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Urinary glycosaminoglycan excretion in normally grown and growth retarded neonates

II. Quantitative distribution of glycosaminoglycans

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The urinary GAG excretion pattern was estimated in normally grown, preterm, and growth retarded infants on the third, tenth, twentieth and in some cases on the thirtieth postnatal days. The cetylpirydinium-chloride precipitable GAGs were fractionated on cellulose-Celite column under standardized conditions.

In this period of extrauterine life a dominancy of nonsulphated GAGs was found in every group of infants, in contrast with children and adults where chondroitin sulphate was excreted in excess. On the third postnatal day the excretion rates of chondroitin sulphates, hyaluronic acid and chondroitin—heparan sulphate fractions were closely related to gestational age, birth weight and body length.

Postnatal growth activity seems to be an important influencing factor on GAG excretion. The 10 and 20-day-old infants with weight increase excreted significantly more hyaluronic acid and characteristically more chondroitin—heparan sulphate and chondroitin sulphate than those whose body weight did not change or decreased as compared with their birth weight.

While there are many observations concerning the excretion pattern of urinary glycosaminoglycans (GAG) in adults and children [1—3, 5, 6, 12, 13—16, 18, 19, 21], no data are available concerning the different components of GAG in the urine of neonates.

In a previous study we have found that in early extrauterine life total GAG excretion shows a great variation, which is affected by intrauterine or extrauterine growth activity [10]. For evaluation of the excretion rate and quantitative distribution of urinary GAG in pathological conditions, normal values are needed. The knowledge of the physiological changes in urinary GAG excretion of neonates would be essential for screening of inborn errors and for studying metabolic disturbances, particularly those affecting the extracellular matrix. Such investigations appeared the more interesting, since it has been reported that the total GAG and collagen content as well as the pattern of GAG fractions in embryonic or young skin, bone, cartilage and aorta differs markedly from that of the adults [4, 7, 9, 17]. ORII [14, 15] found a characteristic difference in non-sulphated

urinary GAG excretion pattern between young children and adults.

In our previous studies we observed a close correlation between intrauterine and extrauterine growth rate and the total amount of GAG and hydroxyproline excreted by the kidney [10, 11]. In order to determine the quantitative characteristics of urinary GAG excretion in the first month of extrauterine life, we studied the excretion pattern of the different GAG fractions in full-term, small-for-dates and premature newborn infants. The results are reported in the present paper.

Materials and Methods

Twentyfour hour urine samples were collected from 51 newborn infants on the 3rd, 10th, 20th and in some cases on the 30th day after birth. The infants were divided into three groups: (i) Normally grown, near term infants; (ii) Intrauterine growth retarded infants; (iii) Preterm infants. The means and ranges of gestational age and body weight of the groups were tabulated in a previous paper [11].

In this examination series, uronic acid was determined by carbazole, and galactose by the orcinol reaction. Otherwise, the isolation and fractionation of GAG as well as statistical analysis were the same as described previously [10]. Each GAG fraction was expressed in units of μmol uronic acid or galactose per 24 hours.

Results

Individual GAG compounds in the urine of neonates

Mean $\pm$ SE urinary GAG obtained in the three groups of infants on the 3rd, 10th and 20th days are shown in Fig. 1. From the results obtained the following conclusions have been drawn.

a) Dermatan sulphate excretion at this early age was very low, in every case less than 1% of total urinary GAG excretion.

b) The proportion of non-sulphated or under-sulphated fractions of urinary GAG compounds (hyaluronic acid, chondroitin) was greater than that of the chondroitin sulphates, which constitute the dominating fractions of GAG in the urine of children and adults.

c) Chondroitin 4-sulphate always exceeded the level of chondroitin 6-sulphate in the urine of infants.

d) In every group of newborns, the chondroitin sulphates showed the smallest fluctuation among all the fractions, as shown by the lowest standard errors.

e) Although the total amount of GAG was different, the relative distribution of the components was similar in the three groups of newborn infants. This distribution pattern is characterized by identical proportions of hyaluronic acid–chondroitin–heparan sulphate (non sulphated GAGs) and chondroitin sulphates.

