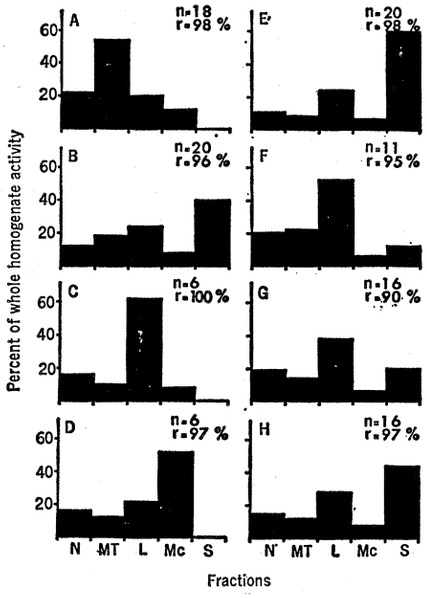

Fig. 1. Distribution of enzymes after differential centrifugation. (A) Succinic dehydrogenase; (B) acid phosphatase; (C) urate oxidase; (D) glucose-6-phosphatase; (E) catalase; (F) D-amino-acid oxidase; (G) α-hydroxyacid oxidase; and (H) allantoinase. Abbreviations are: *N*, nuclear fraction; *MT*, mitochondrial fraction; *L*, lysosome fraction; *Mc*, microsome fraction; and *S*, soluble fraction. Percentage values are given in terms of the ratio of the activity recovered in the fraction to the activity of the whole homogenate; *n*, number of experiments; *r*, mean total recovery (the ratio of the sum of the activities in fractions to a reference aliquot of the whole homogenate × 100).

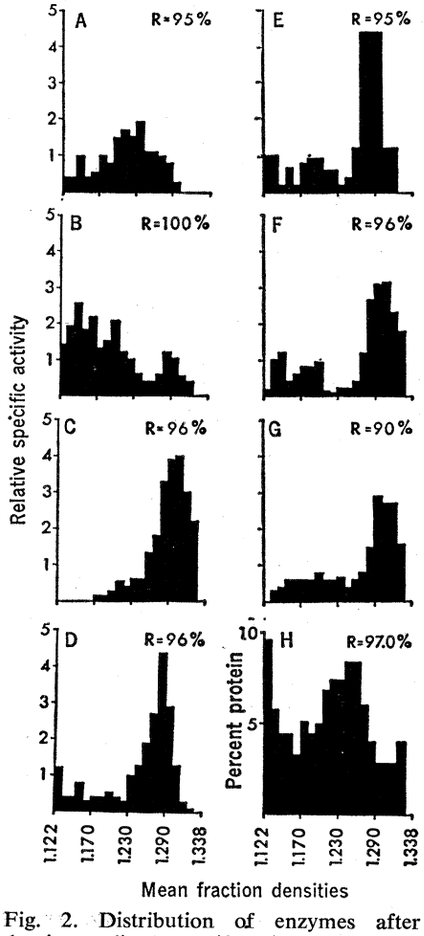

Fig. 2. Distribution of enzymes after density-gradient centrifugation in sucrose-water gradients of density 1.122 to 1.338 at 124,000*g* for 0.5 hour. (A) Succinic dehydrogenase; (B) acid phosphatase; (C) urate oxidase; (D) catalase; (E) D-amino-acid oxidase; (F) α-hydroxyacid oxidase; (G) allantoinase; and (H) protein nitrogen. Relative specific activity equals the ratio of percent activity per fraction to percent protein nitrogen per fraction. Data based on the mean obtained from three separate experiments; *R*, mean total recovery (the ratio of the sum of the activities in the fractions to a reference aliquot of whole homogenate × 100).

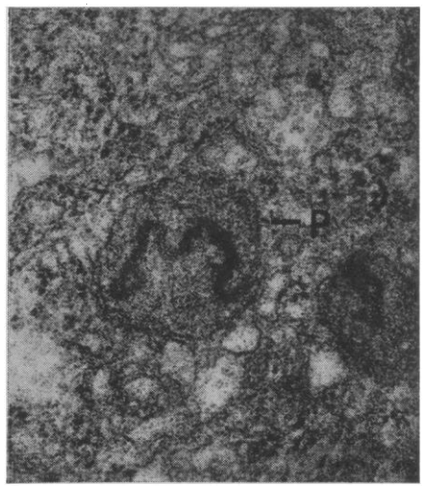

Fig. 3. Typical peroxisome derived from hepatic tissue of the frog, exhibiting a subcrystalloid core in the form of a complex tubular inclusion. Tissue fixed in glutaraldehyde and postfixed in osmium, followed by staining with uranyl acetate and lead citrate (× 47,000).

oxidase was 1.266. Particles with which urate oxidase and α-hydroxyacid oxidase were associated displayed a medium density of 1.278. These values were in contrast to median densities of 1.183 for particles containing acid phosphatase and 1.229 for particles containing succinic dehydrogenase. Thus, particles containing allantoinase resemble peroxisomes rather than mitochondria or lysosomes in their density distribution.

