CHAPTER I.

GENERAL INTRODUCTION
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The sulphate esters present in the cells are hydrolysed by a group of enzymes, the sulphatases according to the general reaction.

\[ R.O. \text{SO}_3^- + H_2O \rightarrow R. CH + H^+ + \text{SO}_4^{2-} \]

The most thoroughly investigated group of sulphatases is the arylsulphatases. They catalyse the hydrolysis of arylsulphates or the sulphate monoesters of phenolic hydroxyl groups. They are widespread in their distribution and have been found in all animal species studied, in several micro-organisms, higher plants and in molluscs. Two groups of arylsulphatases type I and type II have been distinguished, by their substrate specificity, response to inhibitors and subcellular localization in Cx, Rat, and Human livers (1-3).

Type I Arylsulphatases:

The type I arylsulphatases are more active towards simple substrates such as p-nitrophenyl sulphate and p-acetylphenyl sulphate. They are much less active...
chain of a fatty acyl group

Fig. 1. Structure of (a) 3-P-Nitroacetate-

(b) Cerebroside 3-Sulfate and (c) Cerebroside 3-Sulfate

\[
\begin{align*}
\text{CH}_3 - (\text{CH}_2)_2\text{CH} = \text{CH} - \text{CH} = \text{CH} - \text{CH}_2 \\
\text{NH} \\
\text{R} - \text{CO} \\
\end{align*}
\]
towards p-nitrocatechol sulphate (Fig. 1). In general these enzymes are inhibited by sulphite, cyanide, phenylhydrazine and hydroxylamine but not by chloride, fluoride, phosphate and sulphate ions. The most detailed specificity studies have been made on the arylsulphatase of *Alcaligenes metalcaligenes* using a series of substituted phenyl sulphates (4). In this case it has been shown that the introduction of an electrophilic substituent into the phenyl sulphate nucleus increases both the rate of hydrolysis of the substrate and its affinity for the enzyme; the introduction of a nucleophilic substituent induces the opposite effects. The sulphatase of *A. metalcaligenes* furnishes an example of a relatively rare type of inhibition (anti-competitive) in which the inhibitor (cyanide or hydrazine in this case) combines with enzyme-substrate complex but not with the enzyme (5). This enzyme also hydrolyses tyrosine-C-sulphate, an unusual substrate as it is not split by mammalian arylsulphatase C, although theoretically it should be (6). Benkovic, Vergara and Hevey (7) purified an arylsulphatase from *Aspergillus oryzae*. The purified enzyme is inhibited by sulphite, fluoride and phosphate but not by sulphate, chloride and cyanide. On the basis of classification suggested by Dodgson and
Spencer (2) it is neither type I nor type II. At pH 4.8 p-nitrophenyl phosphate acts as a competitive inhibitor of the enzyme activity towards p-nitrophenyl sulphate. It is interesting to note, however, that at pH 7.5 p-nitrophenyl phosphate is not an effective inhibitor of either A. oryzae or A. aerogenes enzyme. Fowler and Rammeler (8) also reported that the phenol sulphatase of A. aerogenes does neither fall in type I nor type II category because it is inhibited by fluoride, cyanide and phosphate but not by sulphate ions. Apte and Siddiqi (9) also have purified an arylsulphatase from Aspergillus nidulans. The enzyme was resolved into two distinct fractions designated as fraction I and fraction II by DEAE-cellulose column chromatography. These fractions differ each other in their Km values for p-nitrophenyl sulphate and p-nitrocatechol sulphate, sensitivity to some inhibitors, electrophoretic mobility and heat stability. On the basis of their substrate specificity and sensitivity to sulphate, phosphate and cyanide both fractions can be classified as type I arylsulphatases.

