Aryl Sulphatases: Properties and Subcellular Distribution in Rat Liver[1]

Francis E. Wilkinson, Sally E. Nyquist,[2]
William D. Merritt and D. James Morre'[3]
Department of Biological Sciences
Purdue University, Lafayette, Indiana 47907

Abstract

Enzymes of the arylsulphatase complex (Arylsulphatases A, B and C) were shown to be widely distributed throughout the endomembrane system of rat liver. Arylsulphatase C, a microsomal enzyme, was localized primarily in rough endoplasmic reticulum. Arylsulphatases A and B were more widely distributed. Electron microscope localization combined with cell fractionation studies showed the activity to be most concentrated in focal specializations of the bulk smooth endoplasmic reticulum or in lysosomes. Less concentrated enzyme activity was associated with rough endoplasmic reticulum, bulk smooth endoplasmic reticulum, the nuclear envelope and the Golgi apparatus (especially in secretory vesicles of the Golgi apparatus). Arylsulphatase A-B activity was not detected in mitochondria, microbodies, the plasma membrane and the ground cytoplasm and was low or absent from smooth endoplasmic reticulum of the Golgi apparatus zone. The results indicate a difference between Golgi apparatus-associated and the bulk of the smooth endoplasmic reticulum and raise the possibility of direct transfer of arylsulphatase A-B from rough endoplasmic reticulum to forming secretory vesicles of the Golgi apparatus.

The enzymes of the arylsulphatase group (aryl-sulphate sulphohydrolases, E.C. 3.1.6.1) are widely distributed among animals (1, 3, 4, 7, 8, 10-13, 15, 17, 28, 29, 31, 32, 34), microbial (16) and plant (29) tissues. The importance of sulphuric acid esters in many metabolic processes and disorders in animals (2-5, 8, 9, 17, 27, 28) adds considerable significance to the widespread distribution of the arylsulphatases. At least in animal tissues, the activity is largely confined to substrates in which sulphuric acid is conjugated with hydroxyl groups of phenols (Fig. 1). The inorganic sulfate released during the reaction can be precipitated with lead to form an electron dense reaction product. The latter provides a means for localizing the enzyme activity at the electron microscope level (1, 15, 18, 29, 34).

At least three arylsulphatases have been distinguished on the basis of their kinetic properties. In rat liver, two of the enzymes, Arylsulphatase A and Arylsulphatase B are considered to be localized primarily in the lysosome fraction (7, 8, 34). The third, Arylsulphatase C, is found in the microsome fraction (10, 13, 18). Yet, the precise subcellular distribution of these enzymes in liver has never been established. The purpose of this study was to investigate the subcellular distribution of Arylsulphatases A, B and C in rat liver with particular emphasis on components of the endomembrane system (25).

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[2]Present address: Dept of Biology, Bucknell Univ., Leesburg, Pa. 17837.

[3]Appointed jointly between the Department of Botany and Plant Pathology and Department of Biological Sciences.

Materials and Methods

Male rats, 200 to 250 g, 50 days old of the Holtzman strain, provided with laboratory chow and drinking water *ad libitum*, were sacrificed and the livers drained of blood. The livers were removed, minced at room temperature and used either for determination of arylsulphatase activity, cell fractionation studies or enzyme cytochemistry.

Golgi apparatus (20) and endoplasmic reticulum (6) were prepared as described previously. The heavy particulate fraction of Table 1 was obtained by collection of the band at the 1.5/1.6 M interface on a discontinuous sucrose gradient used for preparation of Golgi apparatus fractions (22). The band was removed, resuspended in homogenization medium and pelleted at 3,000 *g* for 30 min. This fraction is largely a mixture of mitochondria and rough-surfaced endoplasmic reticulum.

TABLE 1. *Arylsulphatase activities of isolated cell components of rat liver.*

Fraction	Enzyme Activity	
	Arylsulphatase C (mumoles *p*-hydroxyacetophenone/ mg protein/hr)	Arylsulphatases A and B (mumoles *p*-nitrocatechol/ mg protein/hr)
Total Homogenate	59 ± 6	90 ± 13
Rough Endoplasmic Reticulum	385 ± 88	668 ± 58
Golgi Apparatus	87 ± 6	604 ± 13
Heavy Particulate	— —	574 ± 13

The substrate for Arylsulphatase A-B was *p*-nitrocatechol sulphate (Fig. 1) purchased from Sigma Chemical Company, St. Louis, Missouri. The reaction was carried out as described by Roy (31, 32) using a substrate concentration of 30 mM and a pH of 6.0 with a reaction time of 30 min unless indicated otherwise.

