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ISOLATION AND CHARACTERIZATION OF GLYCOSAMINOGLYCANS IN HUMAN BRAIN OF DIFFERENT AGE GROUPS

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Abstract—Five distinct glycosaminoglycan fractions have been isolated from human brain of various age groups, by employing an improved fractionation procedure. Analysis of these fractions showed that human brain contains hyaluronic acid, chondroitin-4-sulphate, chondroitin-6-sulphate, dermatan sulphate, heparan sulphate and two unidentified low sulphated fractions. The pattern of variation of these compounds with age, indicates that they may be playing an important role in the process of myelination and brain maturation.

STUDIES from several laboratories have established the presence of glycosaminoglycans (GAG) in the tissues of the central nervous system. But two important points still remain to be established: (i) the exact nature of GAG and (ii) the physiological role of these compounds in brain.

There is general agreement on the occurrence of hyaluronic acid and chondroitin sulphates in the brain; but a complete characterization of these compounds has yet to be achieved. SZABO and ROBOZ-EINSTEIN (1962) isolated hyaluronic acid and chondroitin-4-sulphate from bovine brain, while CLAUSEN and HANSEN (1963) demonstrated the presence of hyaluronic acid, dermatan sulphate and chondroitin-6-sulphate in human brain. On the other hand, MEYER, HOFFMAN, GRUMBACH and SAMPSON (1959) reported the presence of dermatan sulphate and heparan sulphate in the brain of a gargoyle patient; and hyaluronic acid and heparan sulphate in the brain of a child used as the normal control in this study. Earlier work from this laboratory on GAG of sheep brain (SINGH and BACHHAWAT, 1965) showed the presence of some minor components of GAG fractions, not fully characterized, along with hyaluronic acid, chondroitin-6-sulphate and chondroitin-4-sulphate. In the present investigation, an attempt has been made to get a profile of uronic acid-containing GAG in human brain, of various age groups, as completely as possible, employing improved techniques of isolation.

Although the postulation of BRANTE (1958), as to the possible role of these compounds in the process of myelination has attracted the attention of many workers in this field, very little is known regarding the functional aspect. Earlier work from this laboratory (GUHA, NORTHOVER and BACHHAWAT, 1960; BALASUBRAMANIAN and BACHHAWAT, 1964; SINGH and BACHHAWAT, 1965) provided evidence of the importance of these compounds in the processes of myelination and brain maturation. Work of SZABO and ROBOZ-EINSTEIN (1962) also indicated that hyaluronic acid may be one of the building blocks of the myelin sheath.

Abbreviations used: GAG, glycosaminoglycans; CPB, cetyl pyridinium bromide.

MATERIALS AND METHODS

Glucosamine, galactosamine, hyaluronic acid from human umbilical cord, bovine testicular hyaluronidase, yeast hexokinase type IV and ATP were obtained from Sigma Chemical Co., U.S.A. Chondroitin sulphate from bovine nasal septa and cetyl pyridinium bromide were purchased from Mann Research Laboratories, U.S.A. Sephadex G-25 and DEAE-Sephadex A-25 were supplied by Pharmacia, Upsala, Sweden. Chondroitin-6-sulphate, dermatan sulphate and heparan sulphate were gifts from Dr. A. DORFMAN of LaRabida University of Chicago-Institute, Chicago, U.S.A. Papain (crude) was a gift from Central Food Technological Research Institute, Mysore, India, whereas Pronase-B was obtained from California Biochemicals Corp., U.S.A. Carbazole (British Drug House) was recrystallized from benzene. Other chemicals used were of analytical grade.

Human brain of various age groups, viz., foetal, neonatal, child (1-year-old) and adult, were autopsy samples made available through the courtesy of the Pathology Department of this Institute. Care has been taken to eliminate specimens having any apparent neurological abnormality. Tissues were cleaned free from meninges and adhering blood vessels as far as possible and used as such or kept frozen at −20° till required.

Acetone drying, lipid extraction and GAG *extraction.* Tissues were acetone-dried by homogenizing them in 10 vol. of acetone in a Servall Omni-Mixer and lipids were extracted from the dried tissues by further homogenization in 19 vol. of chloroform–methanol (2:1) and the residues after filtration were used for the extraction of GAG.

