Studies on Sulphatases

9. THE ARYLSULPHATASES OF MAMMALIAN LIVERS

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Kinetic data for the hydrolysis of potassium *p*-acetylphenyl sulphate by fresh rat liver suspensions suggested that the arylsulphatase activity so observed could be attributed to a single enzyme (Dodgson, Spencer & Thomas, 1953) which was later found to be localized mainly in the microsomes of the liver cell (Dodgson, Spencer & Thomas, 1954*a*). Preliminary attempts to purify the microsome enzyme (unpublished data) were handicapped by its insolubility in various buffers and salt solutions of widely differing pH even after treatment of the liver by acetone-drying, alternate freezing and thawing, or use of the tissue disintegrator (Mickle, 1948). Sulphate and phosphate ions had little effect on the enzyme.

These results contrast sharply with the arylsulphatase pattern reported for ox liver (Roy, 1953*a, b*; 1954). Using dipotassium 2-hydroxy-5-nitrophenyl sulphate as the assay substrate, Roy was able to separate two arylsulphatases from the soluble material of acetone-dried ox liver and both enzymes were strongly inhibited by sulphate and phosphate ions. With the same substrate the arylsulphatase activity of mouse liver was found to be mainly concentrated in the mitochondria (Roy, 1953*a*).

The present work, a preliminary report of which has already appeared (Dodgson, Spencer & Thomas, 1954*b*), shows how the occurrence of three different arylsulphatases in mammalian livers enables these contrasting findings to be reconciled.

METHODS

Arylsulphates. Potassium *p*-acetylphenyl sulphate (APS) and potassium *p*-nitrophenyl sulphate (NPS) were prepared by the method of Burkhardt & Lapworth (1926) and dipotassium 2-hydroxy-5-nitrophenyl sulphate (nitrocatechol sulphate, NCS) according to Roy (1953*a*).

Measurement of arylsulphatase activity. The method previously used for APS (Dodgson, Lewis & Spencer, 1953) was also adopted for NPS but using the $\lambda_{\max}$ and $\epsilon_{\max}$ determined for *p*-nitrophenol (Dodgson & Spencer, 1953). The use of NPS was restricted to liver extracts, as recoveries of *p*-nitrophenol from fresh liver suspensions are low (Dodgson & Spencer, 1953). The faint turbidity of the final solutions was the same in both test and controls and duplicate determinations were satisfactory. Turbidity only inter-

fered, by raising control spectrophotometer readings to a high base level when very concentrated liver suspensions and extracts were used and in such cases the original ethanol precipitation method (Dodgson, Spencer & Thomas, 1953) was adhered to. The method of Dodgson, Melville, Spencer & Williams (1954) was used when NCS was the substrate. Although fresh liver suspensions can reduce liberated 4-nitrocatechol (Dodgson & Spencer, 1953), the high activity of rat liver against nitrocatecholsulphate allowed the use of liver suspensions and fractions separated in isotonic sucrose at concentrations at which 4-nitrocatechol could be quantitatively recovered over an incubation period of 1 hr.

In all enzyme assays sodium acetate-acetic acid was the buffer used at a final concentration in the incubation mixture of 0.5M. The buffer, substrate and enzyme solutions were adjusted independently to the required pH with acetic acid or NaOH before mixing. Incubation was for 1 hr, at 37.5° in all cases and the concentration of enzyme solution was such that the spectrophotometric readings corresponded to an extinction E ($\log_{10} I_0/I$) of less than 1.

RESULTS

The experimental methods used by Roy (1953*a*) differed from those of Dodgson, Spencer & Thomas (1953) in three main respects, namely, animal species enzyme substrate and the use of acetone-dried preparations of ox liver as opposed to fresh suspensions of rat liver. The significance of each of these differences was examined in relation to the contrasting findings of the two groups of workers.