The type I arylsulphatases of mammalian liver are generally inhibited by cyanide and sulphite ions but hardly affected by the phosphate and sulphate ions. The type I arylsulphatases of mammalian livers, the
arylsulphatases C, differ from the corresponding enzymes of micro-organisms in their extreme insolubility (10-12). They are localized in the microsomes (13-15) and very difficult to solubilise. The only soluble arylsulphatase C which has been obtained was prepared from rat liver microsomes by treatment with crude pancreatic enzymes in the presence of a non-ionic detergent (10). Similar preparations of the corresponding human (16) or Cx (12) enzymes could not be obtained. The hydrolysis of arylsulphates by the type I arylsulphatases involves fission of the O- bond, as has been shown by the use of $^{18}$O enriched water (17), but there is little information on the nature of the active centres of these enzymes. Studies on the variation of $K_m$ with pH have shown that the active centres of the type I enzymes from Cx liver (12) and from A. metalcaligenes (18) contain grouping with pK values consistent with their being aminogroups, in agreement with the results obtained using specific inhibitors (18) which also suggest the occurrence of carbonyl group therein (18, 19).

**Type II Arylsulphatases:**

Type II arylsulphatases have been detected in plants, animal tissues and micro-organisms. The most studied
examples are the arylsulphatases A and B of mammalian tissues, some of which have been highly purified (20-25). Another group comprises the arylsulphatases of mollusc tissues (26-29). The mollusc arylsulphatases are seen to represent a transition between the type I enzymes and the type II of vertebrates, although they are definitely more closely related to the latter. In plants thoroughly investigated type II arylsulphatases are from the seeds of the mustard, *Sinapis niger* (30). A type II arylsulphatase, very similar in its properties to a mammalian arylsulphatase B, has been isolated from *Proteus vulgaris* by Dodgson (31) and studied in some detail. This is the first example of a type II arylsulphatase in micro-organisms.

The type II arylsulphatases are characterised by their high activity towards p-nitrocatechol sulphate and much less activity towards simple substrates. These enzymes are strongly inhibited by sulphite, phosphate, sulphate, and fluoride ions but not by cyanide ions. The mammalian liver has been shown to contain a microsomal arylsulphatase C belonging to the type I and arylsulphatase A and B belonging to the type II (11,12,24). The enzymes arylsulphatase A and B are localised in lysosomes and can be readily solubilised.
They differ from each other in their reaction kinetics, pH optimum, affinity towards p-nitrocatechol sulphate and electrophoretic mobilities.

The kinetics of arylsulphatase A are extremely complex, because the reaction velocity shows abnormal relationship with the enzyme concentration and time of incubation (32). The anomalous reaction kinetic of arylsulphatase A is manifested as a time-dependent loss of hydrolytic rate during incubation with p-nitrocatechol sulphate, followed by a partial recovery of the initial rate. Baum and Dodgson (32) have proposed "during the interaction of enzyme and substrate a new site capable of binding with substrate as well as the reaction products (nitrocatechol and sulphate) and certain other inhibitory compounds is slowly exposed to the enzyme. When the substrate is bound to the new site of the modified enzyme the latter is virtually inactive. On the other hand when the reaction products or other inhibitory compounds are bound to the new site, the modified enzyme is active, although the active centre may still be inhibited if these compounds are present in excess". This hypothesis is recently confirmed by Nicholls and Roy (33) who actually isolated the so-called "inactive modified enzyme" from incubation
mixture. Apparently "inactive modified enzyme" is strongly activated by sulphate, but only slightly activated by nitrocatechol. Phosphate and pyrophosphate, the inhibitors of the native enzyme, also activate the modified enzyme. The low activity of the modified enzyme was found due to a powerful substrate inhibition which is decreased in the presence of sulphate. Furthermore it was postulated that sulphate displaces the equilibrium in favour of the native enzyme primarily through the formation of a "native enzyme-substrate complex". The involved kinetics of arylsulphatase A depend strongly upon the pH, the observed pH optima depend upon the time of incubation. In the case of human liver arylsulphatase A, Baum, Dodgson and Spencer (20) have reported two pH optima (pH 4.4 and 5.0) for short incubation periods which over long incubation periods or at higher enzyme concentrations, merge into a single pH optimum of 4.7. Balasubramanian and Bachhawat (34) and Harinath and Robins (35) reported only one pH optimum for human brain arylsulphatase A.

