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THE REGIONAL DISTRIBUTION, AGE DEPENDENT VARIATION AND SPECIES DIFFERENCES OF BRAIN ARYLSULPHATASES

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Abstract—The relative proportions of arylsulphatase A and B were determined by the method of BAUM, DODGSON and SPENCER (1959) in brains of various animal species and it was found that there was a considerable variation in the concentration of these two enzymes.

Arylsulphatase A and B of various animal species including rat, man, monkey, sheep and chicken were partially separated using zinc acetate fractionation procedure and gel electrophoresis. The chicken brain arylsulphatase A had a similar electrophoretic mobility to that of arylsulphatase B of other species. Further, chicken brain arylsulphatase A precipitated at a zinc acetate concentration of 0·005 M, a condition under which arylsulphatase B from the brain of other species precipitated.

Kinetic properties such as K_m value and inhibitory effect of sulphite and phosphate ions indicated that chicken brain arylsulphatase A was similar to arylsulphatase A of other species.

The results on regional distribution of arylsulphatase A and B activities in monkey brain and in developing rat brain suggest a relationship between arylsulphatase A and sulphatides and arylsulphatase B and mucopolysaccharides.

THERE are several lines of evidence indicating a relationship between arylsulphatases and mucopolysaccharides and sulphatides. The occurrence and importance of mucopolysaccharides and sulphatides in brain have already been established (AUSTIN, 1960; BRANTE, 1957; GUHA, NORTHOVER and BACHHAWAT, 1960; YOUNG and ABOOD, 1960). A significant decrease in arylsulphatase A activity and accumulation of sulphatides was observed in various tissues of metachromatic leucodystrophy patients (AUSTIN, 1960; AUSTIN *et al.*, 1963). MEHL and JATZKEWITZ (1968) have recently demonstrated that cerebroside-3-sulphate is a naturally occurring substrate for arylsulphatase A. BALASUBRAMANIAN and BACHHAWAT (1963*a*) have reported a relationship between high arylsulphatase activity and the sulphatide content of some regions of brain. Collective evidence thus indicates that arylsulphatase A may have a role in regulating the sulphatide content in brain. A marked increase in arylsulphatase B activity compared to A was observed in Hurler's disease (AUSTIN *et al.*, 1964; ABRAHAM *et al.*, 1969). Furthermore, ABRAHAM *et al.* (1969) found that although there was an 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.

The present communication describes (1) the determination of arylsulphatase A and B in brains of various animal species, (2) the partial separation of arylsulphatases A and B of brain by fractionation with zinc acetate and comparison of properties of arylsulphatase A from avian and mammalian species (3) the determination of arylsulphatases A and B in different regions of monkey brain and (4) the variation of arylsulphatase A and B activities in developing rat brain.

MATERIALS AND METHODS

Dipotassium salt of nitrocatechol sulphate was purchased from Sigma Chemical Co., U.S.A. Acrylamide and *N-N'*-methylene bis acrylamide were obtained from Eastman Organic Chemicals, Distillation Products Industries, U.S.A., Triton X-100 was purchased from Rohm and Haas, Philadelphia, Pa. Coomassie Brilliant Blue was obtained from Sigma Chemical Co., U.S.A. and all other chemicals used were of analytical grade.

The brains of various animal species studied were taken out after decapitation, immediately chilled in ice and were homogenized with 2 ml/g of 0·05 M-tris-HCl, pH 7·4 in a Potter–Elvehjem homogenizer for 2 min at 0°C. Homogenates were centrifuged at 800 *g* for 30 min and the supernatant fluid suitably diluted, was used for the assay of enzyme activity. Since the original supernatant was diluted 40 times, the possibility of inhibition by phosphate ions present in the supernatant fluid was negligible.

Arylsulphatase assay. Arylsulphatases A and B were assayed by the method of BAUM, DODGSON and SPENCER (1959) with slight modification. Assay mixture for arylsulphatase A consisted of 0·1 ml of Reagent A (0·01 M-nitrocatechol sulphate in 0·5 M-sodium acetate-acetic acid buffer containing 5×10^{-4} M-sodium pyrophosphate and 10% (w/v) NaCl, pH 5·0), 0·1 ml of Triton ×-100 and enzyme in a total volume of 0·3 ml. After incubation at 37°C, for 1 h the reaction was stopped by the addition of 2·7 ml of 0·11 M-NaOH and the red colour developed was measured in a Klett Summerson Colorimeter using filter 50. Assay mixture for arylsulphatase B consisted of 0·1 ml of reagent B (0·05 M-nitrocatechol sulphate in 0·5 M-sodium acetate buffer containing 10^{-2} M-barium acetate, pH 6). The tubes were incubated at 37°C for 0·5 and 1·5 h and the reaction stopped as indicated above. The amount of nitrocatechol formed was calculated for 1 h as described by BAUM, DODGSON and SPENCER (1959). The specific activity was expressed as μmol nitrocatechol formed/mg protein/h.