The effect of acetone. Most of the known facts could be explained by assuming that the two enzymes separated from ox liver were artifacts due to the acetone treatment (Roy, 1953*a*), whereas in the fresh rat liver only one enzyme was present. However, the kinetic behaviour of rat liver arylsulphatase towards APS was not affected by acetone treatment, suspensions of whole acetone-dried rat liver showing substrate concentration (Fig. 1) and pH/activity curves which were the same as those previously obtained with fresh liver (Dodgson, Spencer & Thomas, 1953). Neither the curves for fresh rat liver nor those for acetone-dried rat liver indicated the presence of more than one arylsulphatase. Acetone treatment was not therefore responsible for the difference between rat and ox arylsulphatases. This conclusion was subsequently

strengthened by the demonstration of the presence of the same two sulphatases in both fresh ox liver and acetone-dried preparations (Roy, 1954).

Species difference. Substrate concentration/activity curves for suspensions of acetone-dried liver of the rat, ox, mouse and rabbit (Fig. 1) with APS as the substrate showed a species difference. Whereas the rat and rabbit enzymes gave smooth Michaelis curves, the rabbit showing marked inhibition with excess substrate, ox and mouse livers gave anomalous discontinuous curves similar to those found by Roy (1953*a*) for the soluble material of acetone-dried ox liver when NCS was the substrate. The curves for ox and mouse could be interpreted as showing the presence of two arylsulphatases.

Substrate difference. To confirm this apparent species difference the substrate concentration/activity curves were repeated using NCS at pH 6.0. The smooth curves obtained with the whole acetone-

Fig. 1. Substrate concentration/activity curves for the suspensions of acetone-dried liver of rat, ox, mouse and rabbit and for the residue and supernatant obtained by extraction of the acetone-dried rat liver with water for 1 hr. at 37.5°. The substrate was potassium *p*-acetylphenyl sulphate in 0.5M sodium acetate, pH 7.2, and incubation was for 1 hr. at 37.5°. The curves are not quantitatively related to each other. ○—○, rat; ×—×, ox; ■—■, mouse; △—△, rabbit; □—□, residue after extraction of acetone-dried rat liver with water; ●—●, supernatant after extraction of acetone-dried rat liver with water.

dried livers of the four animal species (Fig. 2) were of the normal pattern and gave straight line graphs when $1/v$ was plotted against $1/S$ (Lineweaver & Burk, 1934) except in the cases where inhibition by excess substrate occurred. The experiment was repeated at pH 5.3, the pH at which Roy (1953*a*) had obtained an anomalous curve with ox liver, but the curves obtained were quite smooth. Since these findings differed from those obtained with APS, the curves were repeated with a third substrate, NPS. With this substrate the acetone-dried livers of all four species gave anomalous Michaelis curves (Fig. 3) which suggested the presence of two arylsulphatases in each case, and that the species difference observed when APS was the substrate was only apparent. The findings with APS and NCS emphasize that Michaelis curves of normal type can readily be given by mixtures of two 'isodynamic' enzymes. Whereas discontinuous curves may be safely interpreted as showing the presence of more than one enzyme, the normal type of curves may sometimes be ambiguous. The choice of the rat as the animal species and APS as the substrate in earlier work (Dodgson, Spencer & Thomas 1953) was unfortunate in that no indication of the presence of more than one arylsulphatase was given; the use of other species or substrate would have revealed the complex nature of mammalian arylsulphatase.

Fractionation of acetone-dried liver. The sulphatases measured by Roy (1953*a*) with NCS could be extracted from acetone-dried ox liver with

Fig. 2. Substrate concentration/activity curves for the suspensions of acetone-dried liver of rat, ox, mouse and rabbit and for the residue and supernatant obtained by extraction of the acetone-dried livers with water for 1 hr. at 37.5°. The substrate was dipotassium 2-hydroxy-5-nitrophenyl sulphate in 0.5M sodium acetate, pH 6.0, and incubation was for 1 hr. at 37.5°. The curves are not quantitatively related to each other. □—□, whole acetone-dried liver; ○—○, residue after extraction of acetone-dried liver with water; △—△, supernatant after extraction of acetone-dried liver with water.

Table 1. *Fractionation of acetone-dried rat liver*

The activity of each fraction arising from 1 g. of acetone-dried liver was measured with APS 0.007M, pH 7.2, NPS 0.006M, pH 7.0 and NCS 0.04M, pH 6.0.