For determination of Arylsulphatase C activity, the substrate was potassium *p*-acetylphenylsulphate synthesized from *p*-hydroxyacetophenone and chlorosulfonic acid by an adaptation of the simplified method of Figenbaum and Neubert (14) for preparation of aromatic sulphuric acid esters. This substrate was chosen in preference to some readily available substances due to its relative stability toward metabolism by rat liver homogenates (11). Arylsulphatase C was assayed as described by Dogson *et al.* (12) with the exception that reactions were stopped after 30 min rather than after 1 hour. Proteins were determined by the biuret method.

Tissues and cell fractions subjected to cytochemical assay for arylsulphatases A-B were processed according to the method of Goldfischer (15), except that they were fixed overnight in 2% glutaraldehyde instead of being perfused as with the whole tissue experiments of Goldfischer (15). The tissues and fractions were then embedded in Epon

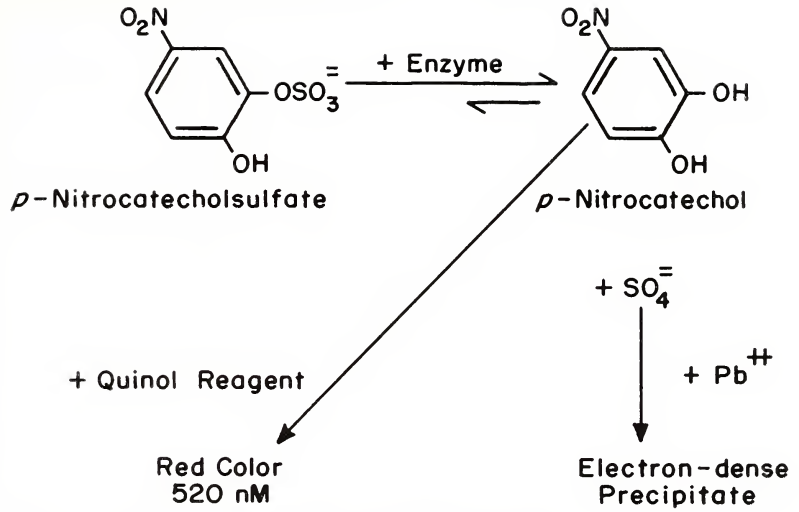


FIGURE 1. *Summary of the detection methods employed for Arylsulphatase A-B. In the presence of the enzymes, the substrate* p-*nitrocatecholsulphate is cleaved to* p-*nitrocatechol plus sulphate. In the presence of quinol reagent, the* p-*nitrocatechol produces a red color absorbing at 520 nM. For cytochemical detection, the sulphate was complexed with lead to yield an electron-dense precipitate observable in the electron microscope.*

(33) and thin sections were examined and photographed with a Philips EM 200.

Results

Whole homogenates of rat liver were used in establishing assay conditions for arylsulphatase A-B in rat liver. The activity was extremely stable and extracts could be stored for several months without loss of enzyme activity. Controls containing the enzyme solution with the substrate added immediately after incubation followed immediately by ethanol, and controls in which the substrate was incubated with buffer alone, were run simultaneously with experimental determinations.

Effect of time and enzyme concentration

Figure 2 shows the effect of incubating rat liver homogenates with the substrate in the presence of varying quantities of protein (Fig. 2A) and for varying times (Fig. 2B) under conditions of optimum pH (Fig. 2C) and a substrate concentration of 30mM. This substrate concentration was near optimal for Arylsulphatase C (12), supraoptimal (inhibitory) for Arylsulphatase A (31) and yielded first order kinetics for Arylsulphatase B (31, Fig. 2D). There was an approximately linear relationship between protein concentration and the reaction velocity over the range 0 to 4 mg of protein in the assay (Fig. 2A) and in subsequent studies a protein concentration of 1 mg or less was employed. In agreement with a previous observation (31), there was a decrease in reaction velocity during the first 10 min of incubation

after which the degree of hydrolysis was proportional to the time of incubation (Fig. 2B).