Partial separation of arylsulphatases A and B. Frozen brain was homogenized with 2 vol. of 0·03 M-tris-HCl pH 7·4 for 2 min in a Waring blender. The homogenate was cooled at − 5°C in a bath of ethylene glycol and then subjected to ethanol fractionation with constant mechanical stirring. Ethanol 20% (v/v) was added dropwise and after 10 min the mixture was centrifuged at 12,000 *g* for 1 h at − 5°C. A small portion (approx. 5 ml) of supernatant was dialysed against 100 vol. of 0·001 M-acetate buffer, pH 5, containing 0·0001 M-$MgCl_2$ for 8 h. The rest of the supernatant was subjected to zinc acetate fractionation. Zinc acetate solution (0·2 M) was added to a final concentration of 0·005 M at − 5°C with constant mechanical stirring. After 15 min the mixture was centrifuged at 12,000 *g* for 1 h at − 5°C. The reddish precipitate was dissolved in 0·1 M-citrate buffer pH 7 and dialysed for 4 h against 500 vol. of 0·001 M-acetate buffer, pH 5, containing 0·0001 M-$MgCl_2$. To the supernatant, zinc acetate was added to a final concentration of 0·02 M. After 15 min it was centrifuged at 12,000 *g* for 1 h at − 5°C. The supernatant was discarded and the white precipitate was dissolved in 0·1 M-citrate buffer pH 7 and dialysed as above.

Gel electrophoresis. Polyacrylamide gel electrophoresis was carried out by the method of ORNSTEIN and DAVIS (1962) in 0·03 M-barbitone sodium buffer pH 8·0 for 10 h using 8 mA/tube using a Buchler instrument. The proteins were stained by Coomassie brilliant blue (CHRAMBACH, REISFELD, WYCKOFF and ZACCARI, 1967). For elution of protein, the whole gel was cut into 1 cm segments and from each segment the protein was eluted by homogenizing the gel in a Potter–Elvehjem homogenizer with 0·03 M-tris-HCl, pH 7·4. The homogenate was centrifuged at 0°C. The supernatant after dialysis against 0·001 M-actate buffer pH 5·0 containing 0·0001 M-$MgCl_2$ was analysed for arylsulphatase A and B activities.

Protein determinations. Protein was determined by the method of LOWRY, ROSEBROUGH, FARR and RANDALL (1951) with crystalline bovine serum albumin as a standard.

RESULTS

The proportion of arylsulphatase A and B in brains of various animal species. Table 1 shows that in a lower vertebrate like frog the ratio of arylsulphatase A:B is 1:1. In birds, like pigeon and chicken arylsulphatase A accounts for greater part of the total arylsulphatase activity. In fact in chicken the activity of arylsulphatase A is exceptionally high compared to B. In mammals like rabbit and sheep arylsulphatase A is high while in rat, monkey and man arylsulphatase B predominates. Zinc acetate fractionation results in partial separation of arylsulphatase A and B in all species studied (Table 2). It should be noted here that arylsulphatase B (0·005 M-zinc acetate precipitate) was contaminated with 6 to 18 per cent of arylsulphatase A, and arylsulphatase A (0·02 M-zinc acetate precipitate was contaminated with 1·8 to 12 per cent

TABLE 1. RATIO OF SPECIFIC ACTIVITIES OF ARYLSULPHATASE A AND B IN BRAINS OF VARIOUS ANIMAL SPECIES

Species	Specific activity A	Specific activity B	Ratio of specific activities A:B
Rat	0·28	0·59	1:2
Man (adult	0·1	0·16	1:1·6
Child (2 years)	0·06	0·07	1:1
Hurler's syndrome patient (7 years)	0·03	0·17	1:5·6
Sanfilippo syndrome patient (8 years)	0·02	0·09	1:4·5
Monkey	0·07	0·12	1:1·7
Sheep	0·16	0·06	2·6:1
Rabbit	0·08	0·06	1·3:1
Pigeon	0·07	0·03	2·3:1
Chicken	0·1	0·003	33·3:1
Frog	0·02	0·02	1:1

The activities of arylsulphatase A and B were measured as indicated in the text.