	Suspension of whole acetone powder			Insoluble fraction			Soluble fraction			Recovery (insoluble + soluble fraction)		
	APS	NPS	NCS	APS	NPS	NCS	APS	NPS	NCS	APS	NPS	NCS
Activity (μ g. phenol liberated)	9180	6870	149500	8170	5670	13200	222	180	137500	8392	5850	150700
% of activity of whole powder	100	100	100	89.0	82.4	8.91	2.4	2.6	92.1	91.4	84.6	101.0
Ratio of activities (units/mol.wt.)	1.38 : 1 : 19.5			1.47 : 1 : 2.09			1.26 : 1 : 685			—	—	—

water, whereas similar treatment of either ox or rat liver failed to bring more than 3% of the arylsulphatase activity into solution when APS (0.007M, pH 7.2) was the substrate (Table 1). This suggested that a preliminary separation of the enzymes of rat liver could be achieved by dividing the acetone-dried liver into soluble and insoluble fractions.

Acetone-dried rat liver (0.1 g.) was incubated for 1 hr. at 37.5° with 10 ml. water and then centrifuged at 20000 *g* for 30 min. at 0°. The residue was washed twice with water (5 ml.), re-centrifuged, the supernatant and washings were combined and the remaining residue was resuspended in water. The activities of the whole acetone-dried liver suspension, the debris and the supernatant were measured in the presence of 0.5M acetate against APS (0.007M, pH 7.2), NPS (0.006M, pH 7.0) and NCS (0.04M, pH 6.0). The conditions for APS and NCS were those found to be optimum for the whole powder (Figs. 1, 2), and those for NPS being the substrate concentration at which the Michaelis curve for the whole powder showed a discontinuity (Fig. 3).

The results show that 92% of the activity against NCS appeared in the soluble material, while less than 3% of the activity against APS and NPS was in this fraction. Furthermore, repetition of the fractionation procedure on the debris resulted in the extraction of more NCS activity without removing an equivalent amount of APS activity (Table 2). Similar findings were made when 0.5M acetate, pH 6.8, replaced water as the extracting medium.

Substrate concentration/activity curves for APS, NPS and NCS (Figs. 1-3), using the soluble and insoluble fractions of rat liver, prepared as described above, showed that separation of the arylsulphatases had been achieved. With APS and NPS the insoluble material had optimum substrate concentration similar to those found for the whole acetone-dried rat liver (Figs. 1, 3), and fresh liver suspensions (Dodgson, Spencer & Thomas, 1953). The soluble fraction had only about 3% of the activity of the insoluble fraction at the optimum substrate concentration for the latter, but showed

Fig. 3. Substrate concentration/activity curves for the acetone-dried liver of rat, ox, mouse and rabbit and for the residue and supernatant obtained by extraction of the acetone-dried livers with water for 1 hr. at 37.5°. The substrate was potassium *p*-nitrophenyl sulphate in 0.5M sodium acetate, pH 7.0, and incubation was for 1 hr. at 37.5°. The curves are not quantitatively related to each other. □—□, whole acetone-dried liver; ●—●, residue after extraction of acetone-dried liver with water; ▲—▲, supernatant after extraction of acetone-dried liver with water.

Table 2. *Fractionation of once-extracted acetone-dried rat liver*

Acetone-dried rat liver which had been extracted once with water for 1 hr. at 37.5° was dried with acetone and then re-extracted with water. The activity of the soluble and insoluble fractions towards APS (0.007M, pH 7.2) and NCS (0.04M, pH 6.0) are quoted as % of the activity of the once-extracted material.

Fraction	Activity	
	APS	NCS
Whole powder (once-extracted)	100	100
Insoluble fraction	95.7	84.6
Soluble fraction	1.8	16.2
Recovery	97.5	100.8

considerable activity at higher substrate concentrations. The optimum substrate concentration of the soluble material with APS and NPS was shown, in a separate experiment, to be greater than 0.175M, but the curves could not be carried to higher concentrations owing to the insolubility of the substrates. The NCS curves for soluble and insoluble fractions showed little difference when measured at pH 6.0.