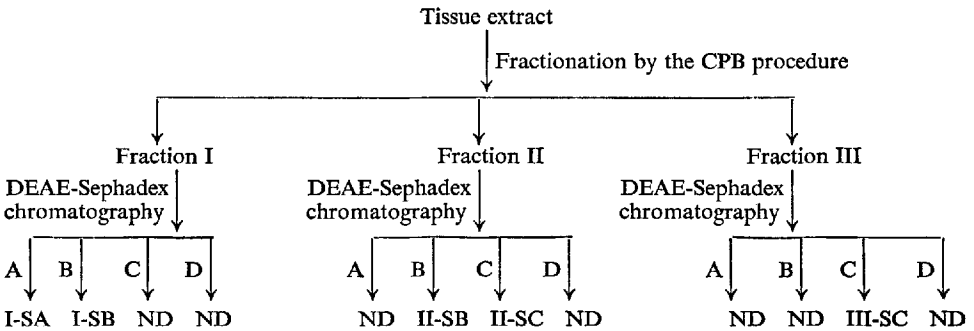
The dried lipid-free material was homogenized in 10 vol. of 0·1 M-acetate buffer, pH 5·0, and heated for 20 min in a boiling water bath. After cooling to room temperature, it was digested with papain (5 mg/g of tissue) at 60° for 24 hr in the presence of 0·005 M-cysteine (freshly prepared) and 0·005 M-EDTA.

The papain digest was dialysed overnight and M-tris–HCl buffer, pH 7·6, was added to make it 0·1 M, along with alcohol (0·001 M) and $CaCl_2$ (0·001 M) and further digested with Pronase-B (5 mg/g of tissue) for 48 hr at 37° under a layer of petroleum ether (60–80).

To the Pronase digest 2 N-NaOH was added to make it 0·1 N and stirred in the cold for 24 hr. It was then neutralized with 30% TCA and deproteinized by further addition of TCA (final concentration 5%), centrifuged and dialysed overnight against running tap water.

GAG were precipitated by CPB and fractionated into three fractions by differential extraction with increasing concentrations of NaCl according to the method of SCHILLER, SLOVER and DORFMAN (1961), and finally precipitated with alcohol (80%).

Further fractionation by DEAE-Sephadex column chromatography. Each fraction obtained by the CPB procedure was subjected to DEAE-Sephadex A-25 column chromatography according to the method of SCHMIDT (1962) using the following eluants: (A) 0·5 M-NaCl; (B) 0·9 M-NaCl; (C) 1·5 M-NaCl in 0·01 M-HCl; and (D) 2·0 M-NaCl in 0·01 M-HCl. The fractionation pattern can be summarized as:



(ND—Not detectable)

In all the cases studied, five distinct fractions were obtained by adopting this procedure.

Paper electrophoresis. Paper electrophoresis of the isolated fractions was carried out with lithium sulphate of 0·1 ionic strength as the electrolyte (FOSTER and PEARCE, 1961) and then stained with 0·5 per cent alcian blue in 3% acetic acid.

Hyaluronidase digestion and estimation of chondroitin-6-sulphate. Various GAG fractions isolated from human brain of different age groups were separately digested with testicular hyaluronidase in 0·1 M-acetate buffer, pH 5·0, for 16 hr at 37° and the amounts of hyaluronidase-resistant material as well as chondroitin-6-sulphate in these fractions were determined by gel filtration in Sephadex G-25

columns by the method of SCHMIDT and DMOCHOWSKI (1964) and by acylhexosamine assay procedure of MATHEW and INOUYE (1961) respectively, as has been described earlier (SINGH and BACHHAWAT, 1965).

Constituent analysis. Uronic acid was determined by Dische's carbazole method (DISCHE, 1947) as modified by BITTER and MUIR (1962) using glucuronolactone as the standard. For the identification of dermatan sulphate, the carbazole reaction was carried out, with and without borate, since iduronic acid gives a very low colour yield without borate in the reaction mixture.