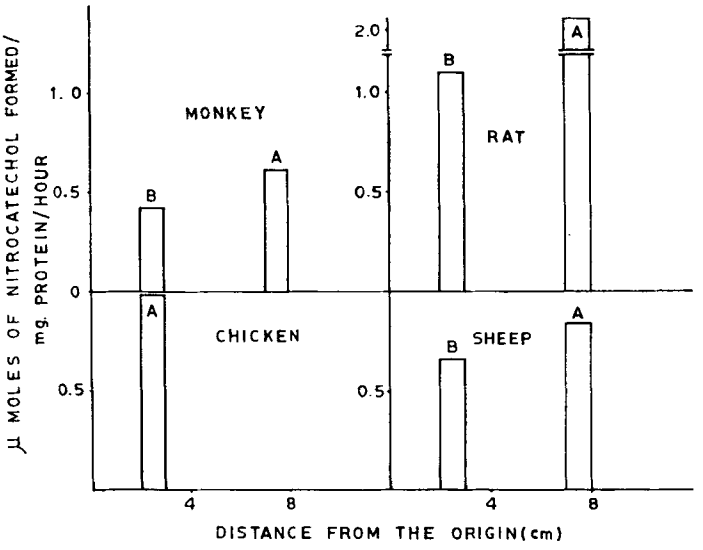

FIG. 1.—The electrophoretic mobility of monkey, rat, chicken and sheep brain arylsulphatase A and B. 7·5 % Polyacrylamide gel in 17 × 1 cm tube was used. The conditions for electrophoresis and the determination of arylsulphatase A and B activities were the same as indicated in the text.

TABLE 2.—PARTIAL SEPARATION OF ARYLSULPHATASE A AND B FROM SHEEP, CHICKEN AND MONKEY BRAIN

Enzyme fraction	Total volume (ml)	Total protein (mg)	Arylsulphatase A			Arylsulphatase B		
			Total enzyme units*	Specific activity	Yield (Per cent)	Total enzyme units*	Specific activity	Yield (Per cent)
Sheep:								
Homogenate	266	9842	400	0·04	100	200	0·02	100
Extract (20% Ethanol supernatant)	200	3000	240	0·08	60	192	0·06	96
Zinc acetate fraction I (0·005 M)	53	1961	27	0·01	7	152	0·07	76
Zinc acetate fraction II (0·02 M)	29	696	177	0·25	44	20	0·02	10
Chicken:								
Homogenate	112	2128	100	0·05	100	3·7	0·002	100
Extract (20% Ethanol supernatant)	64	1312	73	0·06	73	3·7	0·003	98
Zinc acetate fraction I (0·005 M)	17	586	71	0·12	71	0·0	0·0	0
Zinc acetate fraction II (0·02 M)	12	213	16	0·07	16	2·6	0·012	69
Monkey:								
Homogenate	200	5000	260	0·05	100	240	0·05	100
Extract (20% Ethanol supernatant)	100	1460	186	0·12	71	100	0·07	42
Zinc acetate fraction I (0·005 M)	40	1120	44	0·04	17	88	0·08	37
Zinc acetate fraction II (0·02 M)	17	367	129	0·35	50	4	0·01	18

All fractions were assayed after dialysis.

*One unit of enzyme is defined as the amount of enzyme required to liberate 1 μmol of nitrocatechol from nitrocatechol sulphate in 1 h under usual assay conditions.

of arylsulphatase B. In the case of human brain, arylsulphatase A obtained by this procedure was found to be free from arylsulphatase B.

It is interesting to note that arylsulphatase A of chicken brain is quite different from arylsulphatase A of other species since under zinc acetate fractionation most of chicken arylsulphatase A precipitates out at a concentration of 0·005 M while in other cases arylsulphatase A precipitates out only at a concentration of 0·02 M. Furthermore, the electrophoretic mobility of chicken brain arylsulphatase A is similar to that of arylsulphatase B of other species but quite different from arylsulphatase A of other species (Fig. 1). This finding is comparable with the finding of ROY (1958) who has indicated the presence of both arylsulphatase A and B in hen liver although arylsulphatase A could not be detected on paper electrophoresis. It is clear from the above observation that chicken arylsulphatase A behaves like arylsulphatase B under zinc acetate fractionation as well as in electrophoresis. This may explain why ROY (1958) was unable to detect the presence of arylsulphatase A electrophoretically in a crude extract of hen liver. Zinc acetate fraction I, and II from rat, monkey, sheep and chicken brain were subjected to gel electrophoresis (Fig. 1). The arylsulphatase A and B eluted from the gel were found to be free from each other. The increase in specific activity and recovery of arylsulphatase A and B is shown in Table 3.

TABLE 3.—RECOVERY AND SPECIFIC ACTIVITY OF ARYLSULPHATASE A AND B BEFORE AND AFTER ELECTROPHORESIS

Arylsulphatases from various species	Specific activity Before electrophoresis	Specific activity After electrophoresis	Recovery (per cent)
Sheep arylsulphatase A	0·254	0·84	32
Sheep arylsulphatase B	0·07	0·66	48
Chicken arylsulphatase A	0·12	1·0	46
Monkey arylsulphatase A	0·35	0·61	37
Monkey arylsulphatase B	0·078	0·47	38
Rat arylsulphatase A	2·3	2·6	38
Rat arylsulphatase B	0·54	1·1	42

Zinc acetate I and II fraction from various animal species were subjected to the gel electrophoresis. The conditions for electrophoresis are as described in the text. After elution from the gel, the activities of arylsulphatase A and B were estimated as indicated in the text.

