Technical Papers

Intracellular Distribution of Rat-Liver Arylsulfatase as Compared with That of Acid Phosphatase and Glucose-6-Phosphatase

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It has been reported by Dodgson, Spencer, and Thomas, that the arylsulfatase of rat liver is localized mainly in the microsomes (*1*). These authors used potassium *p*-acetylphenylsulfate as substrate. Roy, employing potassium 2-hydroxy-5-nitrophenyl sulfate (4-nitrocatechol sulfate) as substrate, found that some 70 percent of the sulfatase activity was present in the mitochondria of the mouse-liver cell (*2*). More recently Roy reported that the bulk of the sulfatase (43 to 62 percent) is also present in the mitochondrial fraction of rat liver (*3*).

However, none of these authors has studied the intracellular localization of rat-liver sulfatase as compared with that of other enzymes whose intracellular distribution has been well established. Since it has been reported by de Duve *et al.* (*4*) that acid-phosphatase-bearing granules are different from those containing cytochrome oxidase and require larger centrifugal fields for complete sedimentation, it was decided to carry out separation of cytoplasmic granules according to a new fractionation scheme developed by Appelmans, Wattiaux, and de Duve (*5*). In this fractionation procedure, the cytoplasmic granules are separated into a heavy mitochondrial fraction, a light mitochondrial fraction, and a microsomal fraction.

The livers of 15 hours-fasted rats were homogenized in isotonic sucrose containing 0.001*M* versene and fractionated according to the afore-mentioned procedure. The fractions thus obtained were treated for a period of 2 min in a Waring Blendor, usually after a tenfold dilution with glass-distilled water. This was done to cause complete activation of acid phosphatase (*6*). β-glycerophosphate 0.05*M* adjusted to *p*H 5.5 with HCl and buffered with 0.05*M* acetate was used for the acid phosphatase assays. Arylsulfatase-activity tests were made concomitantly, using a slight modification of the method of Dodgson *et al.* (*7*). Assays were made in duplicate, and suitable controls were always run.

The results of a typical experiment are reported in Table 1, which shows that the intracellular distribution of these two enzymes is completely different. Acid phosphatase is localized mainly in the light mitochondrial fraction, whereas 70 percent of the arylsulfatase activity is recovered in the microsomes. The heavy mitochondrial fraction contains only a negligible amount of sulfatase. A larger percentage of this enzyme is present in the light mitochondrial fraction but is probably due to a greater contamination by microsomes. That the nuclei contained a larger percentage of sulfatase than acid phosphatase is easily explained by the fact that nuclei are more readily contaminated by microsomes than by mitochondria. Furthermore Dodgson *et al.* (*1*) have reported that uncontaminated nuclei isolated according to the method of Wilbur and Anderson (*8*) had negligible arylsulfatase activity.

The localization of arylsulfatase in the rat-liver cell was also studied by comparison with that of glucose-6-phosphatase, which has been shown by Hers *et al.* (*9*) to be a typical microsomal enzyme. The same fractionation procedure was used, except that the heavy and light mitochondrial fractions were centrifuged down together. Glucose-6-phosphatase activity was estimated by the amount of inorganic phosphate liberated at 37°C in the presence of 0.008*M* glucose-6-phosphate and 0.05*M* tris (hydroxymethyl) aminomethane buffer *p*H 6.8. Assays were run in duplicate and suitable blanks were always made.

The results of a typical experiment are reported in Table 2. It is evident that sulfatase and glucose-6-phosphatase are both contained in the microsomal fraction of rat liver, the other fractions being contaminated to the same extent by the microsomes. The reconstituted homogenate was made at the end of the fractionation procedure, by recombining aliquots from the different fractions equivalent to the same amount of liver. This preparation still exhibited some 90 percent of the arylsulfatase and glucose-6-phosphatase activities present in the original homogenate.

The foregoing results therefore confirm the observations of Dodgson *et al.* (*1*) that liver sulfatase is contained in the microsomes.

It was found in this laboratory that 2-hydroxy-5-nitrophenyl sulfate is an unsuitable substrate for the study of the intracelluar localization of rat-liver sulfatase, since no proportionality between the quantity

Table 1. Intracellular distribution of rat-liver arysulfatase and acid phosphatase.