These results indicate that at least two enzymes are present in acetone-dried rat liver. The 'insoluble' enzyme has high affinity for APS and NPS, whereas the 'soluble' enzyme has a low affinity for these substrates. On the other hand, the bulk of the activity towards NCS is associated with the soluble rather than the insoluble fraction (Table 1), although the affinities of these fractions for this substrate do not appear to differ greatly.

Work on the inhibition of the arylsulphatases with phosphate ions described in the latter part of this paper suggests that under the experimental conditions used most of the NCS activity of the insoluble portion is actually due to residual soluble enzyme.

Similar patterns existed for the ox, mouse and rabbit livers (Figs. 2, 3), although from the shape of the curves for the whole acetone powders it may be inferred that the proportion of 'insoluble' and 'soluble' enzymes differs with each species.

Intracellular distribution of rat liver arylsulphatase. With APS as the substrate, Dodgson, Spencer & Thomas (1954a) showed that the arylsulphatase activity of rat liver was predominantly localized in the microsomes of the liver cell, while in the mouse 70% of the activity against NCS was present in the mitochondria (Roy, 1953a). It was probable that the use of different substrates rather than a species difference was responsible for these contrasting findings, since the localization of the arylsulphatase activity of rat liver was similar to that of the mouse when NCS was the assay substrate.

The components of rat liver cells were fractionated by the isotonic sucrose technique (Schneider, 1948) and the fractions simultaneously assayed with NCS (0.04M in 0.5M acetate, pH 6.0) and APS (Dodgson, Spencer & Thomas, 1953). Sucrose (0.025M) had no effect on the arylsulphatase activity when either NCS or APS were the substrates. The figures for APS activity (Table 3) were substantially the same as those previously recorded (Dodgson, Spencer & Thomas, 1954a) and showed that the greatest activity was present in the microsomes whilst the mitochondria and final supernatant were only weakly active. With NCS the mitochondria were predominantly active although both the microsomes and the final supernatant fractions retained considerable activity (Table 3).

From Fig. 1 and Table 1 it would appear that assay with APS (0.007M at pH 7.2) measures only

the 'insoluble' enzyme, and that the activity of and competition by the 'soluble' enzyme is very small. On the other hand, the 'soluble' enzyme is responsible for the bulk of the activity measured when NCS (0.04M, pH 6.0) is the substrate (cf. Fig. 2 and Table 1).

The 'insoluble' enzyme is therefore localized in the microsomes and the 'soluble' enzyme in the mitochondria and to a lesser extent in the microsomes and final supernatant fraction. Confirmation of this was obtained by preparing substrate concentration/activity curves with APS (Fig. 4) for the

Table 3. *The localization of the arylsulphatase activity of the rat liver cell*

The fractions separated by the method of Schneider (1948) were assayed against APS (0.007M, pH 7.2) and NCS (0.04M, pH 6.0). The results are expressed as a percentage of the activity of the initial whole tissue suspension and are the average values obtained from four different experiments.

Fraction	Activity (% of initial whole tissue suspension)	
	APS	NCS
Nuclear	12.5	9.1
Mitochondrial	6.0	62.0
Microsomal	61.7	21.5
Supernatant	2.6	16.6
Recovery	82.8	109.2

Fig. 4. Substrate concentration/activity curves for the mitochondrial, microsomal and supernatant fractions obtained by differential centrifuging of the cellular components of rat liver in isotonic sucrose solution according to the method of Schneider (1948). The substrate was potassium *p*-acetylphenyl sulphate in 0.5M sodium acetate, pH 7.2, and incubation was for 1 hr. at 37.5°. The curves are not quantitatively related to each other. ●—●, mitochondrial fraction; △—△, microsomal fraction; ○—○, supernatant fraction.

fractions isolated using isotonic sucrose (Schneider, 1948) when it was seen that the microsomal fraction gave a curve similar to that of the insoluble material of acetone-dried liver (Fig. 1) and that both the mitochondria and the final supernatant gave curves similar to that of the 'soluble' enzyme. These curves show that the 'soluble' and 'insoluble' enzymes do exist in fresh liver and are not just artifacts arising from acetone treatment. The NCS curves for the mitochondria microsomes and final supernatant were almost identical and similar to those shown in Fig. 2.