Arylsulphatase A and B activities in developing rat brain. It appears from Fig. 2 that arylsulphatase A and B activities are present in rat brain on the first day after birth and there is an increase in enzyme activity with age. It is interesting to note that there are two prominent peaks of arylsulphatase B corresponding to 5 and 20 days. The activity of arylsulphatase A is also high during the myelination period.

Regional distribution of arylsulphatase A and B activities in monkey brain. The data on regional distribution (Table 4) suggest that arylsulphatases A and B are present in all the regions of monkey brain. It is apparent that the activity of arylsulphatase A is high in white matter not only in cerebrum but also in regions like medulla and corpus callosum. The activities of arylsulphatase B are high in grey matter compared to white matter in most of the regions.

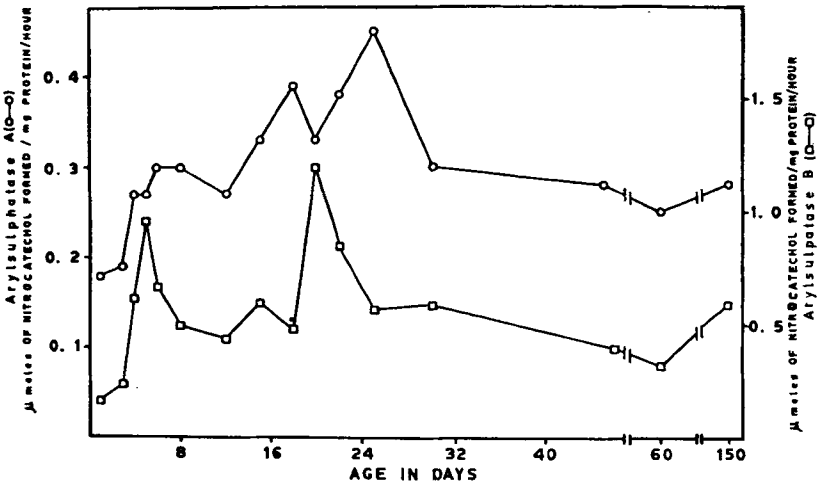

FIG. 2.—Variation of arylsulphatase A and B activities in developing rat brain. The activities were estimated as indicated in the text.

TABLE 4.—RATIO OF SPECIFIC ACTIVITIES OF ARYLSULPHATASE A AND B IN DIFFERENT REGIONS OF MONKEY BRAIN

Regions	Specific activity Arylsulphatase A	Specific activity Arylsulphatase B	Ratio of specific activities A:B
Cerebrum:			
Frontal grey	0·04	0·07	1:1·75
Frontal white	0·1	0·09	1·1:1
Parietal grey	0·05	0·11	1:2·2
Parietal white	0·05	0·12	1:2·4
Occipital grey	0·08	0·15	1:1·8
Occipital white	0·1	0·08	1·2:1
Cerebellum	0·04	0·06	1:1·5
Quadrigeminal bodies	0·07	0·16	1:2·2
Pons	0·1	0·14	1:1·4
Medulla	0·12	0·08	1·5:1
Corpus Callosum	0·09	0·06	1·5:1

Activities of arylsulphatase A and B were measured as indicated in the text.

Properties of arylsulphatases A and B in brains of different species

pH optimum. Figures 3 and 4 show the pH activity curves of arylsulphatases A and B of various animal species. The pH optimum for arylsulphatase A in acetate buffer of rat, monkey, sheep and chicken was found to be 5·0, 4·5, 5·5 and 4·5 respectively. The reported optimum pH for human brain arylsulphatase A is 4·5 (BALASUBRAMANIAN and BACHHAWAT, 1963*b*). The pH optimum for arylsulphatase B in acetate buffer of rat, monkey and sheep was 5·5, 5·5 and 4·5 respectively. When tested with zinc acetate fractions I and II, Triton X-100 had no effect on arylsulphatase A and B activities of the various animal species.

