Excretion of Urinary Glycosaminoglycans During the Menstrual Cycle in the Baboon[1] (35418)

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(Introduced by L. B. Jaques)
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The significance of urinary glycosaminoglycans (GAG)[2] in relation to metabolic changes in connective tissue is still obscure. Under normal conditions the amount of GAG excreted in human urine is about 5 to 10 mg/24 hr (2) and consists mainly of chondroitin sulfate (3). However, changes in the quantity and type of urinary GAG have been observed in a number of pathological (3) and experimental situations (4, 5). Such observations raise the possibility that urinary GAG may reflect the metabolic status of connective tissue in health and disease.

Kaplan and Meyer (4) have studied urinary excretion in man and dog following the intravenous injection of sulfated GAG. The excretion of endogenous sulfated GAG when mobilized by experimental means has been studied in the rabbit by Smith and Kirby (5). However, the fate of nonsulfated GAG has received little attention. The present studies were undertaken to provide information on the excretion of endogenous hyaluronic acid, when this substance is mobilized from tissue by normal regulatory mechanisms in the body.

The female baboon is ideal for studies of this kind. The menstrual cycle in this animal is associated with cyclic fluctuations in the size of the perineal sex skin. Turgescence of the perineum begins immediately after menstruation and reaches a maximum in about 3 to 4 weeks. Ovulation occurs at the peak of turgescence and this is followed by a sudden rapid deturgescence which is complete within 6 to 8 days. Of particular interest is the observation that turgescence is accompanied by a large net increase in the hyaluronic acid content of the sex skin whereas during deturgescence, much of the hyaluronic acid disappears from the tissue (6). Little change in sulfated GAG was noted. The magnitude of the changes in hyaluronic acid suggest a considerable turnover during the menstrual cycle. It was therefore of interest to examine whether the disappearance of GAG from the sex skin, occurring in response to alterations in hormone balance, is accompanied by changes in urinary excretion of GAG. Furthermore, a number of studies suggest that an increased concentration of hyaluronic acid may bring about retention of fluid while depolymerization leads to decreased water retention (7–9). The present study was therefore designed to determine as well, the relationship if any, between urinary GAG and the rapid water loss which occurs from the sex skin during its deturgescence.

Materials and Methods. Adult female baboons of the species *Papio anubis* and *Papio cynocephalus* were used in this study. The animals were housed in separate cages and fed primate chow with daily supplements of fresh fruit and vegetables.

Daily urine collections were made at room temperature with toluene as preservative. Collections were made over a 16-hr period between 4:30 p.m. and 8:30 a.m. Prior to collection, the cages were washed, food was withdrawn, and the urine containers were covered with a double layer of gauze to minimize contamination of the urine sample. Water was available at all times. A receptacle placed under the spout of the water bottle

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[2] The nomenclature suggested by Jeanloz (1) has been used.