The complex nature of the 'soluble' enzyme

The substrate concentration/activity curves with APS, NPS and NCS for the soluble fraction of acetone-dried rat liver (Figs. 1-3) gave no indication of the presence of more than one enzyme. However, as previously explained, such curves may be given by mixtures of enzymes. In view of Roy's claim (1953*a, b*; 1954) to have separated two sulphatases from the soluble material of acetone-dried ox liver, attempts were made to detect the presence of more than one 'soluble' arylsulphatase in rat liver.

Fractionation of rat liver by the method of Roy (1953*b*, 1954) to the stages A-2 and B-2 gave fractions which showed substrate concentration/activity curves at pH 4.75 and 5.75 which were not significantly different. However, a satisfactory demonstration of the presence of two enzymes was obtained by a simple fractionation of acetone-dried rat liver.

A 10% (w/v) suspension of acetone-dried rat liver in water was incubated at 37.5° for 1 hr. and then centrifuged at 20000 g. The clear supernatant was fractionated with acetone between the limits 0-30 and 50-60% acetone (v/v) observing the precautions detailed by Askonas (1951). The precipitates were dried with acetone under ice-cold conditions. The fractions were suspended in water, clarified by centrifuging at 0° and re-fractionated between the limits 0-30 (fraction I), and 50-60% (fraction II) acetone, respectively. The precipitates were dissolved in water, dialysed against running water for 36 hr., clarified by centrifuging and kept in the frozen state.

The NPS and NCS substrate concentration/activity and pH/activity curves were constructed for fractions I and II (Figs. 5, 6). The fractions showed obvious differences, particularly for the NCS substrate and pH curves, although with NPS the difference was less marked in the case of the pH curve and not apparent in the substrate curves. In no case was the difference as great as that found for the two soluble enzymes of ox liver (Roy, 1953*a*). The difference in kinetic behaviour between the fractions does, however, suggest that at least two enzymes are present in the soluble material of acetone-dried rat liver. The great difference between the curves for fractions I and II and

similar curves for the insoluble material of acetone-dried rat liver (Figs. 5, 6) makes it improbable that either of the two enzymes of the soluble material is identical with the main constituent of the insoluble material. This was confirmed by the effect of phosphate ions on the activities of the various

Fig. 5. Substrate concentration/activity curves for fractions of rat liver showing the presence of three different arylsulphatases. The 'insoluble' enzyme fraction was the residue from acetone-dried rat liver after repeated extraction with water, while enzyme fractions I and II were prepared as described in the text (p. 33). The substrates used were dipotassium 2-hydroxy-5-nitrophenyl sulphate (in 0.5M sodium acetate, pH 5.75) and potassium *p*-nitrophenyl sulphate (in 0.5M sodium acetate, pH 6.5). ●—●, fraction I enzyme; ○—○, fraction II enzyme; ■—■, 'insoluble' enzyme.

Fig. 6. pH/Activity curves for three fractions of rat liver showing the presence of three different arylsulphatases. The 'insoluble' enzyme fraction was the residue from acetone-dried rat liver after repeated extraction with water, while enzyme fractions I and II were prepared as described in the text (p. 33). The substrates used for fractions I and II were dipotassium 2-hydroxy-5-nitrophenyl sulphate (0.02M) and potassium *p*-nitrophenyl sulphate (0.05M). The insoluble enzyme was measured using 0.04M dipotassium 2-hydroxy-5-nitrophenyl sulphate and 0.0025M potassium *p*-nitrophenyl sulphate. In each case enzyme activity was measured in the presence of 0.5M sodium acetate-acetic acid buffer adjusted to the required pH. ●—●, fraction I enzyme; ○—○, fraction II enzyme; ■—■, 'insoluble' enzyme.

fractions. The degree of inhibition was determined using the optimum conditions of substrate concentration and pH (Figs. 5, 6) except when NPS was the substrate for fractions I and II, in which case a substrate concentration of 0.05M was used. The arylsulphatase activity of fractions I and II was completely inhibited by 0.025M phosphate when either NPS or NCS was the substrate, but the insoluble material obtained from acetone-dried liver was only slightly inhibited (approx. 10%) by the same concentration of phosphate when NPS and APS (0.007M, pH 7.2) were the substrates.