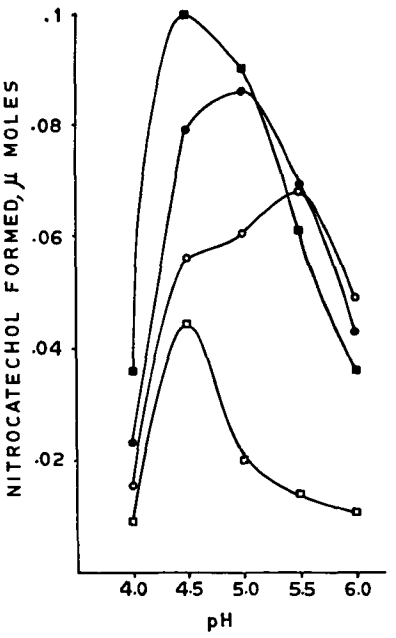

FIG. 3.—pH activity curves of rat, monkey, sheep and chicken arylsulphatase A. The incubation mixture consisted of 50 μmol of acetate buffer of various pH values, 1 μmol of nitrocatechol sulphate, and enzyme (zinc acetate fraction II except in the case of chicken where zine acetate fraction I was used) in a total volume of 0·3 ml. The reaction mixture was incubated at 37°C for 1 h and the reaction was stopped as indicated in the text. Enzyme protein used: rat (-●-●-) 0·05 mg, monkey (-■-■-) 0·076 mg, sheep (-○-○-) 0·24 mg, and chicken (-□-□-) 0·345 mg.

The effect of the length of the incubation period. The time–activity curves (Figs. 5 and 6) for arylsulphatase A from various animal species show anomolous time–activity relationship (BAUM and DODGSON, 1958; BALASUBRAMANIAN and BACHHAWAT, 1963*b*) at various protein concentrations. It should be noted in the case of chicken arylsulphatase A the last two stages (stage II and stage III) are less pronounced compared to other species. Moreover, at high enzyme concentration in all the species stage II is not maintained and the curves tend towards a straight line. Arylsulphatase B of rat, monkey and sheep exhibits normal time–activity relationship.

K_m value and effect of sulphite and phosphate ions. Table 5 compares the K_m values of arylsulphatases A and B in various animal species. The effect of sulphite and phosphate ions was studied on sheep and chicken brain arylsulphatase A (Figs. 7 and 8). The K_i values for sheep and chicken brain enzymes using sulphite ions as inhibitor are $0{\cdot}7 \times 10^{-5}$ M and $0{\cdot}8 \times 10^{-5}$ M respectively. The K_i values for phosphate ions in sheep and chicken brain are $2{\cdot}57 \times 10^{-4}$ M and $0{\cdot}8 \times 10^{-4}$ M. The reported values of K_i for human brain arylsulphatase A for sulphite and phosphate ions are $1{\cdot}98 \times 10^{-6}$ M and $3{\cdot}5 \times 10^{-5}$ M respectively (BALASUBRAMANIAN and BACHHAWAT, 1963*b*).

As mentioned above, the gel electrophoresis of zinc acetate fraction I and II

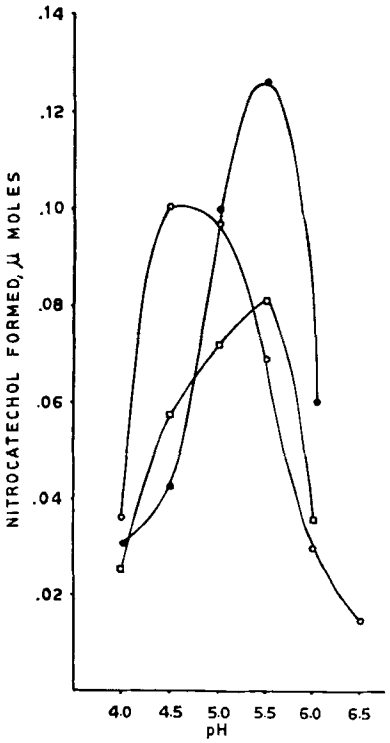

FIG. 4.—pH–activity curves of rat, monkey and sheep arylsulphatase B. The incubation mixture consisted of 50 μmol of acetate buffer of various pH values, 5 μmol of nitrocatechol sulphate and enzyme (zinc acetate fraction I) in a total volume of 0·3 ml. The reaction mixture was incubated at 37°C for 1 h and the reaction was stopped as indicated in the text. Enzyme protein used: rat (–●–●–) 0·04 mg, monkey (–■–■–) 0·3 mg, and sheep (–○–○–) 0·32 mg.

TABLE 5.—COMPARISON OF K_m VALUES OF ARYLSULPHATASE A AND B FROM VARIOUS ANIMAL SPECIES

Animal species	K_m value	
	Arylsulphatase A	Arylsulphatase B
Rat	$1{\cdot}8 \times 10^{-3}$ M	$4{\cdot}3 \times 10^{-3}$ M
Monkey	$1{\cdot}47 \times 10^{-3}$ M	$5{\cdot}7 \times 10^{-3}$ M
Sheep	$4{\cdot}5 \times 10^{-3}$ M	$9{\cdot}09 \times 10^{-3}$ M
Chicken	$3{\cdot}1 \times 10^{-3}$ M	—
Human*	$1{\cdot}5 \times 10^{-3}$ M	—