Quantitative distribution of GAG components in relation to maturity and postnatal age

As it is shown in Fig. 2, in each group of infants the hyaluronic acid excretion rate was high. The maximum appeared between the 3rd and 10th day in the normally grown in-

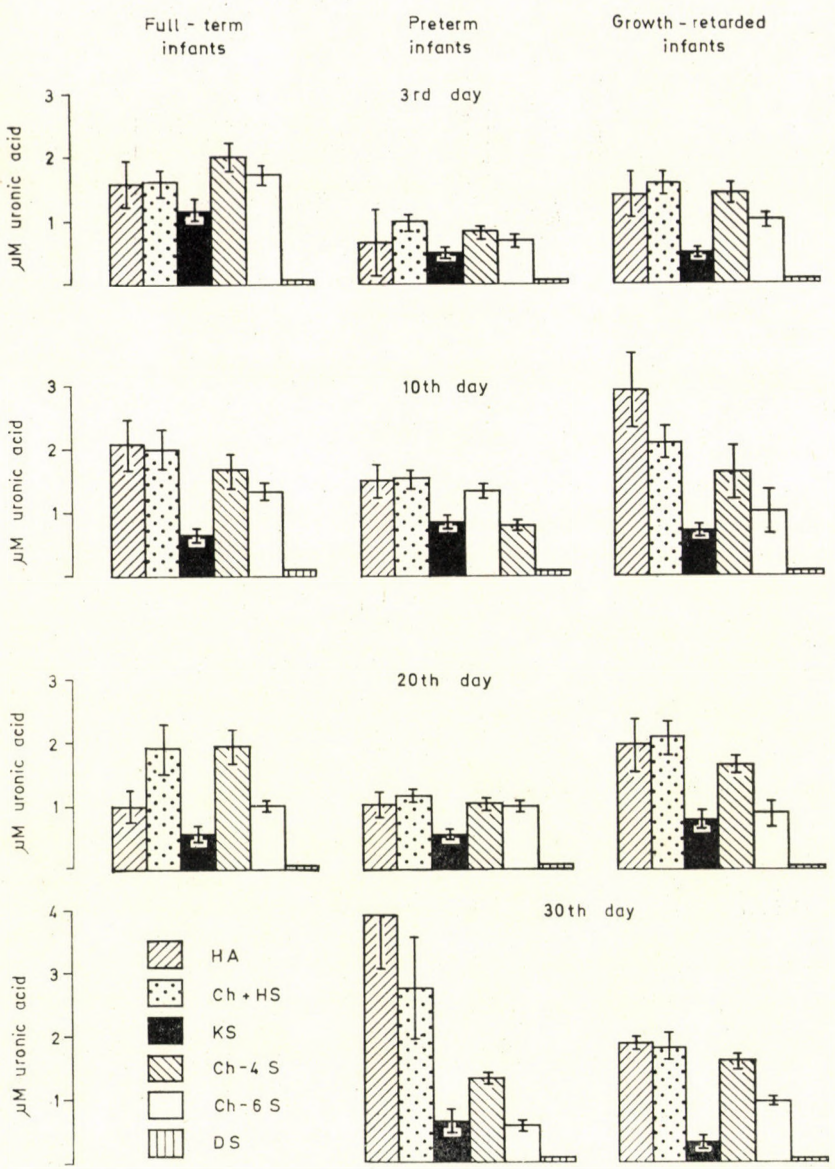


FIG. 1. Urinary excretion of individual GAG compounds in preterm, full-term and growth-retarded neonates on the 3rd, 10th and 20th postnatal days

fants, on the 10th day in the intrauterine growth retarded and on the 30th postnatal day in the preterm infants. Hyaluronic acid excretion was significantly lower in the three-day-old babies ($p < 0.01$) than in the growth retarded or in the normal newborns.