For the analysis of hexosamines, the various GAG fractions were hydrolysed with 6 N-HCl at 100° in sealed tubes for 8 hr and the hydrolysates dried over fused $CaCl_2$ and NaOH pellets in a vacuum desiccator. The hexosamines were assayed by the method of GATT and BERMAN (1966) employing glucosamine and galactosamine as the respective standards and the total hexosamine was expressed as the sum of glucosamine and galactosamine after estimating them separately.

For the separation of glucosamine and galactosamine, a portion of the aminosugar was phosphorylated with yeast hexokinase and glucosamine-6-phosphate was separated from galactosamine by Dowex-1-acetate column chromatography. Galactosamine was not held up in this column and as such appeared in the breakthrough and the aqueous washings while glucosamine-6-phosphate was eluted with 0·025 M-acetic acid.

The hexosamines were further identified by descending paper chromatography according to the method of MUKHERJI and SRIRAM (1964). After drying the paper, the reducing sugar spots were identified by staining with alkaline silver nitrate (TREVELYAN, PROCTOR and HARRISON, 1950).

The total sulphate content of the isolated GAG fractions was determined by $BaCl_2$–gelatin method of DODGSON and PRICE (1962).

N-sulphate residues (or to be more precise, non-acylated hexosamines) in the isolated GAG fractions were determined by the method of LAGUNOFF and WARREN (1962) using glucosamine as the standard.

RESULTS

Variation of GAG *fractions with age in human brain.* The levels of three GAG fractions (obtained by the CPB procedure) in human brain of four different age groups, are presented in Table 1. GAG are present at the foetal stage and at birth,

TABLE 1.—LEVELS OF VARIOUS GAG FRACTIONS IN HUMAN BRAIN OF VARIOUS AGE GROUPS

Age group	mg uronic acid/g dry defatted tissue				
	Fraction I	Fraction II	Fraction III	Total	I/II
Foetal	0·782	0·495	0·026	1·303	1·58
Neonatal	1·66	0·82	0·081	2·561	2·05
Child (1-yr-old)	1·27	1·31	0·04	2·62	0·89
Adult	0·612	0·798	0·075	1·485	0·78

A large batch isolation was carried out using the dried lipid-free material. Extraction of GAG was done by digestion with papain, pronase and alkali. Fractionation was performed by the CPB method.

the amount is quite high. It slightly increases at the age of 1 year and returns almost to the foetal level at the adult stage. During the pre-myelination stage (foetal and neonatal) the values of Fraction I are much higher than those of Fraction II. But as the brain develops, the relative proportion of these two fractions changes. During the peak period of myelination (1-year-old) the ratio of I/II is 0·89 and at the adult stage it is 0·78. These findings agree well with our earlier findings in the developing rat brain (SINGH and BACHHAWAT, 1965).

The results of further subfractionation of the CPB fractions by DEAE-Sephadex column chromatography show (Table 2) that I-SA is highest in the neonatal, I-SB and II-SB, in the child and II-SC in the adult. In all the cases, I-SA is higher than I-SB and the ratio of I-SA/I-SB is lowest in the foetal (1·25) and highest in the neonatal

TABLE 2.—FURTHER FRACTIONATION BY DEAE-SEPHADEX OF GAG FRACTIONS OF HUMAN BRAIN OF VARIOUS AGE GROUPS

Age group	mg uronic acid/g dry defatted tissue				I-SA / I-SB	II-SB / II-SC
	I-SA	I-SB	II-SB	II-SC		
Foetal	0·488	0·294	Not done	Not done	1·25	—
Neonatal	1·495	0·165	0·205	0·615	9·00	0·33
Child	0·94	0·33	0·226	1·084	2·86	0·21
Adult	0·528	0·084	0·296	0·502	6·25	0·59

The subfractionation of the GAG fractions (obtained by the CPB procedure) was carried out on DEAE-Sephadex columns by stepwise elution as described in the text.

(9·09), age group. In general II-SC is higher than II-SB and the ratio of II-SB/II-SC is highest in the adult.

Hyaluronidase digestion of various GAG *fractions of human brain of different age groups.* Table 3 presents the results of testicular hyaluronidase digestion and chondroitin-6-sulphate estimation of some of the GAG fractions. II-SB contains considerable amounts of hyaluronidase-resistant material (30–40 per cent) while II-SC is almost completely digested. In all the cases I-SB was found to contain some undigestible material (7–18 per cent).