* For comparison, this value was taken from BALASUBRAMANIAN and BACHHAWAT (1963*b*).

results in the complete separation of arylsulphatase A and B from each other. The kinetics of monkey brain arylsulphatase A and B eluted from the gel were also studied. It was found that the pH optimum and K_m value of the arylsulphatase A and B were

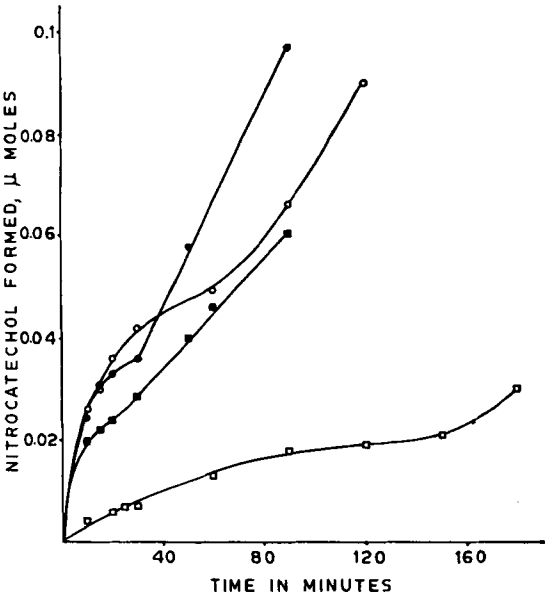

FIG. 5.—Time-activity curves of rat, monkey, sheep and chicken brain arylsulphatase A. The incubation mixture consisted of 50 μmol of acetate buffer with appropriate pH for each animal species, 1 μmol of nitrocatechol sulphate and enzyme (zinc acetate fraction II except in case of chicken where zinc acetate fraction I was used) in a total volume of 0·3 ml. The reaction mixture was incubated at 37°C and the reaction was stopped as indicated in the text.

Enzyme protein used: rat (-●-●-) 0·05 mg, monkey (-■-■-) 0·038 mg, sheep (-○-○-) 0·095 mg and chicken (-□-□-) 0·095 mg.

the same before and after electrophoresis. This indicates that even though the arylsulphatases A and B from the zinc acetate fraction I and II are contaminated with each other to a minor extent, it did not significantly affect the pH optimum and K_m value.

DISCUSSION

The results reported in this paper on arylsulphatase A and B activities are based upon the differential assay procedure of BAUM, DODGSON and SPENCER (1959). The results indicate that this differential assay method is valid for all species. The assay conditions used were optimal for arylsulphatase A and B of chicken, monkey, human and rat whereas the optimal conditions for pigeon, frog and rabbit were not determined. In the case of sheep brain arylsulphatase A and B, the substrate concentration employed was not optimal.

The results of comparative studies on the activity of arylsulphatase A and B in different species show that the proportions of these enzymes vary from one species to another. It is interesting to note that there is a relationship between the mucopolysaccharide content and the amount of arylsulphatase B. Thus in rat, monkey and man,

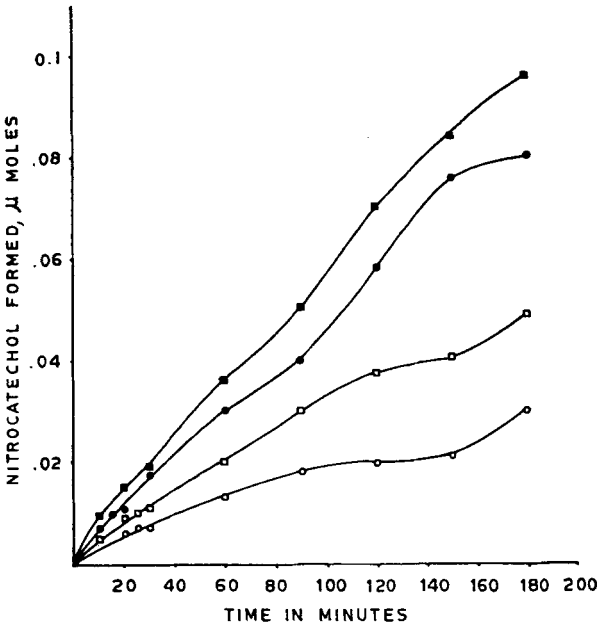

FIG. 6.—Time–activity curves of chicken brain arylsulphatase A at different protein concentration. The incubation mixture consisted of 50 μmol of acetate buffer pH 4·5, 1 μmol of nitrocatechol sulphate and enzyme (zinc acetate fraction I) in a total volume of 0·3 ml. The tubes were incubated at 37°C and the reaction was stopped as indicated in the text.

Enzyme protein used: 0·095 mg (–○–○–), 0·19 mg (–□–□–), 0·304 mg (–●–●–) and 0·38 mg (–■ –■–).