The disparate denstiy distribution shown by particles containing allantoinase, catalase, and D-amino-acid oxidase, when compared to those containing urate oxidase and α-hydroxyacid oxidase, is puzzling. The association of urate oxidase with the crystalloid core of peroxisomes has been advanced as an explanation of the similar behavior of these particles when derived from rat liver (*8*). It is possible, in frog liver, that both urate oxidase and α-hydroxyacid oxidase are associated with the peroxisome core. Peroxisomes from this source show a well-developed subcrystalloid core (Fig. 3) that often appears as a complex tubular inclusion exhibiting lateral extensions. They are closely associated with the endoplasmic reticulum, and occasionally the membrane of the particle appears as a direct saclike extension of the latter.

The presence of allantoinase in peroxisomes of amphibian liver suggests that these particles participate in the degradation of uric acid to glyoxylic acid and urea as well as in the removal of hydrogen peroxide by catalatic oxidation. Our investigations indicate that allantoincase, the enzyme acting in the cleavage of allantoic acid, is associated primarily with the soluble fraction derived from hepatic tissue of the adult frog.

Evidence indicates that peroxisomes of mammals are responsive to hypolipedemic agents (*9*) as well as to irradiation (*10*). This evidence and our data raise the question of the role of the peroxisome in cells of animals undergoing natural environmental pressures and fluctuating life cycles. In plants and *Tetrahymena* the peroxisome has been identified as the site of gluconeogenesis from 2-carbon compounds by virtue of its content of glyoxylate cycle enzymes (*5*). The role of the peroxisome in any unified metabolic scheme in mammals is obscure. In these animals peroxisomes have been described as "fossil organelles" (*8*), serving as important sites of peroxide catabolism and extramitochondrial oxidations. The association of allantoinase with amphibian peroxisomes and the position of this enzyme in the uricolytic sequence leading to glyoxylic acid and urea suggest that the peroxisome, at least in more primitive organisms, may play a role in uric acid catabolism.

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7. Differential centrifugation was carried out as follows: nuclear fraction, 120*g* for 10 minutes followed by three washes and centrifugations at 120*g* for 10 minutes; mitochondrial fraction, 1475*g* for 10 minutes followed by one wash and centrifugation at 1475*g* for 10 minutes; lysosome fraction, 14,500*g* for 20 minutes; microsome fraction, 150,000*g* for 30 minutes. A Spinco SW50 rotor operated at 124,000*g* for 0.5 hour was used for density-gradient centrifugation. Gradients were prepared by the procedure of R. Martin and B. Ames [*J. Biol. Chem.* **236**, 1372 (1961)]. Enzyme assay was as follows: α-hydroxyacid oxidase and D-amino acid oxidase, F. Leighton, B. Poole, H. Beaufay, P. Baudhuin, J. W. Coffey, S. Fowler, C. de Duve [*J. Cell Biol.* **37**, 482 (1968)]; allantoinase, G. Brown, Jr. [*Amer. Zool.* **4**, 310 (1964)]; succinic dehydrogenase, T. Singer and E. Kearney [in *Methods of Biochemical Analysis*, D. Glick, Ed. (Interscience, New York, 1957), vol. 4, p. 307]; acid phosphatase, J. Allen and J. Gockerman [*Ann. N.Y. Acad. Sci.* **121**, 616 (1964)]; catalase, P. Baudhuin, H. Beaufay, Y. Rahman-Li, O. Z. Fellinger, R. Wattiaux, P. Jacques, C. de Duve [*Biochem. J.* **92**, 179 (1964)]; uricase, H. Tsukada, Y. Mochizuki, T. Konishi [*J. Cell Biol.* **37**, 231 (1968)]; glucose-6-phosphatase, M. Swanson [*Meth. Enzymol.* **2**, 541 (1955)]. Protein nitrogen was determined by a biuret reaction according to L. Bailey [*Techniques in Protein Chemistry* (Elsevier, New York, ed. 2, 1967), p. 340].
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1-Δ⁹-Tetrahydrocannabinol: Neurochemical and Behavioral Effects in the Mouse

Abstract. *Administration of pure 1-Δ⁹-tetrahydrocannabinol to mice had the following dose-dependent neurochemical and behavioral effects: a slight but significant increase in concentrations of 5-hydroxytryptamine in whole brain; a decrease in concentration of norepinephrine in brain after administration of low doses and an increase after high doses; diminished spontaneous activity, moderate hypothermia, hypersensitivity to tactile and auditory stimuli, and ataxia after low doses; and sedation, pronounced hypothermia, and markedly diminished spontaneous activity and reactivity after high doses. The duration of the effects on body temperature and spontaneous activity correlated generally with the changes in brain amines. The characteristic changes in brain amines do not correspond exactly to those observed with other psychotropic drugs.*

The availability (*1*) of pure, synthetic 1-Δ⁹-tetrahydrocannabinol (Δ⁹-THC) has permitted study of its effects both on amine metabolism in the brain and on gross behavior in the mouse. In the past, study of the psychopharmacological effects of marihuana has been complicated by the fact that extracts in different laboratories contain varing mixtures of tetrahydrocannabinol isomers, along with other constituents. The effects of low doses (50 to 120 μg) of Δ⁹-THC per kilogram of body weight in man (*2*) have been described as similar to those of marihuana; that is, euphoria, alterations in sense of time