TABLE 3.—TESTICULAR HYALURONIDASE DIGESTION OF THE VARIOUS GAG FRACTIONS OBTAINED FROM HUMAN BRAIN OF DIFFERENT AGE GROUPS

Age group and fractions	% Hyaluronidase undigestible	% Chondroitin-6-sulphate*	% Chondroitin-4-sulphate (by difference)
Foetal			
I-SB	14·6	61·2	24·2
Neonatal			
I-SB	16·4	44·7	38·9
II-SB	40·0	32·7	27·3
II-SC	1·1	34·0	64·9
Child			
I-SB	11·5	Not done	—
II-SB	34·8	53·5	11·7
II-SC	4·8	16·0	79·2
Adult			
I-SB	7·7	80·9	11·4
II-SB	30·0	44·3	25·7
II-SC	1·3	13·2	85·5

* The estimation was based on the hyaluronidase method of MATHEW and INOUYE (1961) as described in the text.

Constituent analysis of the GAG *fractions.* Uronic acid analysis of the various GAG fractions by the carbazole method showed significant lowering in the colour yield in the absence of borate in the case of III-SC indicating the presence of dermatan sulphate in this fraction only.

The analytical data of the various GAG fractions of neonatal and adult brain are provided in Table 4. The hexosamine analysis shows that I-SA of the adult contains only glucosamine whereas in the neonate, there is a small amount of galactosamine also along with glucosamine. The ratios of galactosamine/glucosamine in I-SB and II-SB of the neonatal brain is higher than those of the corresponding fractions of the

TABLE 4.—ANALYTICAL DATA OF THE VARIOUS GAG FRACTIONS OF NEONATAL AND ADULT HUMAN BRAIN

Age group and Fraction	Hexosamines*	Galactosamine/ Glucosamine†	Sulphate*	*N*-Sulphate*
Neonatal				
I-SA	0·91	0·14	Traces	Not done
I-SB	0·86	0·61	0·42	Not done
II-SB	0·89	1·3	0·56	Not done
II-SC	0·93	Galactosamine only	0·98	Not done
Adult				
I-SA	0·98	Glucosamine only	Not detectable	0·01
I-SB	0·89	0·26	0·39	0·33
II-SB	0·95	0·9	0·72	0·17
II-SC	0·93	24·3	0·92	Traces
III-SC	1·07	Galactosamine only	0·96	Not done

* Expressed as molar ratio of uronic acid.

† Separation of glucosamine and galactosamine was carried out by the hexokinase method as described in the text.

adult. Neonatal II-SC contains only galactosamine, whereas the adult II-SC contains a small amount of glucosamine also. III-SC of the adult contains only galactosamine. The presence of more galactosamine in the various GAG fractions of the neonatal brain than in the corresponding fractions of the adult brain is of considerable interest. Total sulphate was estimated for the GAG fractions of foetal and child brain also. In general, I-SA contains only traces of sulphate and no age-dependent change in sulphate content could be observed.

Further identification of hexosamines. Further identification of the hexosamines has been carried out by paper chromatography. The results of these studies for the hexosamines of the adult brain GAG fractions showed the presence of some slow-moving faint spots in the case of II-SB and II-SC, which may be due to slight incomplete hydrolysis. In general, under the conditions adopted for the present study, the hydrolysis of GAG for the release of hexosamines is almost complete for most of the GAG fractions and the results of chromatographic analysis agree with those obtained by the hexokinase reaction, followed by Dowex-1-acetate chromatography. Paper chromatographic studies of the hexosamines of the neonatal GAG fractions show that I-SA and II-SC contain only glucosamine and galactosamine respectively, whereas both the amino sugars are present in I-SB and II-SB. Although the presence of galactosamine has been observed in I-SA of the neonatal brain (Table 4), it could not be detected in the chromatogram, which may be due to this aminosugar being only a minor component.

The results of *N*-sulphate analysis carried out for the various GAG fractions of the adult human brain show that I-SB and II-SB definitely contain *N*-sulphate residues, whereas in II-SC, it is almost negligible. However, I-SA, which does not have any sulphate at all, shows positive indole-HCl reaction. Presence of certain compounds, like protein and peptides, are known to interfere with this method (LAGUNOFF and WARREN, 1962). The low value (0·01) of *N*-sulphate for I-SA, may be due to this reason.

