THE INTRACELLULAR DISTRIBUTION OF SULPHATASE

by

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In a recent publication DODGSON, SPENCER AND THOMAS[1] have reported that the bulk of the arylsulphatase activity of rat liver is present in the microsomes, some 70% of the total recovered sulphatase activity being present therein after their isolation in isotonic sucrose[2]. This is at variance with preliminary qualitative results from this laboratory[3] which indicated that in the case of mouse liver the bulk of the sulphatase was of mitochondrial origin. Because of this discrepancy it appeared necessary to reinvestigate the problem and the results reported below confirm the mitochondrial origin of the enzyme.

To avoid possible species differences rat liver was used in the present investigation. Cell fractionation was carried out according to SCHNEIDER AND HOGEBOOM[4], the nuclear, mitochondrial, and microsomal fractions being defined as those sedimenting at 700 *g*, 5500 *g*, and 25,000 *g* respectively. Sulphatase activity was estimated by the method already described[3] using dipotassium 2-hydroxy-5-nitrophenyl sulphate (nitrocatechol sulphate) as substrate. Preliminary investigation had shown that the optimal conditions for the assay of sulphatase in a whole homogenate of rat liver were a substrate concentration of 0.03 *M* nitrocatechol sulphate and a pH of 5.9 in 0.15 *M* acetate buffer. These conditions were therefore used in the present study with an incubation period of 1 h at 37°.

The results are given in Table I which clearly shows that the bulk of the sulphatase is present in the mitochondrial fraction of rat liver although considerable amounts also occur in the microsomal fraction. Table I also shows that the recovery of added nitrocatechol is virtually quantitative from all fractions under the above assay conditions. The total recovery of sulphatase activity was approximately 80%: this rather low value is comparable to that of DODGSON *et al.*[1], but the reason for the loss of enzyme is not clear. The loss may, however, be apparent rather than real as it has already been shown[5] that the activity of ox liver sulphatase A is not linearly related to the enzyme concentration and that an enzyme fraction with electrophoretic properties similar to those of sulphatase A exists in rat liver[6].

The above results therefore confirm the earlier observation[3] that liver sulphatase is predominantly of mitochondrial origin and do not support the view of DODGSON *et al.*[1] that the enzyme has its origin in the microsomes. The reason for this discrepancy is not yet obvious but it may be due, in part at least, to the use of a different substrate, potassium β-acetylphenyl sulphate, by the latter workers as DODGSON *et al.* (private communication) have recently confirmed the results from this laboratory when using nitrocatechol sulphate as the substrate in their assays.

TABLE I

INTRACELLULAR DISTRIBUTION OF SULPHATASE IN RAT LIVER

The results are expressed as percentages of the total sulphatase recovered and are the values for seven animals. The recovery of added nitrocatechol is quoted as a percentage.

	Sulphatase activity		*Recovery of 36 μg added nitrocatechol %*
	Range %	*Mean %*	
Nuclei	9–20	15	98
Mitochondria	43–62	50	98
Microsomes	20–23	22	99
Soluble fraction	11–21	14	99

REFERENCES

1 K. S. DODGSON, B. SPENCER AND J. THOMAS, *Biochem. J.*, 56 (1954) 177.
2 W. C. SCHNEIDER, *J. biol. Chem.*, 176 (1948) 259.
3 A. B. ROY, *Biochem. J.*, 53 (1953) 12.
4 W. C. SCHNEIDER AND G. H. HOGEBOOM, *J. biol. Chem.*, 183 (1950) 124.
5 A. B. ROY, *Biochem. J.*, 55 (1953) 653.
6 A. B. ROY, *Biochem. J.*, (1954) (in the press).

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