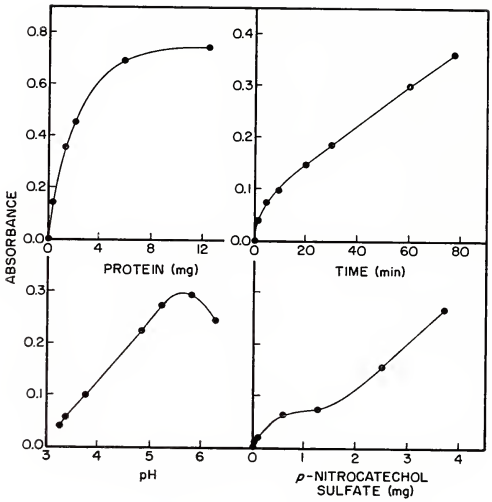


FIGURE 2. *Properties of rat liver Arylsulhatase A-B assayed under standard conditions (31, 32) except for conditions varied showing increased absorbance due to release of* p-*nitrocatechol from* p-*nitrocatecholsulphate as a function of (A) protein concentration, (B) time, (C) pH, and (D) substrate concentration. The enzyme source was total rat liver homogenate (Table 1) frozen and thawed to release structure-linked latent enzyme activity.*

Effect of pH

The effect of pH was determined over the range of 3 to 7. The pH optimum is that of Arylsulphtase B and was determined to be 5.7 in 0.15 M acetate buffer with nitrocatechol sulphate as substrate (31). The pH optimum for arylsulphatase A is 4.7 (31). The optimum for Arylsulphatase C is about pH 7.2 (10, 12)

Effect of substrate concenration

The effect of varying substrate on reaction velocity (Fig. 2D) shows a hump in the curve between 0 and 0.5 mg *p*-nitrocatechol sulphate which is due to the presence of Arylsulphatase A (12) which is saturated at a substrate concentration of approximately 3 mM. As shown previously (12), there is no obvious substrate optimum for arylsulphatase B within the limits studied (Fig. 2D).

Intracellular location

In tissue fractionation studies, the activities of Arylsulphatases A and B as compared to Arylsulphatase C showed distinctly different distribution patterns. Arylsulphatase C appeared to be specifically localized in the rough endoplasmic reticulum (Table 1). Arylsulphatases A-B, however, showed approximately equal distribution in the rough endoplasmic reticulum and the Golgi apparatus (Table 1).