Paper electrophoresis of the isolated GAG *fractions.* Paper electrophoresis shows single spots for the various GAG fractions of the neonatal brain (Fig. 1). I-SA has the same mobility as the standard hyaluronic acid and II-SC, as chondroitin sulphates, while I-SB and II-SB have identical mobility which is slightly lower than that of chondroitin sulphate but higher than that of hyaluronic acid

In the electrophoregram of the various GAG fractions of the adult human brain (Fig. 2) I-SA shows a single spot having the same mobility as hyaluronic acid; I-SB also has a single spot with similar mobility as heparan sulphate. II-SC and III-SC give single spots with mobilities identical to that of chondroitin sulphates. But II-SB resolves into two spots, the mobilities of which are nearer to that of heparan sulphate than those of other standards. The slower moving spot of II-SB (Fig. 3) disappears on digestion with testicular hyaluronidase.

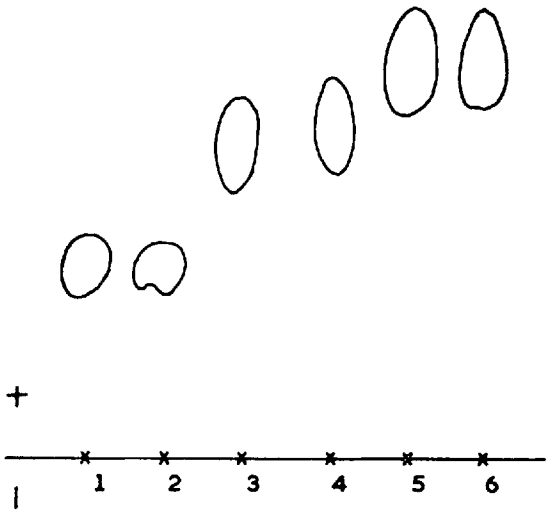


FIG. 1.—Electrophoresis of the various GAG fractions of neonatal human brain. 1. Standard hyaluronic acid; 2. I-SA; 3. I-SB; 4. II-SB; 5. II-SC and 6. Standard chondroitin sulphates.

DISCUSSION

In course of our studies on the isolation and characterization of GAG in the nervous tissues it was observed that the three different fractions obtained by the CPB procedure (SCHILLER *et al.*, 1961) are not composed of single GAG. Further resolution of these fractions has been achieved by DEAE-Sephadex column chromatography (SCHMIDT, 1962). In the present investigation five different GAG fractions have been isolated from human brain. Rechromatography on DEAE-Sephadex columns and re-fractionation by the CPB procedure of these fractions gave rise to the same fractions, indicating that these are not artifacts of the isolation procedure. It is possible that some of these fractions are a mixture of GAG which cannot be further resolved into the individual components by the isolation procedure employed. Although it has not been possible to make a definite identification of each fraction, the analysis of these fractions suggests that they are different from one another.

The results of chromatographic studies, paper electrophoresis and constituent analysis indicate I-SA to be a fairly pure hyaluronic acid fraction. However, the

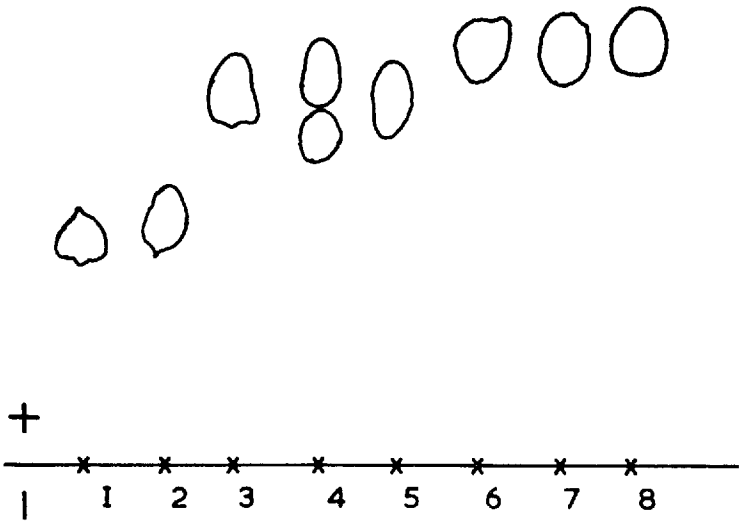


FIG. 2.—Electrophoresis of the various GAG fractions of adult human brain. 1. I-SA; 2. Standard hyaluronic acid; 3. I-SB; 4. II-SB; 5. Standard heparan sulphate; 6. II-SC; 7. III-SC and 8. Standard chondroitin sulphates.