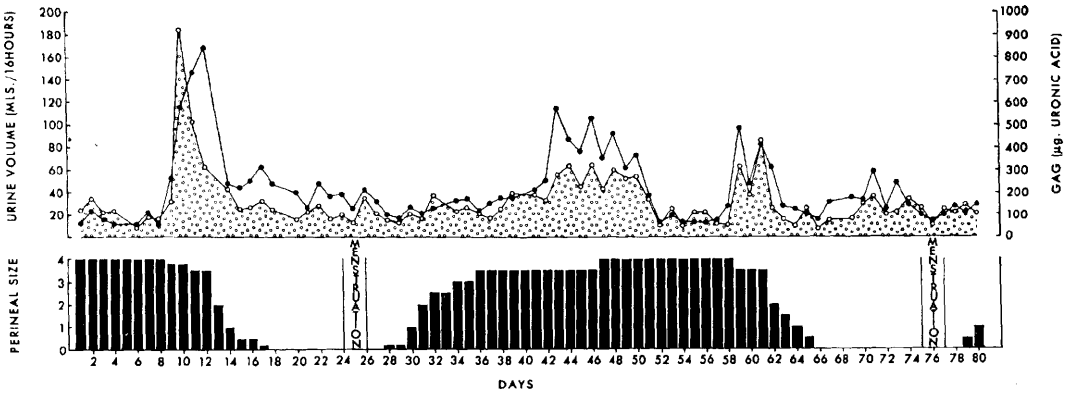

FIG. 1. Changes in urine volume and total urinary glycosaminoglycans (GAG) in relation to perineal size in the baboon (*P. anubis*): (vertical bars) the size of the perineum; (stippled area) total GAG; and (●) the change in urine volume. This baboon was approaching maximal turgescence when the study was begun.

prevented spillage into the urine. Daily visual estimates of perineal size were made using a scale which registered complete collapse as zero and maximum turgescence as +4. This method requires a knowledge of the individual animal, but is a rapid and relatively accurate method of recording changes in the perineum when the animals do not permit handling without sedation.

The urine volumes were measured and then centrifuged at 2000 rpm for 30 min in a refrigerated centrifuge. 25-ml aliquots in duplicate, were taken for analysis of total GAG according to the method of De Ferranti (10). In some instances urinary GAG were separated into sulfated and nonsulfated fractions on microcellulose columns according to Svejcar and Robertson's modification of the method of Antonopoulous *et al.* (11).

Changes in perineal GAG during its growth and regression were measured as follows. Two months after ovariectomy, two baboons (*P. cynocephalus*) were given daily intramuscular injections of estradiol benzoate in arachis oil (400 mg/day) for 9 days. Two days before the last estrogen injection, one of the baboons received in addition daily injections of progesterone in arachis oil (400 mg/day) for 8 days, while the other received daily injections of an equivalent amount of arachis oil. Daily measurements of the size of the sex skin were made as described by Gilman and Gilbert (12). Biopsies of the sex skin were done on alternate days as previously described (13). The biopsy specimens were rinsed in cold, 0.25 *M* sucrose solution homogenized in water (10%; w/v) and filtered through a double layer of gauze. 2.0 ml of homogenate was added to 2.0 ml of 4 *N* HCl and hydrolyzed in a boiling water bath for 8 hr. The GAG content of the hydrolysate was measured as uronic acid according to the method of Bitter and Muir (14). The protein content of the homogenate was measured by the method of Lowry *et al.* (15).

Results. The fluctuations in urine volume, total urinary GAG, and perineal size during two consecutive cycles in three baboons are shown in Figs. 1, 2, and 3. The study shown in Fig. 1 was begun when the perineum was in a state of maximum turgescence. This animal (*p. anubis*) had a 51-day cycle which is at the upper limit of the range (18–52 days) reported by Hendrickx in a study of 105 cycles (16). Urine volume and GAG remained constant except during early deturgescence of the perineum when both parameters increased sharply and returned to almost normal levels well before the complete collapse of the perineum had occurred. Elevations in urine volume and GAG were more marked during the first of the two cycles studied. Neither parameter deviated from normal during menstruation, when the mobilization of uterine GAG might be expected to occur. The slight rise in urine volume and GAG which occurred between day 42 and 50 may be related to elevated room tempera-

