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The Intracellular Distribution of Arylsulphatase C and Oestrogen Sulphatase Activities in Rat Kidney and Spleen

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The arylsulphatase C of rat kidney and spleen is localized predominantly in the microsomal fraction. The distribution pattern is paralleled by that of oestrogen sulphatase, as in rat liver. The general usefulness of arylsulphatase C as a microsomal marker enzyme is indicated.

One of the arylsulphatase enzymes (arylsulphate sulphohydrolase, EC 3.1.6.1), known for many years as arylsulphatase C, is localized in the endoplasmic reticulum of the rat liver cell (Dodgson *et al.*, 1954), where it appears to be a firmly bound membrane component (Dodgson *et al.*, 1957). A simple method for the specific assay of the enzyme in the presence of the other arylsulphatases (A and B) has been developed, and attention has been drawn to its usefulness as a microsomal marker in cell fractionation studies on rat liver (Milsom *et al.*, 1972). The value of this enzyme as a marker in the fractionation of other rat tissues has not previously been determined. Moreover, the distribution of arylsulphatase C in rat liver was shown to be identical with that of oestrogen sulphatase (Dolly *et al.*, 1972), and indeed these two activities are almost certainly due to one enzyme. A comparison of the distribution of these two activities would therefore shed further light on their identity. The subcellular distribution of these two activities has now been determined in rat spleen and kidney.

Materials and Methods

p-Acetylphenyl sulphate (potassium salt) was prepared as described by Milsom *et al.* (1972). Glucose 6-phosphate (sodium salt) was purchased from Boehringer Corp. (London) Ltd., Bell Lane, Lewes, East Sussex BN7 1LG, U.K. α -Glycerophosphate (sodium salt) was obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey KT2 7BH, U.K. Oestrone [³⁵S]sulphate (sodium salt) was prepared (sp. radioactivity approx. 6 mCi/mmol) as described by Dolly *et al.* (1971). All other chemicals were AnalaR grade. Young male Medical Research Council hooded rats (200-250 g body wt.) were used throughout.

Enzyme activities towards *p*-acetylphenyl sulphate

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(arylsulphatase C) and towards oestrone [³⁵S]sulphate (oestrogen sulphatase) were measured by the methods of Milsom *et al.* (1972) and Dolly *et al.* (1972) respectively. The optimal conditions for rat spleen and kidney were found to be identical with those for rat liver, and enzyme activities corresponded to those observed by Dolly *et al.* (1972). Glucose 6-phosphatase was measured by the method used by de Duve *et al.* (1955), except that P_i was measured by the method of Chen *et al.* (1956). Acid phosphatase was assayed by the method used by Gianetto & de Duve (1955) and rhodanese as described by Rosenthal *et al.* (1950). DNA was isolated by the procedure of Munro & Fleck (1966) and assayed by the method of Giles & Myers (1965). Protein was measured by the method of Gornall *et al.* (1949), with bovine serum albumin as the standard.

The kidneys from two rats (14 g wet wt.) were passed through a fine wire mesh to remove connective tissue, and the resulting mince was homogenized in 3 vol. of cold 0.25M-sucrose with the aid of a Tri-R Potter-Elvehjem-type homogenizer (Tri-R Instrument Co. Ltd., Rockville Center, NY, U.S.A.; clearance between tube and Teflon pestle 0.39 nm). The suspension was forced past the rotating pestle (1200 rev./min) once and was then centrifuged at 700g_{av} for 10 min. The pellet was again resuspended in 3 vol. of cold sucrose and the process repeated. A third treatment yielded the washed nuclear fraction and the combined supernatants, which represented the 'cytoplasmic extract'. The volume of the latter was adjusted to represent a 10% (w/v) liver suspension and was then fractionated by the scheme of de Duve *et al.* (1955).

The experiment was first performed by using a decanting procedure which was biased in favour of isolating a 'purified' lysosomal (L) fraction. The post-mitochondrial supernatant was removed carefully with a bent-tipped pipette without taking any of the loosely packed material overlying the pellet. This procedure was also adopted when the pellet was resuspended and washed. In contrast, the post-

lysosomal supernatant and subsequent washings were removed as thoroughly as possible. In a second experiment, the decanting was biased in favour of obtaining a 'purified' microsomal (P) fraction, in which case care was taken to leave all loosely packed material behind in the L fraction when taking the supernatant for isolation of the P fraction and all the final supernatant was removed as thoroughly as possible.