When NCS was the substrate the activity of the insoluble material was inhibited 96% by 0.025M phosphate. This result may be interpreted by assuming that the 'insoluble' enzyme is only slightly inhibited by phosphate, as shown with APS and NCS, and that the activity measured with NCS under the conditions stated is actually due to residual soluble enzymes which are strongly inhibited by phosphate. Table 2 shows that a con-

siderable amount of soluble enzyme remains in once-extracted acetone-dried liver.

Paper electrophoresis. Confirmation of the presence of two separate enzymes in the soluble material of rat liver was obtained by paper electrophoresis. The soluble material was extracted by incubating 20% (w/v) water suspensions of fresh liver and 10% (w/v) suspensions of acetone-dried liver for 1 hr. at 37.5° and centrifuging at 20000 g for 1 hr. Paper electrophoresis of these extracts and fractions I and II was carried out by the technique described by Roy (1954), except that each paper was divided longitudinally after electrophoresis and 1 × 1.5 cm. strips of each half were assayed over 30 min. with either 0.008M NCS, pH 4.8 or 0.02M NCS, pH 6.0. These assay conditions were near optimum for fractions I and II respectively (Figs. 5, 6). Fractions I and II appeared to be substantially free from contamination by each other even when the assay conditions were those optimum for the suspected contaminant (Fig. 7). Fraction I is comparable to sulphatase B of ox liver (Roy, 1954), since it moved towards the anode and its kinetic behaviour followed more closely that of ox sulphatase B than did that of fraction II. Fraction II showed similar points of comparison with ox sulphatase A.

Using either set of assay conditions, the extracts of fresh and acetone-dried liver showed two separate bands of arylsulphatase activity which moved at the same speeds as the enzymes of fractions I and II (Fig. 7). The activity of the bands with the same mobility as fraction I was greatest under the conditions which were optimum for this fraction and a similar relationship existed for the other bands and fraction II. The fraction II enzyme appeared to be present in the liver in small amounts only and the ratio of activity of the two bands was of the order of 1:100 under the respective optimum conditions (cf. Roy, 1954). When the paper strips were assayed over 2 hr. some activity appeared between the two main spots, suggesting that some tailing off of the enzyme bands had occurred. The demonstration of two 'soluble' enzymes in fresh liver confirms Roy's findings (Roy, 1954) that they are not artifacts due to acetone treatment.

Fig. 7. Electrophoretic movement of the arylsulphatases of fractions I and II and the aqueous extracts of acetone-dried and fresh rat liver. Enzyme activity was assayed with dipotassium 2-hydroxy-5-nitrophenyl sulphate using two different sets of experimental conditions (0.02M, pH 6.0, open figures; 0.008M, pH 4.8, closed figures). The activities for the two sets of conditions are approximately related to each other within each fraction only. Electrophoresis in veronal-acetate-HCl buffer, pH 7.1, was for 16 hr. at 200 v using Whatman no. 100 paper.

DISCUSSION

The arylsulphatase activity of rat and ox livers can be attributed to a complex system of at least three arylsulphatases which possess widely different substrate affinities. Fractionation of rat liver in isotonic sucrose solution showed that two of the enzymes ('soluble') were mainly associated with the mitochondria and could be obtained in solution by techniques which rupture these granules, such as acetone-drying and alternate freezing and thawing.

Table 4. *Conditions used by previous workers for the study of mammalian arylsulphatases*

From an examination of these conditions the particular enzyme studied in each case has been inferred and included in the table under the heading 'enzyme studied'. Insufficient details are given by Abbott & East (1949) to enable a decision to be given. The pH and substrate concentrations shown are either those found to be optimum for the enzyme under consideration or those used for assay purposes.