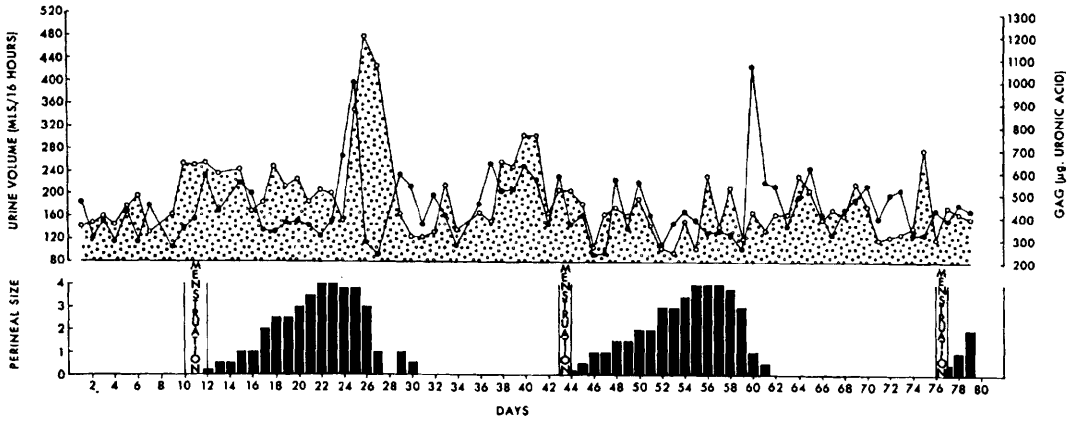

FIG. 2. Changes in urine volume and total urinary glycosaminoglycans (GAG) in relation to perineal size in baboon (*P. cynocephalus*): For details see Fig. 1. The perineum was completely collapsed when the study was begun.

tures during this period resulting from a failure in the air conditioning equipment in the animal quarters.

Figure 2 shows the results of a similar study in another baboon (*P. cynocephalus*) which had a 33-day cycle. In this case the perineum was in a fully collapsed state when the study was begun. Urine volume and GAG were somewhat higher and more variable in this animal. A sharp increase in both parameters occurred in early deturgescence during the first cycle. In this case high levels of urinary GAG were maintained over the greater part of deturgescence. During the second cycle, however, the rise in urine volume was observed but not the change in GAG. Once again no significant changes occurred during menstruation.

Figure 3 shows the results of a study in a baboon (*P. anubis*) which had irregular cycles. During each of the two cycles recorded, this animal menstruated near the peak of turgescence in addition to menstruation occurring after complete perineal collapse. On the whole, the size of the perineum, when fully turgescent, was much smaller than in the other two baboons. During the first cycle the rate of perineal collapse was unusually slow. Thus, after 13 days, the perineum had collapsed to only half its fully swollen size. During the next 2 days, rapid and complete collapse of the residual growth occurred. The

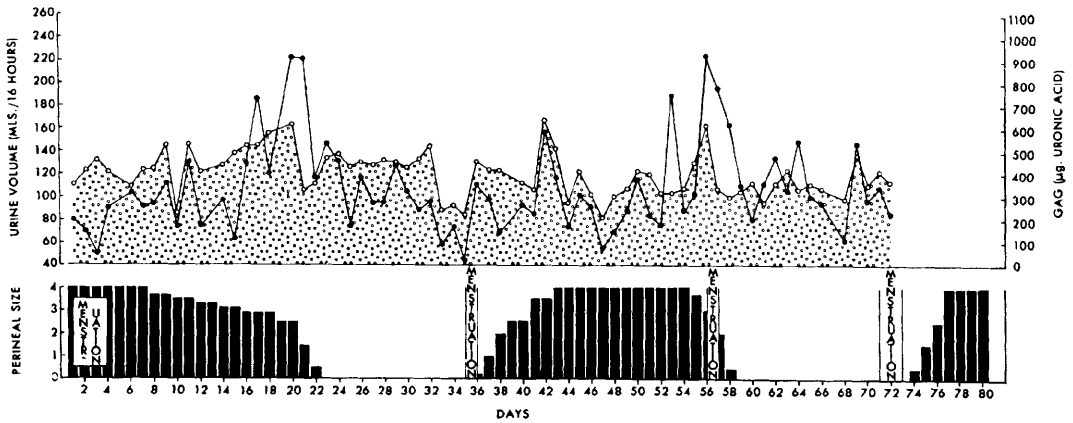

FIG. 3. Changes in urine volume and total urinary glycosaminoglycans (GAG) in relation to perineal size in a baboon (*P. anubis*) with irregular cycles: For details see Fig. 1. The perineum was fully turgescent when the study was begun.