The spleens from ten rats (10g) were treated by a procedure based on the method of Bowers *et al.* (1967). The tissue was diced with scissors and then homogenized in 7 vol. of cold 0.2M-KCl with the aid of a 40ml Dounce-type homogenizer (Kontes Glass Co. Ltd., Vineland, NJ, U.S.A.) by ten strokes of the A pestle followed by two strokes of the B pestle. After centrifuging at $600g_{av}$ for 10min the pellet was re-homogenized, but only with the B-pestle

treatment, and the whole procedure was then repeated a third time as for kidney. The combined supernatants, adjusted with 0.2M-KCl to represent a 20% (w/v) spleen homogenate, were then fractionated exactly as for kidney.

Results and Discussion

The distribution patterns of protein and also the marker enzymes in both tissues (Figs. 1a and 1b) correspond closely to those obtained by other workers and differ from that of rat liver; thus the protein content of the N and S fractions of spleen is higher and that of the M, L and P fractions is lower than for corresponding liver fractions (Bowers *et al.*, 1967). The distribution of protein in kidney shows a rather higher proportion associated with the N

Fig. 1. Intracellular distribution of arylsulphatase C, oestrogen sulphatase and marker enzymes etc. in rat spleen (a) and kidney (b)

The results represent the average of triplicate experiments in which the decanting procedure was biased either towards isolating a 'purified' P fraction (i) or 'purified' L fraction (ii) as described in the text. N, M, L, P, S represent the nuclear, mitochondrial, lysosomal, microsomal and cytoplasmic fractions respectively. A, DNA; B, rhodanese; C, acid phosphatase; D, arylsulphatase C; E, oestrogen sulphatase; F, glucose 6-phosphatase. Relative specific activity is percentage of enzyme activity in fraction/percentage of protein in fraction. Recoveries of the enzymes and DNA are given in parentheses.

fraction, and, from the relatively high relative-specific-activity value for microsomal enzymes in this fraction, this appears to be due largely to contamination with endoplasmic reticulum. Compared with other studies (Wattiaux-de Coninck *et al.*, 1965), a more loosely fitting pestle was used in the present work, and this could account for the observed results.

The relative specific activity of glucose 6-phosphatase in kidney microsomal fraction seems to be unaffected by the decanting procedure (Fig. 1*b*, F), indicating that, when the experiment was biased in favour of obtaining a 'purified' L fraction, the 'fluffy layer' carried over was probably mainly endoplasmic reticulum. In spleen, the opposite situation seemed to obtain (Fig. 1*a*, F), and the relative specific activity of the P fraction was decreased, indicating that the material carried over was lysosomal. These observations are consistent with the existence of a distinctly heterogeneous lysosomal population in spleen, as described by Bowers & de Duve (1967).

In all experiments, arylsulphatase C and oestrogen sulphatase are mainly distributed parallel with the microsomal marker enzyme glucose 6-phosphatase. Moreover, the relative specific activities for the two sulphatase activities follow each other closely, as observed for liver (Dolly *et al.*, 1972). The presence of the activities in other fractions in the liver cell has been shown to be due mainly to contamination with endoplasmic reticulum (Milsom & Rose, 1970), except for the nuclear fraction, where the nuclear membrane is appreciably active [S. B. Thomas & F. A. Rose, unpublished work; cf. glucose 6-phosphatase (Kashing & Kasper, 1969; Gunderson & Nordlie, 1973)].

The precise situation in kidney and spleen remains to be investigated further, but is likely to be difficult to resolve since, compared with liver, these tissues represent a much more heterogeneous collection of cells. Nevertheless the observations add further support to the claims that arylsulphatase C and oestrogen sulphatase are one and the same enzyme. Further,

the claims made by Milsom *et al.* (1972), that arylsulphatase C may represent a useful microsomal marker for rat tissues generally, has been extended to two more rat organs which are often the subject of cell-fractionation studies.

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