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Urinary Glycosaminoglycan Excretion in Normally Grown and Growth Retarded Neonates

I. Total Glycosaminoglycan Excretion

By

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Urinary glycosaminoglycan (GAG) excretion was estimated in normally grown near term, preterm and growth retarded infants on the third, tenth, twentieth and in some cases on the thirtieth postnatal day. Excretion was determined from 24-hour urine samples and was expressed as μM uronic acid/24 hours, μM uronic acid/1.73 sq.m body surface/24 hours; the creatinine ratio was also calculated.

On the third postnatal day, GAG excretion showed a good correlation with intrauterine growth rate. Urinary uronic acid excretion on the third day of life was also closely related to gestational age, birth weight and body length. GAG excretion reaches its peak on the 10th day in term and especially in dysmature infant, and on the 30th day in premature infants.

Postnatal growth also seemed to influence GAG turnover. The 10- and 20-day-old infants gaining in weight excreted twice as much GAG as those whose body weight did not change, or even fell as compared to birth weight.

Since knowledge of the normal excretion rate of glycosaminoglycans (GAG) is a precondition in screening newborns for mucopolysaccharidoses it seemed desirable to determine the normal variation of the daily urinary excretion of GAG in the early postnatal period. In addition, it appeared interesting to collect data on the urinary excretion rates of GAG in normally grown and growth retarded infants, since it has been assumed that in renal hydroxyproline excretion [4] GAG excretion rates might be used as an indicator of intrauterine and neonatal growth activity.

Materials and Methods

Altogether 51 newborn infants have been studied. They were divided into three groups. *(1)* Normally grown near term infants with a birth weight of more than 2200 g and a gestational age of 35 weeks or more. *(2)* Intrauterine growth retarded infants with a gestational age of 35 weeks or more and a birth weight less than 2200 g. All infants of this group were below the 10 percentile curve of our local intrauterine weight chart [3]. *(3)* Preterm infants, born earlier than 35 weeks with a birth weight less than 2200 g. Details of the groups are shown in Table I.

Feeding: All infants were fed on artificial cow's milk formula providing 70 kcal/100 ml.

TABLE I

Postnatal age	3 days			10	
Gestational age	$\geq$35 weeks		< 35 weeks	$\geq$35 weeks	
Birth weigth	>2200 g	<2200 g	<2200 g	>2200 g	<2200 g
n	14	13	24	14	12
Mean birth weight	3320	1830	1707	3248	1688
Mean gestational age	39.5	37	31	38	37
Mean body weight at postnatal age	3258	1808	1661	3420	1832

Samples: 24-hour urine samples were collected on the third, tenth, twentieth and thirtieth day after birth. Samples were kept at 4°C, without adding any preservative.

Isolation of GAG: After collection, the samples were immediately acidified to pH 5 with 1 N hydrochloric acid. Urine was diluted only if its creatinine content was above 50 mg per 100 ml. GAG was precipitated with 2% aqueous cetylpyridinium chloride (CPC). After addition of about 0.3 g Celite, the urine sample was allowed to stand for 2—3 hours at room temperature and overnight at 4°C. The precipitate was collected by centrifugation, resuspended in saturated potassium acetate solution in 96% ethanol. This procedure was repeated until the supernatant was entirely clear. The complex was then resuspended in 0.05 M sodium chloride and precipitated again with CPC. The GAG–CPC–Celite complex was then fractionated by the method of TANAKA and GORE [7].

Determination: Uronic acid was estimated by the carbazole reaction in every effluent tube [1]. In fractions containing keratan sulphate (no uronic acid present) galactose was estimated by the orcinol method [1], creatinine by the alkaline picrate method [5].

The total GAG excretion was calculated as the sum of the quantity of uronic acid and of galactose containing fractions given as μM uronic acid/day or μM uronic acid/1.73 sq.m body surface/day or μM uronic acid/g creatinine.

Statistical methods: The GAG excretion rate on the 3rd postnatal day was related to gestational age, birth weight and length using linear regression analysis. The significance of the difference of the means of urinary GAG output was estimated by Student's *t*-test.

RESULTS

1. Postnatal age and GAG excretion rates

In Fig. 1, mean values of GAG excretion are shown. The standard error is indicated at those sampling periods where the number of examined infants was more than ten. The amount of total GAG expressed in μM uronic acid/24 hours or μM uronic acid/1.73 sq.m body surface or in μM uronic acid/g creatinine showed a similar relationship with postnatal age.