Animal	Organ	Method of preparation of enzyme	Substrate	Substrate concentration (M)	pH	Enzyme studied	Reference
Pig	Kidney	Washed acetone-dried tissue	Potassium phenyl sulphate	0.047	CaCO ₃	'Insoluble'	Hommerberg, 1931
Rabbit	Liver	Sucrose and glycerine extracts	NPS	0.0005	5.9	'Insoluble'	Morimoto, 1937
Rabbit	Liver	Glycerine extract	NPS	0.0005	6.1	'Insoluble'	Tanaka, 1938
Rat	Liver and other tissues	Centrifuged water extracts	NPS	0.001	5.8	'Insoluble'	Huggins & Smith, 1947
Rat	Liver and other tissues	?	NPS	?	6.6	?	Abbot & East, 1949
Rat	Liver and other tissues	Extracts (?)	NCS	0.0025	6.0	'Soluble'	Robinson, Smith & Williams, 1951
Rat, ox, rabbit and pig	Adrenals	Whole tissue	NPS	0.001	5.8	'Insoluble'	Malström & Glick, 1952
Rat	Liver and other tissues	Whole tissue	APS	0.007	7.2	'Insoluble'	Dodgson, Spencer & Thomas, 1953 <i>b</i> , 1954 <i>a</i>
Ox	Liver	Centrifuged extract of acetone-dried liver	NCS	0.003 > 0.06	4.9 5.7	'Soluble', 'Soluble'	Roy, 1953 <i>a, b</i> , 1954
Rabbit	Liver and kidney	Centrifuged NaCl extract	NPS	> 0.15	6.02	'Soluble'	Maengwyn-Davies & Friedenwald, 1954

It was not possible to attribute the appreciable activity of the final supernatant after fractionation to the cytoplasmic fluid of the unbroken cell since it might possibly have been extracted from the mitochondria during the fractionation procedure. The remaining enzyme ('insoluble') occurs predominantly in the microsomes and is only present in negligible amounts in the mitochondria and final supernatant. Techniques used for disrupting mitochondria fail to bring this enzyme into solution, and the only known method of achieving this is by the use of surface-active agents (unpublished data). The complex nature of liver arylsulphatase is not confined to the ox and rat, since mouse, rabbit and human (unpublished) livers have been shown to contain both 'soluble' and 'insoluble' enzymes. No investigation has yet been made into the homogeneity of the 'soluble' enzymes of these species.

It is now obvious that in previous work Dodgson, Spencer & Thomas (1953, 1954a) have measured only the 'insoluble' enzyme, since at the concentration at which APS was used the 'soluble' enzymes have only about 2% of the activity of the 'insoluble' enzyme (Table 1). On the other hand, the work of Roy (1953a, b; 1954) has been confined to the 'soluble' enzyme since, by discarding the insoluble material of acetone-dried ox liver after extraction with water, all the 'insoluble' enzyme was effectively removed from his preparations. The contrasting findings of the two groups of workers can be adequately explained on this basis. It is pertinent to consider how the work of previous authors is affected by these findings. By comparing experimental conditions used in the past (Table 4) with the curves for the 'soluble' and 'insoluble' enzymes (Figs. 1-3) it appears that workers have mainly measured one or other of these enzymes only. In other cases the method of preparation of the enzyme itself has ensured that one of the enzymes has been discarded before estimation. Thus, Hommerberg (1931) found that the enzyme of pig's kidney could not be brought into solution, and from the conditions of substrate concentration and pH used it is obvious that he was concerned with the 'insoluble' enzyme. Morimoto (1937) and Tanaka (1938), using conditions that measured mainly the 'insoluble' arylsulphatase of rabbit liver, found that the enzyme could be obtained in solution, since centrifuged sucrose and glycerol extracts of the livers were active. However, it is probable that the enzyme was not actually in solution, since the high specific gravity of the extracts would ensure that the microsomes, containing the 'insoluble' enzyme, were not removed under the normal conditions of centrifuging. A similar comment is applicable to the centrifuged extracts of rat tissues made by Huggins & Smith (1947) particularly since at the concentration of NPS used for assay only the

'insoluble' enzyme would be measured. On the other hand, centrifuging of sodium chloride extracts of rabbit liver at 16 000 rev. min.⁻¹ for 1 hr. (Maengwyn-Davies & Friedenwald, 1954) effectively removed all the microsomes, and the supernatant solution showed a Michaelis curve similar to that of the 'soluble' enzyme in Fig. 3. The particular enzyme studied by previous authors has been inferred from the conditions used, and a list is presented in Table 4.