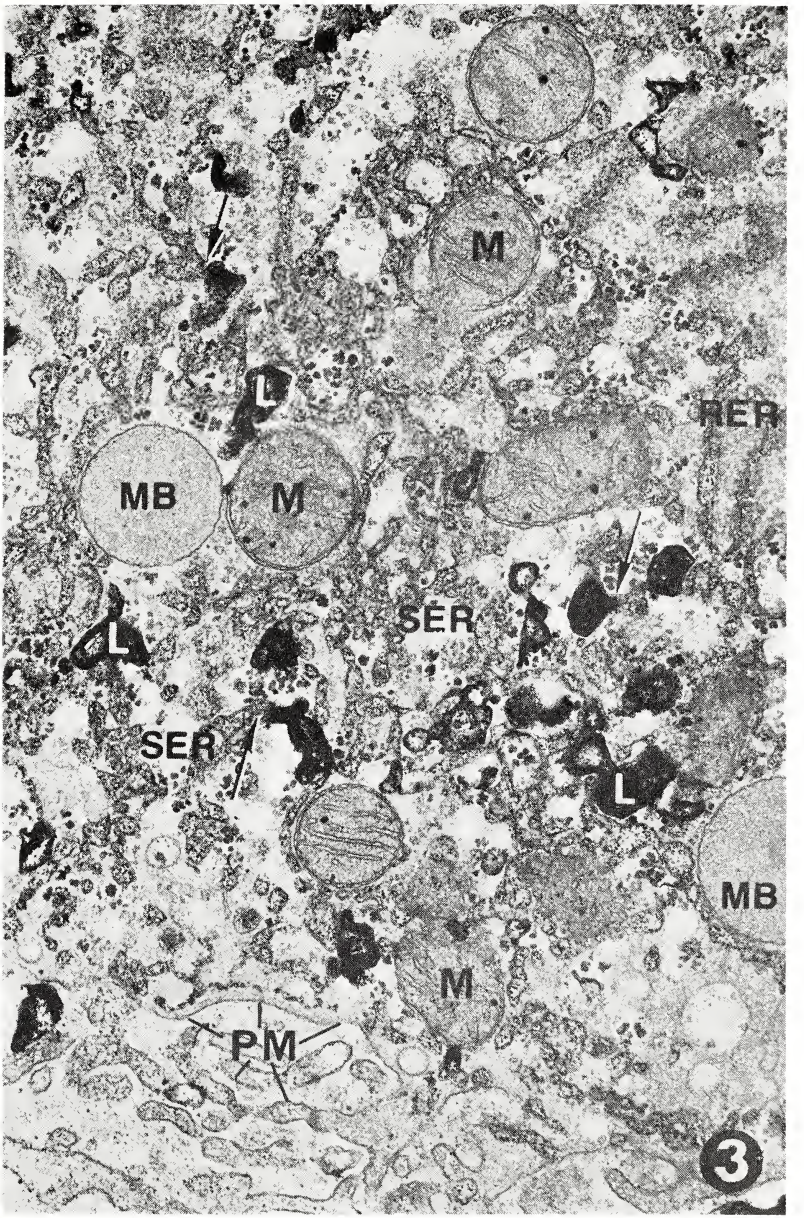


FIGURE 3. *Electron micrograph of rat liver incubated for arylsulphatase activity at pH 5.5 for 30 min at 37°C (15). Thin sections were stained with lead citrate (30). Reaction product is found in specific bodies (L) adjacent to or continuous with (arrows) smooth endoplasmic reticulum (SER). Lighter, granular deposits of reaction product are seen over the lumens and membranes of both rough (RER) and smooth endoplasmic reticulum. The plasma membrane (PM) at the cell border, mitochondria (M), microbodies (MB) and the cytoplasmic matrix are free of reaction product. Magnifications are approximate. X 30,000.*

This distribution was confirmed and extended by the results from cytochemical localization of Arylsulphatases A-B. The heaviest deposits of lead were found over the membranes and contents of bodies attached to and interspersed with fields of smooth endoplasmic reticulum (Fig. 3). These bodies may represent lysosomes but their consistent association with smooth endoplasmic reticulum was unexpected. More important in the context of the present study, lead deposits of a more diffuse nature were found equally distributed over the lumens of both rough and smooth endoplasmic reticulum (Figs. 3 and 4), the nuclear envelope (Fig. 4) and cisternae and vesicles of the Golgi apparatus (Fig. 4). In isolated fractions of rough endoplasmic reticulum, dense reaction products were still present and uniformly distributed but more so over the membranes than within cisternal lumens (Fig. 5). With isolated Golgi apparatus fractions, reaction products were found over secretory vesicles containing lipoprotein secretory products as well as secretory vesicles lacking lipoprotein secretory products and presumed to represent primary lysosomes (Figs. 6 and 7). Only small amounts of reaction product were found over Golgi apparatus cisternae and the smooth system of peripheral tubules (23, 24, 26). Both the isolated endoplasmic reticulum and Golgi apparatus fractions were free of the lysosome-like bodies containing dense lead deposits observed *in situ* (Fig. 3).

Discussion

In the animal tissues investigated by Goldfisher (15) and others (1, 34), Arylsulphatase A-B activity was largely localized in cytoplasmic bodies identified as lysosomes. In contrast, Arylsulphatase C has been generally considered to be a microsomal enzyme (10, 18), but microsomes are a heterogeneous cell fraction containing membrane fragments of many different types of cell components. The analysis of arylsulphatase activity is complicated to some extent by anomalous kinetic properties. These anomalies have been noted previously (31, 32) and no clarification is provided by the present study.

Our studies, however, do show that much of the Arylsulphatase A-B activity of rat liver is localized in cytoplasmic structures not readily identified as conventional lysosomes. Arylsulphatase A-B activity was about equal in endoplasmic reticulum and Golgi apparatus fractions. Both fractions were considerably enriched over that of the homogenate but the specific activity was only 0.1 that reported for lysosome fractions (7, 8). Its presence in the endoplasmic reticulum and Golgi apparatus fractions was not due to lysosomal contamination of the fractions as shown by the cytochemical analyses of the isolated fractions (Figs. 6 and 7).