**Distribution of Arylsulphatases:**

The earlier investigation of this problem has been hampered by the lack of the methods of assaying these enzymes in unfractionated tissue preparations and
much of the published work is unreliable because of the lack of appreciation of the complexity of the problem. The most reliable information on the distribution of the arylsulphatases in mammalian tissues is that of Dodgson and his group for the rat (36) and the human (16). They have shown that, in general, liver is the organ richest in arylsulphatases and that considerable amounts also occur in the kidney, pancreas and adrenal. Arylsulphatases occur in human urine and serum also (37, 38, 39). The soluble arylsulphatase has also been found in epiphyseal, articular and rib cartilages and in metaphysis and bone marrow of rat (40).

Arylsulphatase activity in nervous tissue was first reported by Neuberg and Simon in rabbit brain (41). Arylsulphatase activity in homogenates of whole rat brain was reported by Dodgson, Spencer and Thomas (36). Balasubramanian and Bachhawat (42) studied the regional distribution of arylsulphatase activity in sheep brain and they found that the activity of arylsulphatases was high in those regions which were rich in white matter. Similar results on the regional distribution were obtained by Dzialoszynski and Wenclewski (43) in cow brain. The regional distribution of arylsulphatases in rat brain was studied by Sellinger and Hiatt (44).
In liver the intracellular distribution of arylsulphatases was studied by Roy (15) and Viala and Gianetto (45) according to these authors arylsulphatase A and B occur in lysosomes while arylsulphatase C is found exclusively in microsomes. These findings have been recently confirmed by cyto-chemical staining method (46). Clendenon and Allen (47) studied the subcellular distribution of arylsulphatases in rat brain and they found that the localization of arylsulphatases was the same as in the liver. Roy (48) has reported that arylsulphatase B of ox liver is localized in the framework of the lysosomes while arylsulphatase A is in the sap.

Arylsulphatases have been detected in all animal species (15, 49, 50). The quantitative data are available only for the liver. Here also the separate determination of arylsulphatase A and B has not been carried out, and hence proportion of these enzymes is only approximately known. Roy (15) has studied the proportions of arylsulphatase A and B by separating them on paper electrophoresis and according to him the enzymes corresponding to arylsulphatases A and E are detected in all the species except the guinea-pig, the hen, the frog and the stickleback. In frog and stickletack only arylsulphatase E is present. In the guinea-pig the situation
was quite different, although only one arylsulphatase was detected by paper electrophoresis, the shape of pH curve suggested that more than one such enzyme was present. In the hen also the pH curve suggested that both arylsulphatases A and B were present, although they could not be detected by paper electrophoresis. However, the arylsulphatases of the hen were apparently much less stable during electrophoresis than were those of the other animal species, as the total amount of activity recovered after electrophoresis was only a very small proportion of that originally present. This instability might well account for the apparent absence of arylsulphatase A from hen liver.

Further, it is of interest to compare the situation in the vertebrates with that in the few invertebrate species so far studied. Electrophoretic (29) and kinetic (26, 29) studies have shown that the arylsulphatase of the limpet, *Patella vulgata*, is comparable in many respects with a mammalian arylsulphatase A. Similarly in the snail, *Helix pomatia*, the arylsulphatase seems to be of the A type, as judged by its kinetic properties and its behaviour on electrophoresis (51). Dodgson, Lewis and Spencer (26) did not detect in the limpet any arylsulphatase C. Thus in the few invertebrates
so far studied the sole arylsulphatase present seems to be of the type of mammalian arylsulphatase A, in rather striking contrast with the situation in the lower vertebrates where the arylsulphatase is of the B type. It should be noted however, that the invertebrate sulphatases do not show the kinetic anomalies of mammalian arylsulphatase of the A type (32).