The mean GAG excretion rate, expressed in the generally used term (uronic acid/creatinine) varied greatly in each group and therefore at no postnatal age could a statistically significant difference be observed between the three groups of infants. However, if uronic acid excretion obtained on

days	20 days			30 days	
< 35 weeks	≥35 weeks		< 35 weeks	>35 weeks	< 35 weeks
< 2200 g	> 2200 g	< 2200 g	< 2200 g	< 2200 g	< 2200 g
22	4	9	19	3	6
1612	3121	1809	1565	1778	1510
32	39	36.5	30	36.5	30
1584	3485	2028	1614	2420	1911

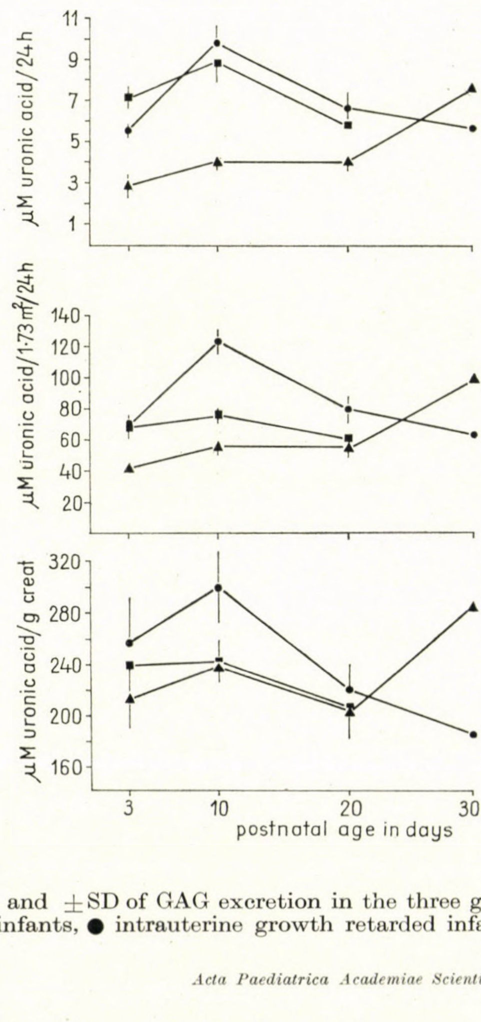

FIG. 1. Mean values and ±SD of GAG excretion in the three groups of infants. ■ Normally grown, term infants, ● intrauterine growth retarded infants, ▲ preterm infants

the 3rd day was expressed as the daily total output, or referred to 1.73 sq.m body surface/24 hours, the normally grown and growth retarded infants turned out to excrete significantly more GAG than premature infants. Between normally grown and malnourished infants only the absolute amount/24 hours differed significantly.

Mean GAG excretion rate on the tenth postnatal day was higher than on the third day in all the three groups. The increase was most pronounced in the growth retarded groups, whether the value for μM uronic acid/24 hours or that per 1.73 sq.m/24 hours was used for comparison ($p < 0.001$). Thereafter, while the uronic acid excretion rates decreased in normally grown and growth retarded infants, in premature infants a striking increase could be observed. Unfortunately, during the third ten-day period observation only a few premature infants could be examined, so that the results could not be analysed statistically.

2. Relationship between gestational age, birth weight, body length and GAG excretion rate on the third postnatal day

Regression analysis showed a significant correlation between gestational age and total daily GAG excretion observed on the third day and given in μM uronic acid (Fig. 2). Although there was a significant linear correlation ($r = 0.832$), the distribution of individual values seemed to be logarithmic. Thus, log μM uronic acid per day plotted against log gestational age showed the best correlation (Fig. 3). The gestational age showed good correlation both with the daily uronic acid excretion, and the same referred to 1.73 sq.m body surface on the third day (Fig. 4).

The relationship between urinary uronic acid excretion found on the third day, and birth weight as well as body length was also examined. In Figs 5 and 6 it can be seen that both parameters correlated significantly with GAG excretion per 24 hours.

3. Extrauterine growth and urinary GAG excretion rate

While no relationship could be observed between urinary GAG excretion on the 10th and 20th postnatal days and gestational age as well as birth weight, it seemed interesting to study whether postnatal growth activity affected the renal output of GAG. For this purpose the 10 and 20-day infants were divided into two groups, (i) the non-growing group included infants whose body weight did not change or even fall; (ii) the growing group consisted of infants who showed a definite weight gain after an initial weight loss. The GAG excretion rates in the two groups of infants are shown in Table II. The growing babies excreted significantly more GAG than the nongrowing ones, whether the daily absolute amount or that expressed in 1.73 sq.m/24 hours was compared. However, within each group there was no significant difference between the excretion rates obtained on the 10th and 20th postnatal days.

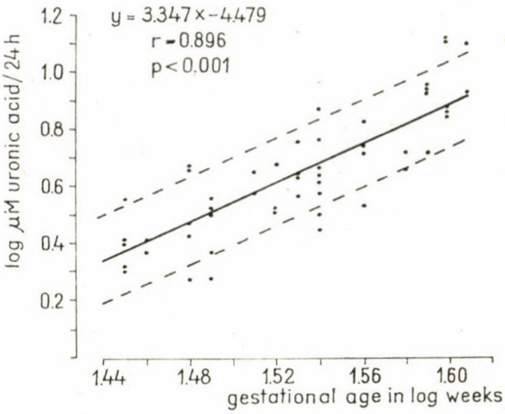

FIG. 2. Correlation between gestational age and daily uronic acid excretion

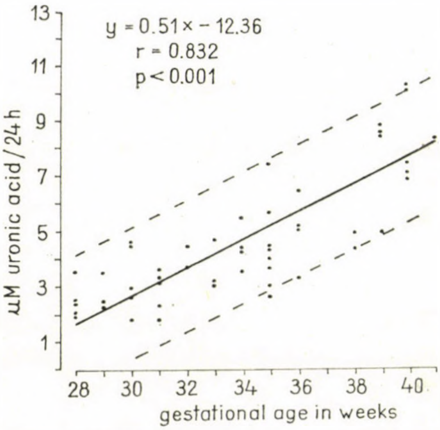

FIG. 3. Relationship between log gestational age and log uronic acid excreted in 24 hours