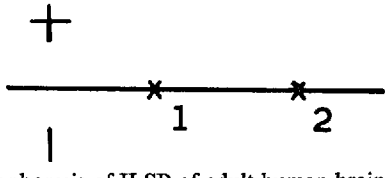


FIG. 3.—Electrophoresis of II-SB of adult human brain before and after hyaluronidase digestion. 1. Before digestion; 2. After digestion.

occurrence of a small amount of galactosamine and the absence of sulphate in this fraction of neonatal brain indicate the presence of chondroitin in this tissue.

Since I-SB has got certain unusual features, as indicated by the results of the present study, no definite conclusion could be made regarding the nature of this fraction. Generally, it is found to contain more glucosamine than galactosamine and its sulphate content ranges from 30–50 per cent of the uronic acid and there is no indication for the presence of iduronic acid. Non-acetylated hexosamine determination by the indole-HCl method (LAGUNOFF and WARREN, 1962) indicates the presence of this sugar to the extent of 33 per cent of uronic acid in this fraction of adult brain. If this value represents *N*-sulphate residues only, it will account for 83 per cent of the total sulphate in this fraction. All these evidences suggest the similarity of this fraction to heparan sulphate, (with a low degree of sulphation). However, on the basis of the available information regarding the substrate specificity of testicular hyaluronidase, the digestion of this fraction to the extent of 83–93 per cent, is not consistent with its identification as heparan sulphate. On the other hand, the digestibility by hyaluronidase and the high glucosamine content (about 80 per cent of the total hexosamine in the case of adult brain) as well as its extraction by 0·4 M-NaCl from the GAG-CPB complex, suggest that a part of the glucosamine might be contributed by hyaluronic acid. But paper electrophoresis shows the presence of a single spot having higher mobility than hyaluronic acid. Moreover, preliminary *in vitro* sulphation studies with 6-day-old rat brain sulphotransferase (BALASUBRAMANIAN and BACHHAWAT, 1964) using this fraction as the acceptor showed that this fraction is a good acceptor of sulphate; and substances behaving like II-SB and II-SC, on CPB fractionation and DEAE-Sephadex chromatography, could be isolated as products of sulphation of I-SB. In the light of all this evidence, further structural investigation will be needed for a conclusive identification of this fraction.

The identification of II-SB also poses similar problems. II-SB resembles I-SB in many respects but for the following differences: (i) a higher concentration of NaCl (i.e. 1·2 M) is needed to extract it from the GAG-CPB complex, (ii) it contains more galactosamine than I-SB, (iii) its degree of sulphation is higher than that of I-SB, (iv) it contains more hyaluronidase resistant material. In the case of adult human brain, II-SB contains 30 per cent hyaluronidase-resistant material. Although this cannot account for the total amount of glucosamine present in this fraction (53 per cent of the total hexosamine), all the other evidences suggest that the undigestible material may be heparan sulphate. As in the case of I-SB, the characterization of the glucosamine containing, hyaluronidase sensitive material in this fraction needs to be further investigated before a definite conclusion is arrived at. However, the presence of chondroitin sulphates with low degree of sulphation can be inferred from the presence of galactosamine (47 per cent of the total hexosamine in the case of the adult) and the absence of iduronic acid. This observation has been further substantiated by the isolation of radioactive II-SC (which is identified as chondroitin sulphates) as a product of II-SB sulphation in a preliminary *in vitro* sulphation study using [^{35}S]PAPS and a sulphotransferase preparation obtained from 6-day-old rat brain (BALASUBRAMANIAN and BACHHAWAT, 1964).

All the evidence suggests that II-SC is primarily composed of chondroitin-6-sulphate and chondroitin-4-sulphate along with a negligible amount of hyaluronidase undigestible material.