The livers of the marsupials *Trichosurus vulpecula* and *Phascolomis mitchelli*, the monotreme *Echidna aculeata*, the lizard *Tiliqua rugosa* and the frog *Rana temporaria* have also been studied with regard to their content of arylsulphatases (50). Type I arylsulphatases were found only in the opossum (*T. vulpecula*) and the lizard, in very small amounts in the latter. Type II arylsulphatases were found in all the species studied. The enzymes had the electrophoretic properties of arylsulphatase B from eutherian mammal but they were not typical of this group as their hydrolyses of p-nitrophenyl sulphate were not greatly activated by chloride ions and they seemed to combine many of the properties of the arylsulphatases A and B of the higher mammals. Thus type II arylsulphatases of lower mammals do not correspond to either arylsulphatase A or arylsulphatase B but rather combine some of the properties of both.
Regarding the mode of action of the type II arylsulphatases Spencer (17) has shown that it is the O-S bond which is splitable. It has been suggested that the active centre of rabbit liver arylsulphatase A probably contains an imidazole group (52). Quite recently, Jerfy and Roy (53) demonstrated by the use of several group specific reagents, that tyrosine and histidine residues are essential for the activity of arylsulphatase A from ox liver. They also reported that neither SH groups nor aminogroups are involved in the reaction catalysed by arylsulphatase A. The active centre of human arylsulphatase B contains grouping with pK values of 5.3 and 6.9 which might be imidazole residues (54). Bleszynski, Leznicki and Lewosz (55) suggested that \( \beta \)-aspartic and \( \gamma \)-glutamic carboxyles and imidazole may be involved in the active centres of arylsulphatase B. According to these authors although the structure of the active centre seems to be very similar for all soluble arylsulphatases of ox brain. The aminoacid composition of arylsulphatase A and B must differ to some extent as their UV-spectra are very different.

**Arylsulphatases in pathological conditions:**

Pronounced changes of the arylsulphatase activity
have been observed in pathological cases. Many diseases are associated with increased excretion of arylsulphatases in urine (56). A thirty-fold increase of arylsulphatase activity was observed in myeloid leukemia (57). Kwashiorkor patients have also shown increased urinary excretion of arylsulphatase A (58). A considerable increase of arylsulphatase activity in urine was observed in case of pulmonary and retinal tuberculosis (59) and in some cases of tumour (57).

Ugazio and his co-workers (60) studied the influence of carbon-tetrachloride, introduced intraperitoneally, on the activity of various enzymes (among them arylsulphatase A and B) in fatty livers induced by that agent in the rat. The authors found increased specific activity of arylsulphatases in the liver homogenate at advance stage of poisoning.

It is suggested that elevated arylsulphatase activity in urine may be connected in some way with observed enlargement of the spleen (61). Various workers have found increased arylsulphatase activity in various tumours as compared with the activity in the parent tissue (62-64). Austin et al (65) found very low arylsulphatase A activity in the brain, kidney, liver and
urine of the patients with metachromatic leukodystrophy. A very marked elevation in arylsulphatase B activity was also observed in liver in gargoylism (65).

**Arylsulphatase A:**

Arylsulphatases A and B can be separated by fractional precipitation with acetone (22), paper electrophoresis (15), gel filtration with sephadex G-200 (66) and ion exchange chromatography (67).

Arylsulphatase A was purified from ox and human livers by Roy (23) and Baum, Dodgson and Spencer (20) but these preparations were not very pure. Moreover, the possibility of alteration in the structure of the enzyme could not be excluded, as these authors have used acetone during the enzyme purification.