In contrast, Arylsulphatase C activity of the Golgi apparatus fraction was only 22% that of the rough endoplasmic reticulum (Table 1). This value is higher than that observed with glucose-6-phosphatase and other endoplasmic reticulum enzymes (13 to 15%, ref. 21). One explanation for this discrepancy lies in the observation that potassium

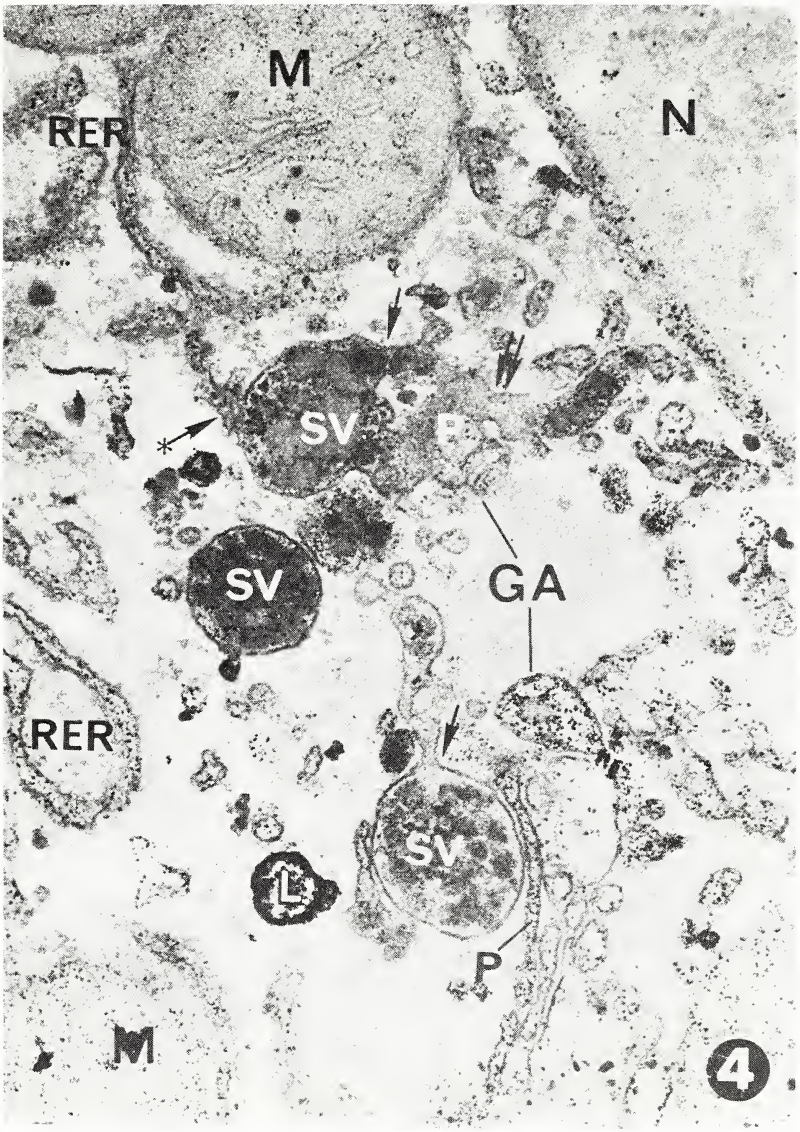


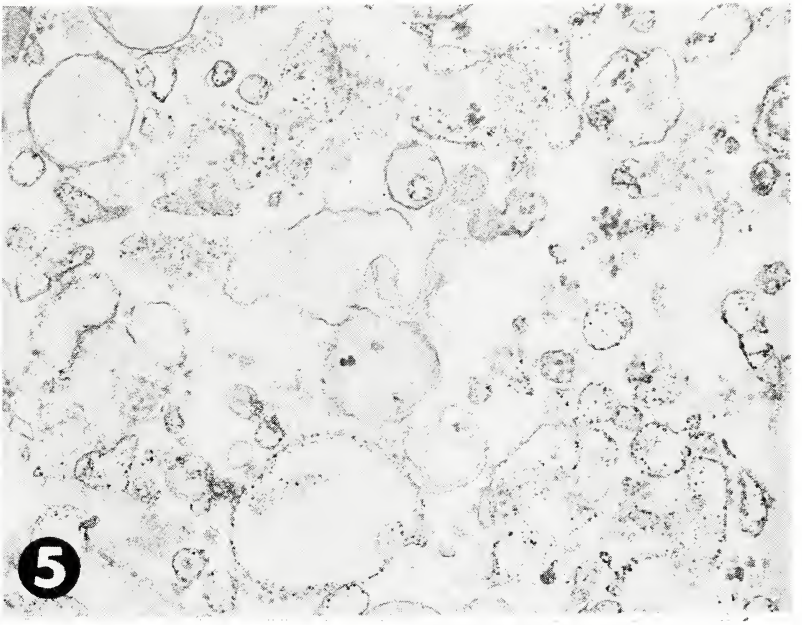
FIGURE 4. *As in Figure 3 showing a portion of the Golgi apparatus (GA) adjacent to the nucleus (N). Granular reaction product is seen within the lumen of the nuclear envelope, within plate-like portions (P) of Golgi apparatus cisternae, within secretory vesicles (SV) of the Golgi apparatus and within the lumens of rough endoplasmic reticulum (RER). One densely staining body next to the Golgi apparatus may represent a primary lysosome (L). Note the connections between lipoprotein-containing, smooth endoplasmic reticulum tubules and the large secretory vesicles of the Golgi apparatus (single arrows), between these tubules and a portion of a Golgi apparatus plate (white P) seen in face view (double arrow) and between a secretory vesicle and rough endoplasmic reticulum (arrow with asterisk). Arylsulphatase reaction product is reduced somewhat in amount from the SER tubules near their point of continuity with Golgi apparatus cisternae. M mitochondrion. X 54,000.*