TABLE I. Fractionation of Urinary Glycosaminoglycans During Turgescence and Deturgescence of Baboon Perineum.[a]

			Baboon no.[b]			
			1		2	
Eluent	HA[c]	ChS[c]	Turg	Deturg	Turg	Deturg
1% CPC	0	0	0	388.2	0	96.3
1.75% NaCl	17.8	0	0	163.8	0	211.9
1.25 *M* $MgCl_2$	0	16.9	281.2	309.1	22.1	125.2
Total	17.8	16.9	281.2	861.1	22.1	433.3
Direct determination	18.3	17.8	—	—	—	—
% Recovery	97.3	94.9	—	—	—	—

[a] Abbreviations: CPC = cetylpyridinium chloride; HA = hyaluronic acid; ChS = chondroitin sulfate; Turg = turgescent; Deturg = deturgescent. The glycosaminoglycan in each fraction is expressed as micrograms of uronic acid.

[b] The distribution of baboon urinary glycosaminoglycans among the various fractions is expressed as the total amount present in a 16-hr urine sample. One of the two urine samples selected from each baboon was obtained during perineal turgescence and the other during early deturgescence. The samples selected were from those cycles in which elevated GAG occurred during deturgescence.

[c] Hyaluronic acid and chondroitin sulfate were purchased from Sigma Chemical Co.

rate of perineal collapse during the second cycle approximated the rates in the other two baboons. Such menstrual irregularities are known to occur in baboons (16). As in the other two baboons an increase in urine volume during deturgescence was observed in both cycles. However, the GAG peaks which were observed in the other animals are barely noticeable in this case.

To determine the type of GAG present in urine at the time when peak excretion occurs, urinary GAG were separated into sulfated and nonsulfated fractions. The results are shown in Table I. One of the two urine samples selected from each baboon was obtained during perineal turgescence and the other during very early deturgescence. It is apparent that during perineal turgescence, all of the GAG appears in fraction 3 whereas during deturgescence about ⅔ was present in fractions 1 and 2. The increase in total GAG during deturgescence is due largely to an increase in fractions 1 and 2. The elution of authentic samples of hyaluronic acid and chondroitin sulfate is also shown for comparison. Svejcar and Robertson (11) have shown that when hyaluronic acid which normally appears in fraction 2 is digested with hyaluronidase, the products appear in fraction 1. It therefore appears that the GAG appearing in fractions 1 and 2 during deturgescence indicate the presence in urine at this time, of nonsulfated GAG, possibly hyaluronic acid and lower molecular weight compounds derived from it.

To obtain further information regarding the source of urinary GAG during the early stages of deturgescence, the fluctuations in uronic acid content of sex skin during hormone induced growth and regression were measured in ovariectomized baboons. The results are shown in Fig. 4. Estrogen injections resulted in rapid swelling of the sex skin and its withdrawal resulted in collapse. Swelling was accompanied by a considerable increase in uronic acid. During deturgescence, uronic acid returned to preestrogen levels. The greatest fall in uronic acid occurred during the early phase of deturgescence and thus coincided with the peak in urinary GAG which was observed in some cycles. When progesterone was injected into an estrogen primed animal, the size and uronic acid content of the sex skin appeared to decline more rapidly. The greatest fall in uronic acid again occurred during the early stages of deturgescence.