The chondroitin–heparan sulphate fraction behaved like hyaluronic acid

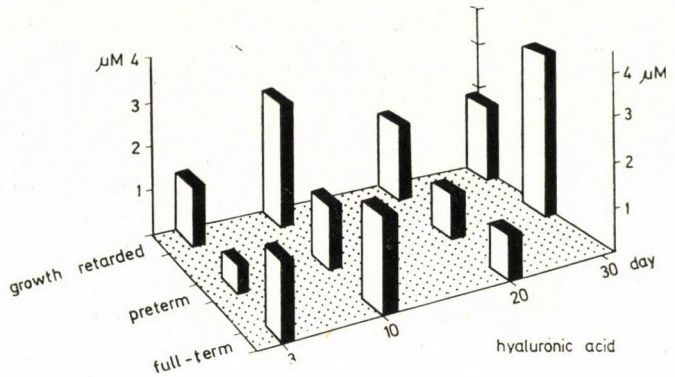


FIG. 2. Urinary hyaluronic acid excretion in preterm, full-term and growth retarded neonates

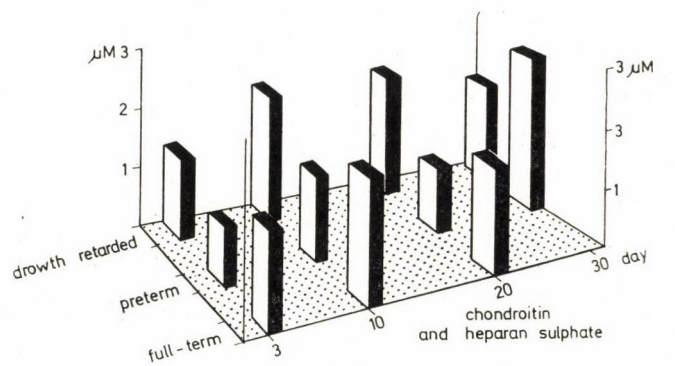


FIG. 3. Urinary chondroitin and heparan sulphate excretion in preterm, full-term and growth retarded neonates

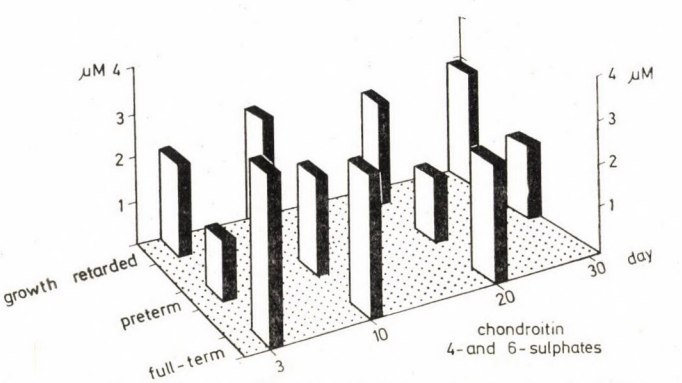


FIG. 4. Urinary excretion of chondroitin sulphates in preterm, full-term and growth retarded neonates

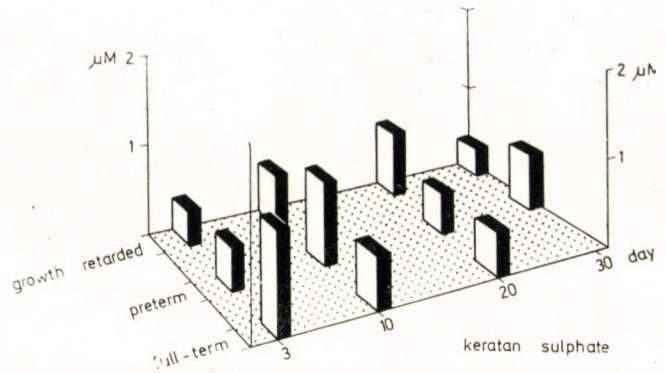


FIG. 5. Urinary keratan sulphate excretion in preterm, full-term and growth retarded neonates

(Fig. 3). Although the quantitative differences observed on the 3rd, 10th and 20th day were not significant between the single groups, a considerable increase in renal excretion rate occurred on the 20th day.