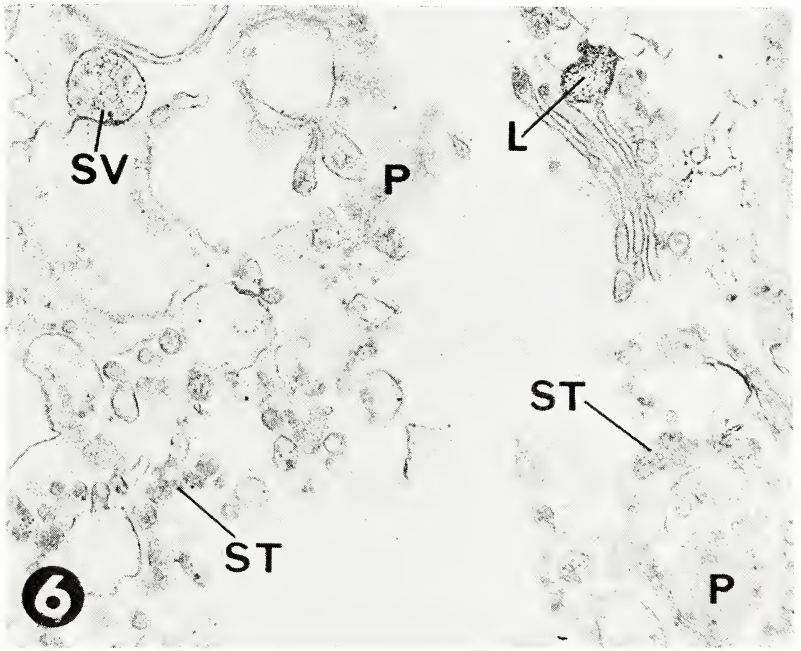
p-acetylphenylsulphate is not a completely specific substrate for Arylsulphatase C under the conditions used, so that the arylsulphatase A and B, shown to be present in the Golgi apparatus, could account for the additional 7 to 10% of the activity. F. A. Rose (personal communication) suggested that phosphate ions increase the specificity of *p*-acetylphenylsulphate for Arylsulphatase C. However, phosphate ions were not added to our reaction mixture.

In the cytochemical analyses, the activity detected may be largely ascribed to Arylsulphatase B. Arylsulphatase A-B show a high degree of substrate specificity hydrolyzing *p*-nitrocatechol sulphate while splitting *p*-acetylphenylsulphate very slowly (31, 32). The pH optima of these two activities also differ. Type C Arylsulphatase has a pH optimum slightly over neutrality and would not be expected to contribute to the cytochemical results. At the acid pH employed, both Arylsulphatases A and B are active although data of Figure 2D suggest that, in future studies, Arylsulphatases A and B could be differentiated cytochemically by varying substrate concentration.