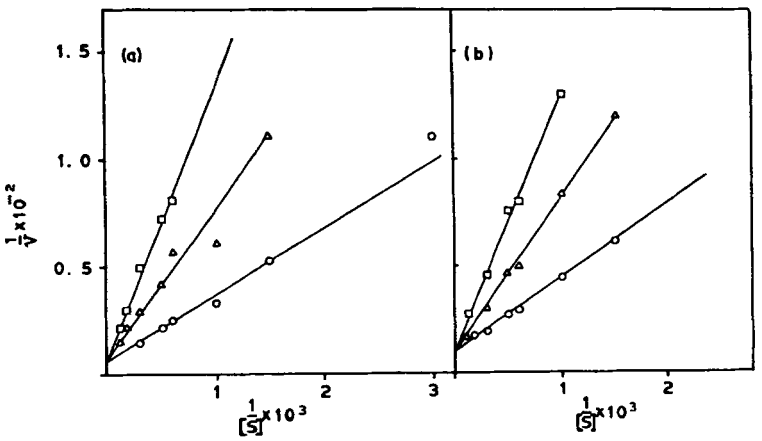

FIG. 7.—Lineweaver–Burk plots showing the effect of sodium sulphite on sheep (a) and chicken (b) brain arylsulphatase A. (a) The assay mixture consisted of 50 μmol of acetate buffer pH 5·5, different concentrations of nitrocatechol sulphate and 0·24 mg enzyme protein (zinc acetate fraction II) in a total volume of 0·3 ml. (b) The reaction mixture consisted of 50 μmol of acetate buffer pH 4·5, different concentrations of nitrocatechol sulphate, 0·345 mg enzyme protein (zinc acetate fraction I) in a total volume of 0·3 ml. The tubes were incubated at 37°C for 60 min and nitrocatechol formed was estimated as indicated in the text. Sodium sulphite concentration –○–○–, none; –△–△–, 0·5 × 10^{-5} M; and –□–□–, 2·5 × 10^{-5} M.

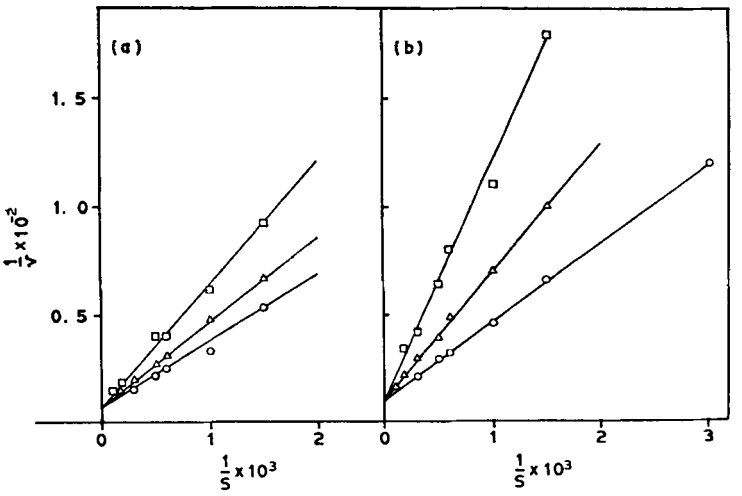

FIG. 8.—Lineweaver–Burk plots showing the effect of potassium phosphate on sheep (a) and chicken (b) brain arylsulphatase A. (a) The assay mixture consisted of 50 μmol of acetate buffer pH 5·5, different concentrations of nitrocatechol sulphate, 0·24 mg enzyme protein (zinc acetate fraction II) in a total volume of 0·3 ml. (b) The assay mixture consisted of 50 μmol of acetate buffer pH 4·5, different concentrations of nitrocatechol sulphate and 0·345 mg enzyme protein (zinc acetate fraction I) in a total volume of 0·3 ml. The tubes were incubated at 37°C for 60 min and nitrocatechol formed was estimated as indicated in the text. Phosphate concentration –○–○–, none; –△–△–, $0{\cdot}5 \times 10^{-4}$ M; and –□–□–, $2{\cdot}5 \times 10^{-4}$ M.

where the mucopolysaccharide contents are very high (SINGH, CHANDRASEKARAN, CHERIAN and BACHHAWAT, 1969) arylsulphatase B accounts for the greater part of the total arylsulphatase activity. On the other hand in birds and sheep where the mucopolysaccharide contents are low and sulphatide concentration is high compared to that in rat and man (CUZNER, DAVISON and GREGSON, 1965) arylsulphatase A is present in higher amount.