Comparing the trends of the quantitative changes in urinary chondroitin sulphates in the three groups of neonates (Fig. 4), in the dysmature infants the excretion rate did not change during the period of observation, in the term infants a marked fall was observed with postnatal age. In premature infants the maximum amount of chondroitin sulphates was excreted on the 10th day, but this was much less than in the other two groups of newborns of corresponding postnatal age.

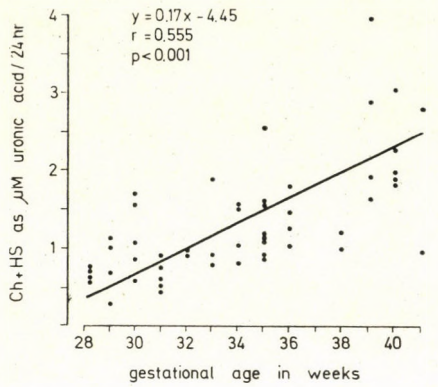
Fig. 5 demonstrates the excretion pattern of keratan sulphate. Its excretion was relatively high in the 3-day old normal infants, in the 10-day old preterm and 20-day old growth retarded infants.

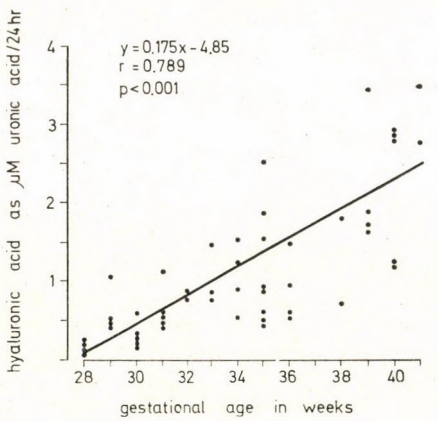
Gestational age, birth weight, body length and their correlation to the GAG compounds excreted on the third postnatal day

Regression analysis showed a statistically significant correlation between gestational age and the excretion of chondroitin–heparin sulphate, hyaluronic acid and chondroitin sulphates (Fig. 6). This relationship was the closest in the case of chondroitin sulphates and hyaluronic acid. No correlation was found for keratan sulphate and dermatan sulphate.

When the logarithm of gestational age in weeks was used for analysis, the correlation proved to be closer, as shown in Fig. 7.

Relating the excretion rates of the different GAG fractions to birth length, it was found that only chondroitin sulphate showed a significant correlation (Fig. 8). From Fig. 9, where values for individual GAG





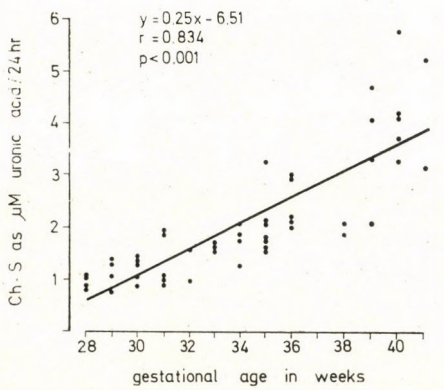


FIG. 6. Different urinary GAG compounds in neonates, in function of gestational age on the 3rd day of life