In addition to the focal specializations of smooth endoplasmic reticulum (Fig. 3) and other lysosome-like bodies, we find arylsulphatase activity distributed throughout the endomembrane system (Figs. 3 to 7). It is less evident in the smooth tubules of the Golgi apparatus (Figs. 4, 6 and 7) and is absent from plasma membrane, microbodies, mitochondria and ground cytoplasm. It is present in the nuclear envelope, bulk rough and smooth endoplasmic reticulum and cisternae and vesicles of the Golgi apparatus. This distribution is similar to that observed in plants where acid arylsulphatase activity was found in the nuclear envelope and endoplasmic reticulum with the lead deposits located in the lumen lying next to the membrane (29). Poux (29) noted lead deposits in a type of cytoplasmic body found in root tips of *Cucumis sativus* which closely resemble the structures illustrated in Figure 3 but which she tentatively identifies as phragmosomes (= microbodies, ref. 19). We found no arylsulphatase activity associated with microbodies in rat liver. Nor did we find arylsulphatases concentrated in pericanalicular dense bodies of liver in contrast to the report by Abraham (1). Arylsulphatase A-B was not found cytochemically in mitochondria. The activity present in crude mitochondrial fractions isolated from liver (31, 32, Table 1) may be due to contamination of the fraction by lysosomes and/or endoplasmic reticulum.

The widespread localization of Arylsulphatase A-B in the endomembrane system of rat liver raises the possibility of an active protein being synthesized in the endoplasmic reticulum and/or nuclear envelope and being transported to the bulk smooth endoplasmic reticulum or to the Golgi apparatus where it then becomes incorporated into lysosomes and other types of vesicles. Of specific interest in terms of the present study





FIGURES 5 and 6. *Isolated cell fractions from rat liver incubated for Arylsulphatase A-B activity as described in Figure 3. The only lysosome-like bodies observed in the preparations were those attached to Golgi apparatus cisternae.* FIGURE 5. *Endoplasmic reticulum fraction showing finely granular reaction product located principally over the membranes or within the cisternal lumens against the membranes. X 40,000.* FIGURE 6. *Golgi apparatus fraction.showing finely granular reaction product primarily restricted to secretory vesicles containing lipiprotein particles (SV) and vesicles lacking the particles and presumed to represent primary lysosomes* (L) *still attached to the Golgi apparatus. In this preparation, both the plate-like portions of cisternae (P) and the lipoprotein-containing SER tubules (ST) lacked reaction product. X 33,000.*

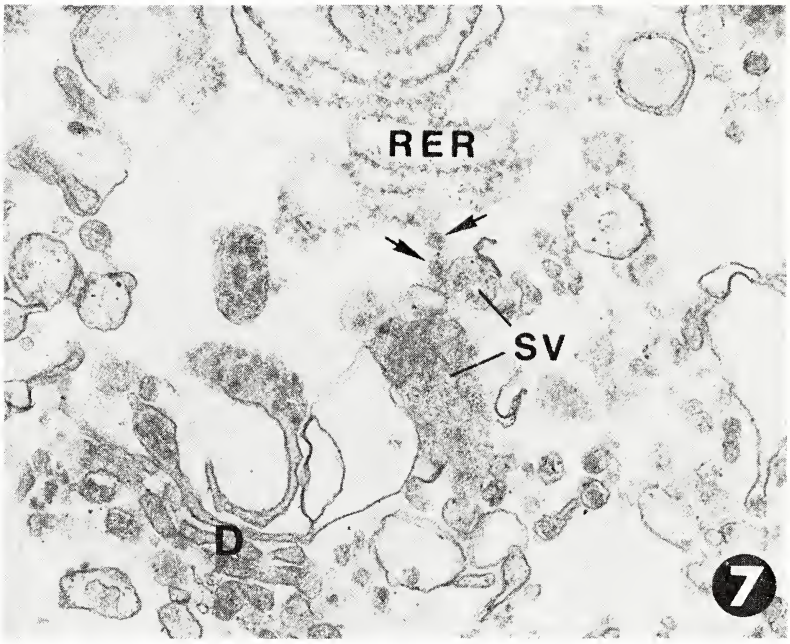


FIGURE 7. *As in Figure 6 showing an intact stack of Golgi apparatus cisternae = dictyosome (D) with finely granular reaction product being restricted to the secretory vesicles (SV) and a fragment of rough endoplasmic reticulum (RER). A region of close association between the RER fragment and a portion of a secretory vesicle is indicated by the arrows. Rather than being represented by a continuous tubule, the region of association has a beaded or vesicular character. X 47,000.*

is the absence of arylsulphatase activity from smooth tubules (= smooth endoplasmic reticulum, refs. 23, 24, 26) of the Golgi apparatus zone. The results show a difference between Golgi apparatus-associated and bulk smooth endoplasmic reticulum and raise the possibility of direct transfer of arylsulphatase A-B from rough endoplasmic reticulum to forming secretory vesicles of the Golgi apparatus.

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