The behaviour of chicken brain arylsulphatase A under zinc acetate fractionation and acrylamide gel electrophoresis suggests that it is similar to arylsulphatase B of other species studied as far as the charge on the protein molecule is concerned. The other properties such as K_m value and the inhibitory effect of phosphate and sulphite ions, suggest that this arylsulphatase is similar to sheep brain arylsulphatase A and human brain arylsulphatase A (BALASUBRAMANIAN and BACHHAWAT, 1963*b*). The time–activity relationship of chicken brain arylsulphatase A shows the same abnormality as seen in all the other species studied except that it is less pronounced.

The regional distribution study on monkey brain shows that arylsulphatase A and B activities are present in all regions. It may be noted that activity of arylsulphatase A is always high in white matter which is the richest source of sulphatides (DAVISON and GREGSON, 1962).

The investigations on the activities of arylsulphatase A and B in developing rat brain indicate a relationship between mucopolysaccharides content and activity

of arylsulphatase B. SINGH and BACHHAWAT (1965) have reported that the mucopolysaccharide content of rat brain decreases rapidly and reaches a minimum on the fifth day after birth; further increase in age results in a sharp peak on seventh day followed by a plateau between 11 and 19 days, after which there is a gradual decrease in mucopolysaccharide content. The activity of arylsulphatase B also increases after birth, reaches a peak on fifth day then falls off. As age increases the activity of arylsulphatase B becomes more or less constant after another peak of activity on the 20th day which is the time when mucopolysaccharides start decreasing.

AUSTIN *et al.* (1968) have reported that arylsulphatase A levels tend to parallel the level of incorporation of [^{35}S]sulphate into sulphatides of rat brain and the levels of arylsulphatase A and sulphatide reach their peak in young rat brain during the myelination period. Our results on the activity of arylsulphatase A in developing rat brain show that the activity is high in the 18- to 25-days old rat. Further, HAUSER (1968) has reported that the sulphatide content of rat brain is high in the same age groups. These observations lend additional support to the idea that there is a relationship between arylsulphatase A activity and sulphatide content of brain.

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REFERENCES

ABRAHAM J., CHAKRAPANI B., SINGH M., KOKRADY S. and BACHHAWAT B. K. (1969) *Indian J. med. Res* **57**, 1761.

AUSTIN J. H. (1960) *Neurology* **10**, 470.

AUSTIN J. H., BALASUBRAMANIAN A. S., PATTABIRAMAN T. N., SARASWATHI S., BASU D. K. and BACHHAWAT B. K. (1963) *J. Neurochem.* **10**, 805.

AUSTIN J. H., MCAFEE D., ARMSTONG D., O'ROURKE M. and BACHHAWAT B. K. (1964) *Biochem. J.* **93**, 15C.

AUSTIN J., ARMSTRONG D., FOUCH S., MITCHELL C., STUMPF D., SHEAPER L. and BRINER O. (1968) *Archs Neurol. Chicago* **18**, 225.

BALASUBRAMANIAN A. S. and BACHHAWAT B. K. (1963*a*) *Ind. J. exp. Biol.* **1**, 179.

BALASUBRAMANIAN A. S. and BACHHAWAT B. K. (1963*b*) *J. Neurochem.* **10**, 201.

BAUM H., DODGSON K. S. and SPENCER B. (1959) *Clinica. chim. Acta* **4**, 453.

BAUM H. and DODGSON K. S. (1958) *Biochem. J.* **69**, 573.

BRANTE G. (1957) In *Metabolism of the Nervous System* (Edited by RICHTER D.) p. 112. Pergamon Press, London.

CUZNER M. L., DAVISON A. N. and GREGSON N. A. (1965) *J. Neurochem.*, **12**, 469.

CHRAMBACH A., REISFELD R. A., WYCKOFF M. and ZACCARI J. (1967) *Analyt. Biochem.* **20**, 150.

DAVISON A. N. and GREGSON N. A. (1962) *Biochem. J.* **85**, 558.

GUHA A., NORTHOVER B. J. and BACHHAWAT B. K. (1960) *J. scient. ind. Res.* **19C**, 287.

HAUSER G. (1968) *J. Neurochem.* **15**, 1237.

LOWRY O. H., ROSEBROUGH N. J., FARR A. L. and RANDALL R. J. (1951) *J. biol. Chem.* **193**, 265.

MEHL E. and JATZKEWITZ H. (1968) *Biochim. biophys. Acta* **151**, 619.

ORNSTEIN L. and DAVIS J. B. (1962) *Disc electrophoresis*, Reprinted by Distillation products, Industries Eastman, Kodak Company.

ROY A. B. (1958) *Biochem. J.* **68**, 519.

SINGH M. and BACHHAWAT B. K. (1965) *J. Neurochem.* **12**, 519.

SINGH M., CHANDRASEKARAN E. V., CHERIAN R. and BACHHAWAT B. K. (1969) *J. Neurochem.* **16**, 1157.

YOUNG I. J. and ABOOD L. G. (1960) *J. Neurochem.* **6**, 89.