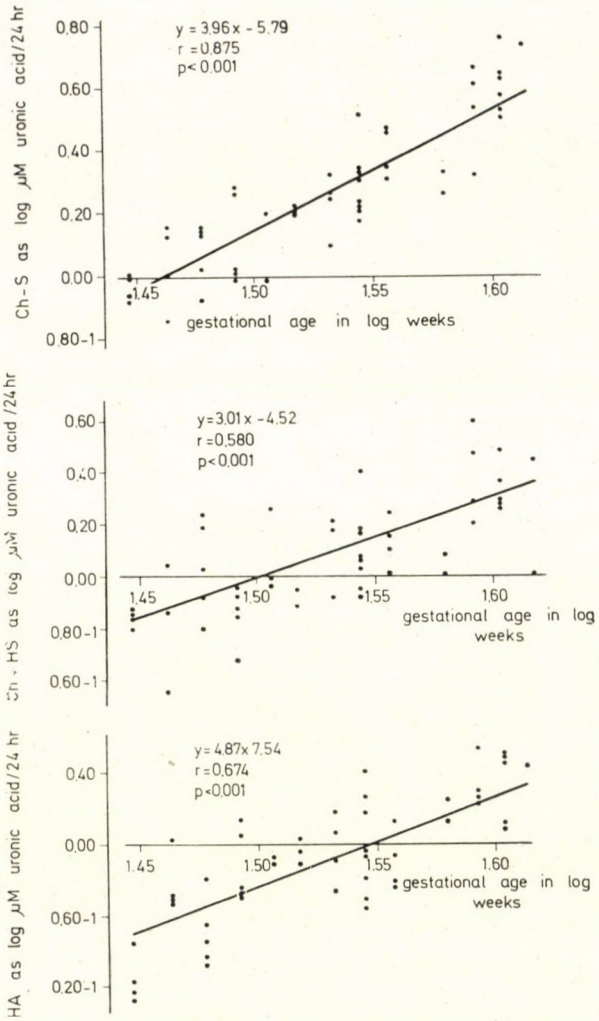


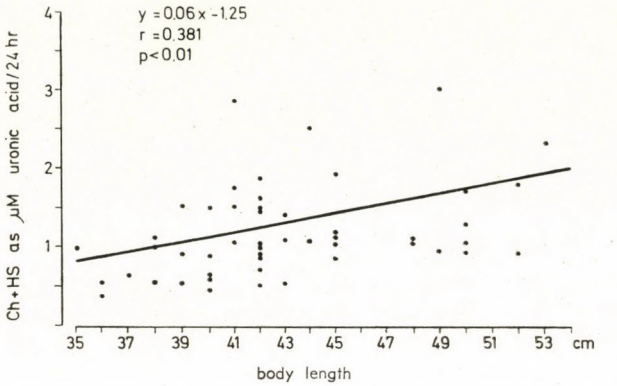
FIG. 7. Different urinary GAG compounds in neonates in function of logarithm gestational age on the 3rd day of life

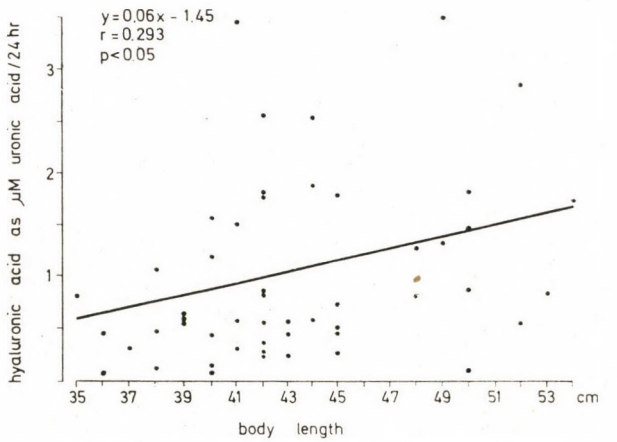
fractions are plotted against birth weight, it can be seen that chondroitin sulphate and chondroitin–heparin sulphate excretion was significantly related to body weight.

Extrauterine growth and the excreted GAG compounds

To study the effect of postnatal growth activity on the excretion rate of GAG fractions, the 10 and 20-day old infants were divided into two groups: 1. Growing group including infants who already showed definite weight gain; 2. Non-growing group comprising infants whose body weight did not change, or even fell during the observation period.