Nichol and Roy (68) described a new purification procedure for arylsulphatase A of ox liver in which the conventional acetone fractionation was omitted, and instead the separation of arylsulphatases A and B was achieved by taking advantage of the fact that the arylsulphatase A will precipitate as an insoluble tetramer at acid pH. These workers (69, 70) have recently used the analytical ultracentrifuge in a detailed study of the
sub-unit structure of the enzyme. The molecular weight of the enzyme at neutral pH was estimated as 107000. The protein is very acidic with an iso-electric point at pH 3.4 and as the pH is lowered towards this value the monomer shows an increasing tendency to associate to produce a tetramer of molecular weight 411,000. Probably, hydrophobic bonds are involved in the aggregation since it is reversed by dioxan, as well as by raising the pH so that the electrostatic repulsion between similarly charged units increases. Conversely treatment of the monomeric units with sodium dodecyl sulphate causes them to break down to components of 24,000 molecular weight which, on the basis of chemical data, may be of more than one kind.

Recently Roy (71) has purified arylsulphatase A from Kangaroo liver. This arylsulphatase A is different from ox liver arylsulphatase A in the sense that it has molecular weight of 100,000, does not polymerise at low pH, has an isoelectric point 5.1 - 5.4 and shows less pronounced anomalous kinetics. Balasubramanian and Bachhawat (34) have purified an arylsulphatase from human brain which resembles arylsulphatase A of human liver. The extraction and purification of arylsulphatase A and its properties from ox and human brain have been recently
reported (35, 72, 73). The method for the preparation of an immunoelectrophoretically pure arylsulphatase A from human placenta has also been recently described (74).

**Arylsulphatase A and metachromatic leukodystrophy:**

According to Roy (3) arylsulphatases are unlikely to be active in living tissues because they would be inhibited by the normal concentrations of phosphate and chloride, even if they had natural substrates but Flynn et al (75) have shown the hydrolysis of \( ^{35}S \) p-nitrocatechol sulphate in perfused rat liver, or anaesthetised rats in which kidney function had been eliminated by ligation of the renal pedicles. It may be that the special micro-environments that exist for the functioning of lysosomal enzymes e.g. the endocytotic vacuoles, are adapted to the requirements of arylsulphatases. The identification of arylsulphatase A as ceretoside sulphatase was made in two ways. Patients with metachromatic leukodystrophy exhibit accumulation of ceretoside 3-sulphate (Fig. 1) in various organs and spillage into urine, from this it seemed likely that the ceretoside 3-sulphate degrading enzyme might be lacking in such individuals. In a series of pioneering studies, Austin (76) showed that these individuals had typical arylsulphatase B activities but little or no arylsulphatase A
activity. Mehl and Jatzkewitz (77) found a lack of activity towards ceretroside 3-sulphate in human leukodystrophic kidney. Moser et al (78) studied the turnover of ceretroside 3-sulphate in human subjects and found that the leukodystrophic subjects exhibited very slow break down of ceretroside 3-sulphate.

A more direct comparison has been made with highly purified arylsulphatase of kidney (79). Electrophoresis yielded two peaks one for the A enzyme and one for the E enzyme. The distribution of the ceretroside sulphatase activity after electrophoresis was found to parallel with the distribution of arylsulphatase A to some extent, although not exactly. This discrepancy was explained by the incomplete separation of arylsulphatase A from so called complementary "heat stable fraction", which moves little ahead of peak A, probably overlapping it. They have further shown that this heat stable material greatly stimulates the ceretroside sulphatase activity although it has no effect on arylsulphatase F activity. The role of this material has not been worked out.

The ceretroside sulphatase activity (80) is inhibited by sulphate, sulphite, phosphate, pyrophosphate and fluoride but not by cyanide, in these respects
resembling arylsulphatase A (2). Furthermore cerebroside 3-sulphatase activity was also inhibited by cerebroside 6-sulphate, galactose 3 or 6-sulphates (79). These findings support the hypothesis of Austin et al (65, 81) who demonstrated that the tissues of patients with metachromatic leukodystrophy contained exceptionally low arylsulphatase A, from this they postulated that the lysosomal enzymes may be essential for the normal turnover of these sulphated glycolipids which accumulate in the congenital disease.