In the earlier communication (SINGH and BACHHAWAT, 1965), Fraction III of sheep brain was tentatively identified as heparin, based on its behaviour in the CPB fractionation procedure and its anticoagulant activity of blood. But the limitation of such an identification without analysing the constituents was pointed out in the same communication. Further purification of this fraction and analysis of the constituents, viz. uronic acid, hexosamines and sulphate, show that this fraction is composed of dermatan sulphate only. However, it is not clear why dermatan sulphate is not extracted by 1·2 M-NaCl along with the chondroitin sulphates from the GAG-CPB complex.

It is clear from the results obtained in this study that hyaluronic acid, chondroitin-6-sulphate, chondroitin-4-sulphate, dermatan sulphate and heparan sulphate are present in human brain, along with some low sulphated fractions, the nature of which is yet to be fully ascertained. The present findings which do not completely agree with any of the earlier reports (SZABO and ROBOZ-EINSTEIN, 1962; CLAUSEN and HANSEN, 1963; MEYER *et al.*, 1959; MARGOLIS, 1967) indicate that a complete profile of GAG of brain has not been obtained by these workers.

The relative amounts and types of GAG present in adult human brain from this study are:

1. Hyaluronic acid comprises about 35 per cent of the total GAG in this tissue.
2. 34 per cent of the II-SC represents the next higher fraction in this tissue, 85 per cent of which is chondroitin-4-sulphate whereas only 13 per cent is chondroitin-6-sulphate.
3. Dermatan sulphate accounts for about 5 per cent of the total GAG.
4. 20 per cent of the total GAG is represented by the low sulphated fraction, II-SB, 30 per cent of which has been identified as heparan sulphate (probably with a lower degree of sulphatation) and another 47 per cent, as low sulphated chondroitin sulphate, the rest being the unidentified glucosamine containing, hyaluronidase sensitive sulphated compound.
5. About 6 per cent of the total GAG is accounted for by the low sulphated fraction, I-SB, the exact nature of which still remains to be established.
6. About 10 per cent of the total GAG remains to be characterized.

The present findings of higher level of hyaluronic acid than those of chondroitin sulphate in the premyelination stages and the changes in the relative proportions of these compounds with the development and maturation of brain, are in good agreement with the earlier findings in the developing rat brain (SINGH and BACHHAWAT, 1965). This further substantiates the earlier evidence from this laboratory (GUHA *et al.*, 1960; BALASUBRAMANIAN and BACHHAWAT, 1964) supporting the postulation of BRANTE (1958) about the possible role of GAG in the process of myelination. Although the recent findings of MARGOLIS (1967) indicate that there is no GAG in the myelin sheath itself, the absence of GAG in the myelin itself does not completely rule out a possible role of GAG in the process of myelination and brain maturation.

The results of chondroitin-6-sulphate estimation by the hyaluronidase method of MATHEW and INOUYE (1961) are difficult to interpret in the case of the unidentified fractions, viz. I-SB and II-SB, as this method is based on slight structural differences between chondroitin-6-sulphate and chondroitin-4-sulphate. It also requires the absence of hyaluronidase sensitive GAG, like hyaluronic acid, which may give rise to

oligosaccharides containing terminal *N*-acetyl hexosamines responding to the Morgan–Elson reaction. The finding of 80·9 per cent of chondroitin-6-sulphate in the adult brain I-SB fraction which has only a small amount of galactosamine (20·6 per cent of the total hexosamine) indicates an inherent limitation of this method, when applied to GAG which is not properly characterized. In view of these considerations, the quantitative values of chondroitin-6-sulphate present in I-SB and II-SB might be lower than indicated, and as such these results need to be interpreted with some reservation. However, in general the values of chondroitin-6-sulphate in II-SC will be representative of the true composition of this fraction.

There is a significant change in the relative amounts of chondroitin-6-sulphate and chondroitin-4-sulphate (in II-SC only) with age. In the neonatal period, chondroitin-6-sulphate represents 34 per cent of II-SC, but in the child and the adult this percentage decreases to 16, and 13, respectively, with a concomitant rise in the percentage of chondroitin-4-sulphate. This finding is of great interest although its implication cannot be explained with the available data.

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