As Table I shows, considerable differences were found between the two





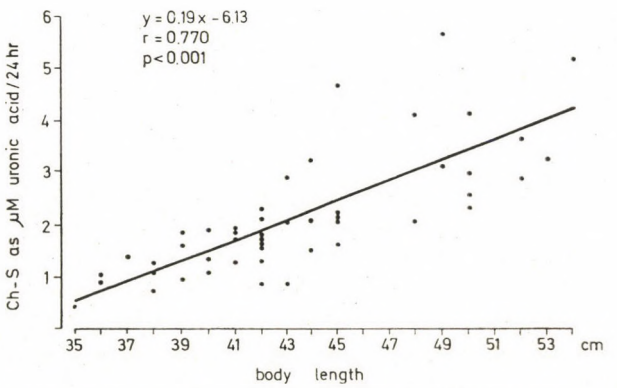


FIG. 8. Different urinary GAG compounds in neonates in function of body length on 3rd day of life

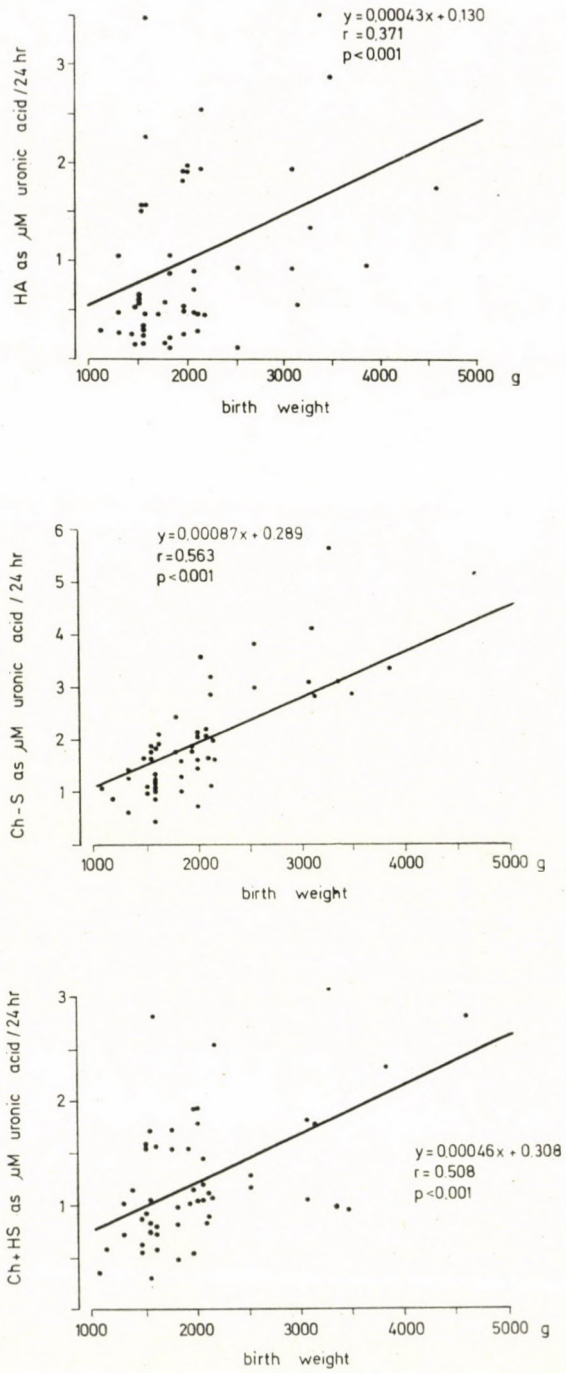


FIG. 9. Different urinary GAG compounds in neonates in function of birth weight on the 3rd day of life

TABLE I

Effect of postnatal growth activity on urinary excretion of different GAG compounds