Very recently Jatzkewitz and Mehl (82) have demonstrated that both arylsulphatase A and cerebroside 3-sulphatase activities were reduced to the limit of detection in the several tissues of metachromatic leukodystrophy patients. Taninguchi and Nanba (83) studied arylsulphatase A activity in the leukocytes from patients had from their close relatives in three families with metachromatic leukodystrophy. The activity of arylsulphatase A in leukocytes of metachromatic leukodystrophy was approximately one-tenth of the control value. The enzyme activity in leukocytes from the parents of affected patients and from some of their relatives was approximately half that found in control subjects. There appeared to be no overlap between the enzyme activities in leukocytes
from the parents of patients and from normal individuals. The deficiency of arylsulphatase A in metachromatic leukodystrophy was also confirmed by the use of skin fibroblast obtained from the metachromatic leukodystrophy patients (84). The cultured fibroblasts derived from patients with late infantile metachromatic leukodystrophy incorporated arylsulphatase A from the growth medium. Upon exposure to cerebroside sulphate, the fibroblasts exhibited patterns of uptake and hydrolysis indistinguishable from cells derived from control subjects. Furthermore, inclusion granules formed in the metachromatic leukodystrophy fibroblasts upon exposure to cerebroside 3-sulphate were cleared by subsequent supplementation of the growth medium with arylsulphatase A (85).

Recently Porter et al (86) studied a correlation of intracellular cerebroside sulphatase activity in fibroblasts with latency in the metachromatic leukodystrophy and reported that despite the absence of cerebroside sulphatase activity in cell-free preparations, fibroblasts in culture derived from patients with metachromatic leukodystrophy, were capable of hydrolysing exogenous cerebroside sulphate. Moreover, the degree of whole cell arylsulphatase activity was directly correlated to the age of onset of clinical symptoms in the patients.
from whom the fibroblasts were derived. In fact fibroblasts from patients with the earliest manifesting form, late infantile metachromatic leukodystrophy, did not hydrolyse and cerebroside sulphate, while fibroblasts from patients with later manifesting forms hydrolysed appreciable amounts of the sulpholipid.

Neuwelt et al (87) have reported that metachromatic leukodystrophy tissue extracts contain immuno-reactive proteins which precipitate anti-arylsulphatase A serum. These findings and the data of Porter et al (86) with fibroblasts provide evidence that arylsulphatase A proteins are produced in some types of metachromatic leukodystrophy. These mutant proteins appear to be similar in that they all exhibit extreme lability under normal extraction procedures but they may differ among themselves in affinity for cerebroside sulphate or in some other parameter which determines latency of clinical onset. The studies by Porter et al (86) and the wide range of reported latencies in clinical manifestations suggest that the metachromatic leukodystrophies result from a broad spectrum of cerebroside sulphatase deficiency ranging from virtual absence to an appreciable percentage of normal levels in those cases of late onset of symptoms.
The studies by Koenig and his co-workers (88) have indicated that essentially all the enzyme proteins of rat kidney and liver lysosomes are glycoproteins and that at least some of these glycoproteins contain sialic acid (88). Recently Goldstone, Konecny and Koenig (89) have claimed that approximately 40% of arylsulphatase A can be converted to arylsulphatase B by neuraminidase treatment and the incubation without neuraminidase produced a smaller conversion of the arylsulphatase A into arylsulphatase B. The arylsulphatase B produced by neuraminidase treatment showed increased binding affinity for p-nitrocatechol sulphate and reacted as arylsulphatase E on biochemical assay (90). But it is really surprising because arylsulphatase A has more affinity for p-nitrocatechol sulphate and arylsulphatase B has less affinity for this synthetic substrate. On the basis of the acidic and basic nature of lysosomal hydrolases the authors have proposed the deficiency of the specific sialyl transferase which produces arylsulphatase A, deficient in metachromatic leukodystrophy, from arylsulphatase B which is present in normal or elevated amounts in this disease.