Discussion. Deturgescence of the baboon

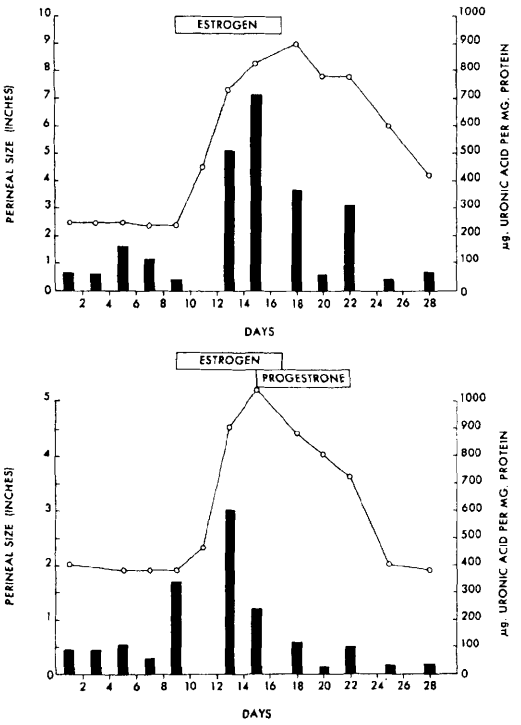

FIG. 4. The effects of estrogen and progesterone on the uronic acid content of perineal sex skin in ovariectomized baboons: (vertical bars) the change in uronic acid; and (○) the change in perineal size.

perineum was, without exception, accompanied by a marked increase in urine volume. This is in accord with observations in the pigtail monkey by Krohn and Zuckerman (17). The results of their water balance studies show that the excess water is derived from the perineum. The present data show that in 3 out of the 6 cycles studied, increased urine volume was accompanied by a marked transient increase in total urinary GAG. In each case the increase occurred during the early stages of perineal deturgescence. The increase in total GAG was due largely to an increase in nonsulfated GAG, presumably hyaluronic acid and lower molecular weight products derived from it. The close correspondence between deturgescence and elevation of urinary GAG suggest that the latter originate in the perineum. This possibility is supported by the observation that the greatest fall in perineal GAG occurs during early deturgescence. Furthermore, Rienits has shown that the fall in perineal GAG during deturgescence is due almost exclusively to a decline in hyaluronic acid (6).

During deturgescence, the excretion of total urinary GAG show considerable variations from one cycle to another in the same animal. This variation ranges from an absence of change in total urinary GAG to as much as a threefold increase. It is noteworthy that even when a relatively large increase in GAG did occur, the total amount excreted during the entire period of deturgescence in that cycle is small compared to the very considerable amount of GAG known to disappear from the perineum during this period (6). This suggests that when large amounts of nonsulfated GAG (hyaluronic acid) are mobilized from baboon connective tissue by hormonally mediated mechanisms, they are not quantitatively reflected in urine. It would appear that by far the greater part of perineal GAG is either degraded and reutilized perhaps during perineal turgescence in the next cycle or excreted in urine as low molecular weight derivatives, which are not precipitated by cetylpyridinium chloride (CPC). The increase in CPC-precipitable GAG observed in some cycles may be due to saturation of degradative mechanisms resulting from the sudden mobilization of large amounts of perineal GAG. Such mechanisms exist in mammalian tissues and are particularly effective against hyaluronic acid (18, 19).

Hyaluronic acid has been implicated in fluid retention in tissues (7–9). In this regard it is noteworthy that an increase in urinary GAG was always accompanied by a rise in urine volume. The present studies and those by Krohn and Zuckerman (17) show that the increased urinary GAG and water during deturgescence derive from the same source namely, the perineum. It appears that the mobilization of GAG from the perineum results in loss of water from this tissue. It is conceivable that the bulk of GAG lost from the perineum actually precedes the water loss, but that this relationship is obscured by extensive degradation of GAG during the early stages of release.

Summary. Total urinary glycosaminoglycans (GAG) and urine volumes were mea-

sured in relation to perineal size during the menstrual cycle in the baboon. Perineal deturgescence was accompanied by a marked increase in urine volume. The increase in urine volume was in some cycles accompanied by a marked transient increase in urinary GAG. This increase was due largely to nonsulfated GAG, tentatively identified as hyaluronic acid and lower molecular wt products derived from it. Evidence is presented, showing that these GAG originate in the perineum. The results show that when nonsulfated GAG are mobilized from the baboon perineum by normal regulatory mechanisms, they are not quantitatively reflected in urine, suggesting the presence of effective mechanisms for their degradation.

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