Postnatal age		10 days		20 days	
Groups of infants		Growing	Not growing	Growing	Not growing
Hyaluronic acid	μM uronic acid/24 hours ± SE	3.03 ± 0.35	0.47 ± 0.08	1.89 ± 0.21	0.37 ± 0.07
Chondroitin-heparan sulphate		2.05 ± 0.19	1.11 ± 0.13	1.86 ± 0.15	0.92 ± 0.10
Keratan sulphate		0.70 ± 0.03	0.44 ± 0.08	0.70 ± 0.10	0.42 ± 0.08
Chondroitin 4-sulphate		1.50 ± 0.11	1.13 ± 0.18	1.41 ± 0.14	1.02 ± 0.08
Chondroitin 6-sulphate		1.06 ± 0.10	0.55 ± 0.11	0.78 ± 0.18	0.58 ± 0.09
Dermatan sulphate		0.11 ± 0.06	0.03 ± 0.004	0.06 ± 0.009	0.04 ± 0.014
n		27	16	19	13

groups. The growing group on the 10th and 20th postnatal days excreted more of every GAG compound than the non-growing group did. The difference was significant statistically in the case of hyaluronic acid ($p = 0.01$). Within both groups, no significant difference was, however, observed between the excretion rates on the 10th and 20th postnatal days.

DISCUSSION

The normal urinary excretion rates of individual GAG compounds reported by different authors showed a great variation. The following reasons may account for these differences. *1.* The different procedures used by the authors for isolation and fractionation appears to be a major factor in causing such differences. *2.* The GAGs in urine are not as homogeneous as those in the tissues, as far as the molecular weight and the degree of sulphatation are concerned [16, 20]. *3.* The composition and concentration of urine might also affect the distribution of GAG fractions.

It is certain that all kinds of GAG molecules present in the extracellular matrix are excreted with urine, and in this respect the chondroitin sulphates are the most prominent ones [5, 12, 19, 21].

Most authors used pooled urine samples from different subjects of similar age, and analyzed the isolated samples by various chemical or enzymatic methods, thus creating an adequate basis for comparative examinations [14, 19, 18].

Contrary to most authors who used pooled urine samples from different subjects of similar age for analysis, we performed individual and serial examinations on infants in the first month of extrauterine life. This kind of analysis may offer more information about the individual variation of urinary GAGs and allows a more reliable comparison between normal and abnormal conditions. The method, however, involves some risk of ana-

lytical inaccuracy. To reduce this source of error, we first of all standardized the procedure of isolation. The urine was diluted to an identical creatinine concentration, and during the precipitation process particular attention was paid to avoid excess cetylpyridinium chloride. The application of Celite ensured uniform precipitation and sedimentation, and time and temperature of precipitation were always identical. With this standardized isolation procedure we could achieve a complete recovery of GAG molecules over a certain chain length (CPC precipitable GAG).

As regards the fractionating technique, we made great efforts to create and maintain standard conditions, e.g. constant flow rates and column temperature. In this way identical fractions could be produced. Comparison of the fractions by chemical analysis and ion-exchange chromatography showed that they were constantly as expected, yet not absolutely free of impurities. The hyaluronic acid and chondroitin–heparan sulphate fractions contained some chondroitin sulphate fragments, but the degree of impurity was always below 10%, not higher than what can be expected from a separation technique of this kind.

Our knowledge concerning the mechanism of human GAG turnover is still insufficient. We do not know the way and mode of excretion and the origin of these metabolic end-products in the urine. As our investigations showed, the quantity of excreted GAGs was related to the growth activity of newborns. This would mean that the changes of urinary GAGs reflect changes of tissue GAG metabolism. In addition to the relatively high excretion rate of GAGs, their urinary pattern at the beginning of the process (the first month of life) is also characterized by the dominance of hyaluronic acid and chondroitin–heparan sulphate. The excretion of dermatan sulphate remains, however, unchanged in this period of extrauterine life. The close correlation observed between the urinary chondroitin sulphate fraction and the intrauterine and extrauterine growth rate indicates that growth activity affects the metabolism of the skeleton's most characteristic GAG compounds.

The high urinary excretion rate of non-sulphated GAGs, particularly that of hyaluronic acid, the major compound of embryonal extracellular matrix, might be related to the immaturity of connective tissue. Although the limited renal function at this age may affect the excretion of different substances, it does not seem to be a causative factor in the high urinary GAG excretion.

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