Arylsulphatase E:

Earlier described preparations (24) of arylsulphatase B were grossly impure. Recently Allen and Roy (91)
have described a new method for the preparation of arylsulphatase B from ox liver. The enzyme was resolved into two fractions, arylsulphatase Bα and Bβ by CM-sephadex chromatography. Each fraction was purified about 2000-fold and obtained in a yield of about 5%. Both fractions have molecular weights of about 25,000 and they are kinetically indistinguishable. The purified enzyme was examined by the technique of isoelectric focussing and it was shown that during this procedure the arylsulphatase B aggregates to mixtures of polymers having molecular weights of up to at least 300,000. The aggregation is probably due to electrostatic interaction because it is reversed by increasing the ionic strength. Arylsulphatase B of human liver was also purified (54). This enzyme had little activity towards potassium phenyl sulphate and its mono-substituted derivatives, but it showed appreciable activity towards disubstituted derivatives. Arylsulphatase B from ox brain (55,72) and human brain was purified by Bleszynski and Harinath and Robins (35). The ox brain arylsulphatase B was resolved into three subfractions by DEAE-cellulose chromatography and these subfractions were designated as E-1, E-2 and E-3. According to Bleszynski (73) these subfractions were similar to Wortman's fractions (92) 'a', 'b' and 'c'
which he obtained from ox cornea.

Arylsulphatase B too is not without its anomalies. This enzyme hydrolyses p-nitrocatechol sulphate, but has often been considered quite without activity against p-nitrophenyl sulphate. Webb and Morrow (25) however, found that a partially purified arylsulphatase B from ox liver hydrolysed p-nitrophenyl sulphate when activated by chloride. On the other hand, the hydrolysis of p-nitrocatechol sulphate by the same enzyme was inhibited by chloride, at high concentrations. The activity against p-nitrophenyl sulphate was inhibited by phosphate, sulphate, and fluoride but not through competition with chloride; the two may have competed with the substrate. Ox liver arylsulphatase B has low affinity for p-nitrocatechol sulphate and is inhibited by citrate (24). Allen and Roy (91) have recently confirmed the requirement of chloride for the hydrolysis of p-nitrophenyl sulphate that was first described by Webb and Morrow (25).

Arylsulphatase E and Mucopolysaccharidoses:

The role of arylsulphatase B is quite obscure. However, a marked increase in arylsulphatase E activity was observed in Hurler's syndrome (93). Further it
was found that although there was increase in heparan sulphate and dermatan sulphate fraction in Hurler's disease compared to normal, the total mucopolysaccharide content in Hurler's and normal brain was the same. Recently Abraham et al (94) reported that there was a marked increase of arylsulphatase B in the biopsy samples of patients with Sanfilippo syndrome compared to arylsulphatase A and the ratio of arylsulphatase B to A was very high compared to that of the normal brain.

Held and Buddecke (95) using $^{35}$S chondroitin 4-sulphate of bovine nasal septum, have purified chondroitin 4-sulphatase free from arylsulphatase and hyaluronidase from bovine arterial tissue and showed that this is a specific enzyme acting at polymer level. The enzyme has pH optimum at 4.4 in acetate buffer. The release of inorganic sulphate from unlabelled chondroitin 4-sulphate could be demonstrated turbidimetrically by converting it to barium sulphate, but this method did not show any action of the enzyme on chondroitin 6-sulphate, dermatan sulphate or keratan sulphate. The above authors also claimed that the addition of hyaluronidase did not stimulate the release of sulphate by this enzyme. Very recently Tudball and Davidson (96) have shown the existence of an enzyme related to that of bovine aorta
and yet distinct in lysosomes of rat liver. Although partially purified, enzyme was contaminated with \( \beta \)-glucuronidase, \( \beta \)-acetylglucosaminidase and arylsulfatase; its substrate specificity was curious in that it was most active with high molecular weight oligosaccharides derived from \( ^{35}S \) chondroitin 4-sulphate of chick embryo by the action of hyaluronidase. The enzyme had less action on low molecular weight oligosaccharides and none on the parent polysaccharide; the removal of the non-reducing glucuronosyl residue of the oligosaccharide substrate was apparently essential for enzymic activity. Lloyd (97) and his associates have shown that mammalian sulfamidase liberates inorganic sulphate from heparan sulphate by the cleavage of sulfamido groupings. Recently, a sulphamidase has been purified from mammalian sources by Dietrich (98).