Fraction	Percentage of activity of homogenate	
	Acid phosphatase	Arylsulfatase
Homogenate	100	100
Nuclei, washed twice	3.6	6.2
Heavy mitochondrial fraction, washed once	20.6	5.2
Light mitochondrial fraction, wash once	37.0	15.8
Microsomes, unwashed	15.9	72.0
Final supernatant	17.4	3.0
Recovery	94.5	102.2

Table 2. Intracellular distribution of rat-liver glucose-6-phosphatase and arylsulfatase.

Fraction	Percentage of activity of homogenate	
	Glucose-6-phosphatase	Arylsulfatase
Nuclei, washed twice	9.7	9.3
Heavy and light mitochondrial fraction, washed once	12.1	10.9
Microsomes, unwashed	75.4	71.8
Final supernatant	3 2	4.7
Recovery	100.4	96.7
Reconstituted homogenate	87.1	90.6

of 4-nitrocatechol liberated and the amount of enzyme used could be obtained. Roy (*2*) reported similar observations and Maengwyn-Davies and Friedenwald have shown that this nonproportionality is attributable to an endogenous inhibitor, which they have shown to be inorganic phosphate (*10*).

References and Notes

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Olfactometric Method Utilizing Natural Breathing in an Odor-Free "Environment"

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Since 1935, when Elsberg and Levy first described the blast- and stream-injection techniques for measuring olfactory sensitivity in human beings (*1*), their suggestion that prepared stimuli be blown into the nostril(s) under pressure has dominated much experimentation in olfaction. The earlier method of using a sniff has frequently been supplanted by the unnatural one of having the subject suspend his breathing while a substitute "sniff" is blown in. After working with variations of the latter method for some time, I have finally abandoned it for many problems, despite my previous endorsement (*2*). The inability of most subjects to perform reliably, even with long training, was one reason for changing; lack of control over the position of internal mouth and throat parts that affect the volume of air admitted was another; and the extreme artificiality of the situation, which raised the question of generalizing to ordinary breathing, was a third.

No artificial mechanism is as efficient as sniffing in carrying air to the olfactory membrane, and there is no reason to believe that it is necessary to control sniff size if concentration of the gaseous mixture being sniffed is controlled so that the number of odorous molecules available, as well as the volume of inodorous air, can be specified.

In 1921, Zwaardemaker (*3*) described what he called a camera inodorata, an unventilated box of glass and aluminum for use with his olfactometer. The subject, with his head inside the box, sniffed through the olfactometer tube that was inserted into his nostril. Thus, the absolute threshold could be measured in an atmosphere relatively free of uncontrolled odors. A much more elaborate "box," actually two glass rooms called an olfactorium, was described in 1950 by Foster *et al.* (*4*) as providing an odor-free, climatically controlled environment for the whole subject. Although neither of these devices has been put to much use by others, the principle appears sound. Accordingly, I have built a modern camera inodorata, avoiding the tremendous cost and space demands of the olfactorium but still achieving the goal of surrounding the subject's head with continually flowing odor-free air during an experimental session. Instead of using a separate olfactometer to test sensitivity, I simply add controlled amounts of odor to the air in the box; the subject is allowed to sniff at will.

The box, in this case, is made of Plexiglas, and has a top and four walls with an inlet near the upper rear corner of one long wall. Inside dimensions are 45.5 cm long by 35.1 cm wide; the walls are 0.6 cm thick. All inside surfaces are perfectly smooth and entirely Plexiglas, yet the box comes apart completely and easily for cleaning. The bottom is loosely closed with a piece of Pliofilm having a slit down the center to serve as an entrance for the head and an exit for the air. The subject's hair and face (except the nostrils) can be covered with plastic materials to eliminate their odors.

The subject is continuously supplied with pure air at a rate of about 13 ft^3/min, enough to insure that positive pressure always exists inside the box so that other air cannot enter. In an adjoining room, a blower draws room air, previously filtered while coming in from outside, through another filter (*5*) of activated carbon, filterdown, and absolute filter paper (*6*), and propels it into the box through a Plexiglas tube 5.3 cm in diameter that passes through the wall and joins the inlet in the side of the box.

The system for odor production, modified from a previous one (*7*), connects with this system for fresh air supply. Odor control is achieved by the saturation