The precise assay of the individual arylsulphatases of mammalian tissues presents some difficulties. Although it may be possible to choose specific substrates and conditions which will assay one of the enzymes preferentially, competition by the other two enzymes might cause some interference. The inhibition of the 'soluble' enzymes by the varying amounts of endogenous phosphate ions is a further complicating factor. However, these factors are at a minimum in the case of the assay of rat tissues with APS (Dodgson, Spencer & Thomas, 1953). Under the conditions used (0.007 M, pH 7.2) the activity of and competition by the 'soluble' enzymes is slight (Table 1) and the 'insoluble' enzyme which is being measured is hardly affected by phosphate ions at the concentration found in tissues. The results previously recorded for the distribution of arylsulphatase activity in rat tissues (Dodgson, Spencer & Thomas, 1953) and the localization in the rat liver cell (Dodgson, Spencer & Thomas, 1954a) may therefore be considered as descriptive of the 'insoluble' (microsomal) enzyme only.

There is as yet no evidence to suggest that the arylsulphatase activity of sources other than mammalian tissues is of a complex nature. The arylsulphatase of *Alcaligenes metalcaligenes* (Dodgson, Melville, Spencer & Williams, 1954) has been shown to be electrophoretically homogeneous (unpublished data), but in the other cases no such criterion is available and their homogeneity must remain in doubt pending further investigation. Indeed, the flat pH optima exhibited by taka-diastase are suggestive of the presence of more than one enzyme (Robinson, Smith, Spencer & Williams, 1952).

SUMMARY

1. The presence of two arylsulphatases in the livers of rat, ox, rabbit and mouse has been shown. The enzymes can be distinguished by the ready solubility of one of them ('soluble') after disruption of the mitochondria by acetone-drying, incubation in hyper- and hypo-tonic solutions or alternate freezing and thawing.

2. In the rat the 'insoluble' enzyme is localized in the microsomes of the liver cell and the 'soluble' enzyme is found mainly in the mitochondria. The

'insoluble' enzyme has a high affinity for *p*-nitrophenyl and *p*-acetylphenyl sulphates whilst the 'soluble' enzyme has a low affinity for these substrates but a high activity towards nitrocatechol sulphate.

3. The 'soluble' enzyme of rat liver has been further separated into two enzymes which are comparable to sulphatases *A* and *B* of ox liver (Roy, 1954).

4. The work of previous authors was discussed in the light of these findings.

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Fate of Porphobilinogen, Administered Enterally or Parenterally, in the Rat

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Porphobilinogen is excreted in the urine in large quantities in acute porphyria and is always found in the livers of fatal cases of this disease. In experimental porphyria in animals induced by sedormid (allylisopropylacetylurea) (Schmid & Schwartz, 1952) or allylisopropylacetamide (Goldberg, 1953), porphobilinogen is likewise found in the urine and liver. Previous reports on the behaviour of this substance when administered to experimental animals are conflicting, probably because the porphobilinogen used was only partially purified or was available in amounts too small for adequate investigation. Thus Waldenström & Wendt (1939) injected partially purified porphobilinogen into rabbits and found it to be excreted in the urine, while Prunty (1945) failed to find any in the urine under similar conditions. Goldberg, Paton & Thompson (1954) injected porphobilinogen, which had been isolated by Westall (1952), intravenously into one rabbit with an external bile fistula and found traces of uro-

porphyrin in the urine and a slight increase in the excretion of bile porphyrin.

The following experiments with porphobilinogen were done in order to clarify its mode of excretion in an experimental animal. It is suggested that the results of these studies in the rat may be relevant to human acute porphyria. It was found that porphobilinogen, when administered parenterally to the rat, is excreted into the urine mainly by glomerular filtration. If it is given enterally or parenterally, it is not found in the livers of rats subsequently killed, at a time when the plasma still contains porphobilinogen. These findings suggest that the porphobilinogen, found in the livers of rats with experimental porphyria, has been formed there and has not been transported to the liver from an extrahepatic site.

Falk, Dresel & Rimington (1953) showed that porphobilinogen behaved as a precursor of certain porphyrins in a haemolysed chicken-erythrocyte