Assay of Arylsulphatases A and B:

There have been difficulties in assaying arylsulphatases A and B when they are present together, as in human urine. A solution to this problem has been worked out by Baum, Dodgson and Spencer (99) by utilising a differential inhibition with chloride ions and other small differences in properties. The determination
of arylsulphatase A in the presence of B is based upon the following principles:

1. 'Normal Kinetics' are exhibited by arylsulphatase A at low substrate concentration in the presence of $2.5 \times 10^{-4}$ M-sodium pyrophosphate.

2. Considerable inhibition of arylsulphatase B occurs under these conditions.

3. Arylsulphatase B is specifically inhibited by chloride ions when p-nitrocatechol sulphate is used as substrate, whereas arylsulphatase A is not.

The determination of arylsulphatase B in the presence of A is based on the fact that arylsulphatase A exhibits only a little residual activity after the first 20 minutes of the reaction when incubated at high substrate concentration in the presence of barium ions and in the absence of interfering ions. Moreover, this residual activity is linear and proportional to the intercept obtained by extrapolating this line back to zero time. Barium ions do not affect the activity of arylsulphatase B. According to Dodgson and his co-workers (67) this assay procedure may not be applied to rat liver arylsulphatases A and B because the differences in the kinetic properties of these enzymes are not sufficiently great to enable the independent assay of one enzyme in the presence of other.
AIMS AND OBJECTS OF THE PRESENT STUDY.

It is clear from the general introduction that there is a good relationship between arylsulphatases and cerebrosides 3-sulphate and mucopolysaccharides. The importance of mucopolysaccharides in the synthesis of myelin in brain has been indicated by various workers (100, 101, 102). The sulphur containing lipids, cerebroside 3-sulphate are the components of the myelin sheaths of the nervous system. As a building-blocks of the myelin they are of great importance and any abnormal changes in their concentration result in extensive damage to the myelin as seen in the disease metachromatic leukodystrophy which is characterised by the deficiency of arylsulphatase A not only in brain and kidney but also in urine (65). Further, Austin et al (103) and Saxena et al (104) have reported that arylsulphatase A levels tend to parallel the level of incorporation of (35S) sulphate into cerebroside 3-sulphate of the rat brain and the levels of arylsulphatase A and cerebroside 3-sulphate reach their peak in young rat brain during myelination period. A study of cerebroside sulphotransferase activity in the developing rat brain in our laboratory (104) has revealed that there was practically no cerebroside 3-sulphate synthesising
activity up to 9 days after birth and there was a peak of activity around 18 to 22 days after birth once again emphasizing the fact that cerebroside 3-sulphate synthesis is maximum at the myelination period. Moreover, a very striking relationship between arylsulphatase activity and cerebroside 3-sulphate content of some regions of the sheep brain was also observed in our laboratory (42). Recently Mehl and Jatzkewitz (79) have shown that cerebroside 3-sulphate is a naturally occurring substrate for arylsulphatase A from pig kidney. However, at present there is no information available as to whether or not the brain arylsulphatase A can degrade cerebroside 3-sulphate.

In the light of these observations a systematic comparative study of arylsulphatases A and B activity was made in the brains of various animal species and also in developing rat brain. The enzyme arylsulphatase A was purified from chicken brain to study its physicochemical and kinetic properties. Some kinetic properties of this purified enzyme were also studied using cerebroside 3-sulphate as substrate.