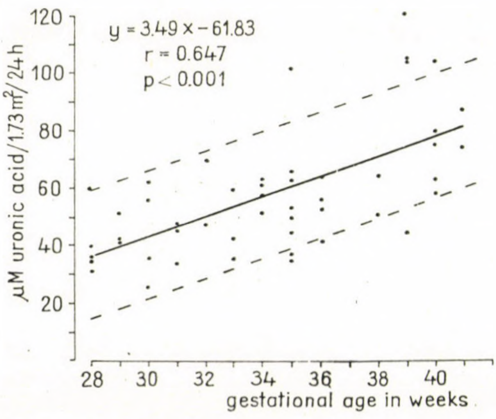

FIG. 4. Relationship between gestational age and uronic acid excretion corrected to 1.73 sq.m body surface

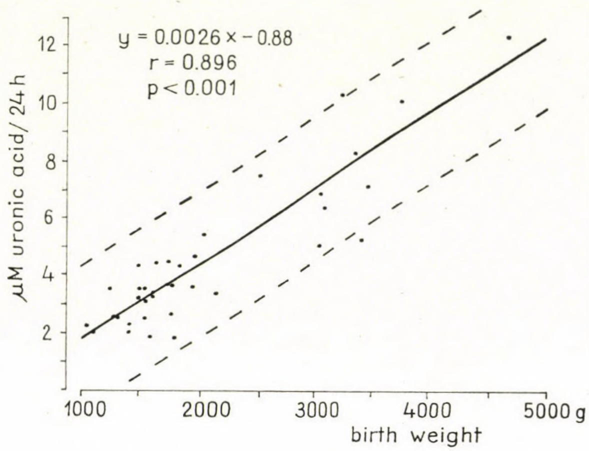

FIG. 5. Relationship between daily uronic acid excretion and birth weight

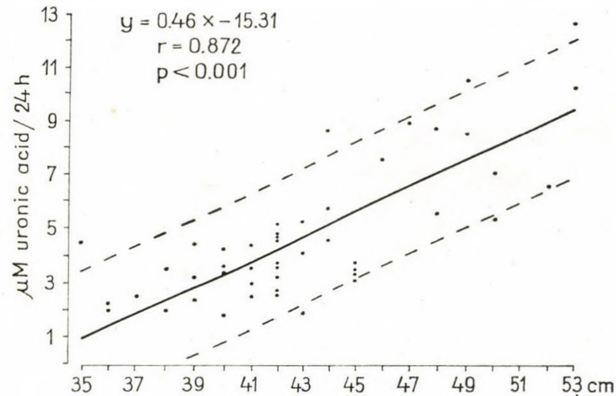

FIG. 6. Correlation between uronic acid excretion and body length

TABLE II

Postnatal age	10 days		20 days	
Groups of infants	growing	not growing	growing	not growing
μM uronic acid/day	8.94 ± 0.60	3.71 ± 0.41	6.63 ± 0.48	3.36 ± 0.29
μM uronic acid/day 1.73 sq. m	96.41 ± 6.88	46.25 ± 3.64	80.63 ± 4.80	42.35 ± 4.41
n	29	19	19	13

Discussion

Since no sufficient data are available concerning the importance and role of GAG excretion in connective tissue metabolism of newborn infants, it is difficult to interpret the present results. Instead of speculation it seems more pertinent to draw some practical conclusions.

First of all, urinary uronic acid content is not generally used for characterizing urinary GAG excretion rate in μM/24 hours. Since, however, uronic acid and galactose fractions are expressed together, we prefer reckoning total GAG as μM uronic acid.

Urinary GAG output corrected to body surface unit is as high in the first month of extrauterine life as in adolescence. The present data are in agreement with those of Pennock et al. [6] who also reported on a high GAG excretion rate expressed as creatinine ratio.

Urinary GAG excretion shows a trend of changes similar to that of hydroxyproline excretion which is regarded as an index of collagen metabolism or growth activity [4]. In the above observations urinary GAG output obtained on the third postnatal day referred to body surface unit was significantly different in newborns exhibiting different intrauterine growth rates. In addition, the correlation observed between uronic acid excretion and gain in body weight on the 10th day in dysmature and the 30th day in premature infants suggested that postnatal growth may also be an important factor in influencing GAG excretion. Comparison of growing and non-growing infants definitely showed that renal GAG output in this period of extrauterine life is related much more to growth than to postnatal age.

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