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Temporal Variations in the Collagen, Noncollagen Protein and Hexosamine of the Uterus and Vagina.* (28142)

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The rat uterus following parturition and undergoing involution showed a rapid loss of collagen (C)(1,2). Harkness, Harkness and Moralee(3), however, have reported that growth and removal of C in the rat uterus during the estrous cycle were small and not significant. The investigation of Morgan(4) indicated that low levels of estrogens administered to castrate rats induced a greater quantity of hexosamine (Hx) and hydroxyproline (OHP) in the uterus and vagina. The present study was designed to reexamine the uterus and vagina of the rat during the estrous cycle for possible changes in C and Hx content. C can be approximated by determining OHP since the latter occurs in significant amounts only in C(5). Hx is characteristic of the mucopolysaccharides (MPS) but is also present in mucoproteins, mucoids and glycoproteins(6). Total nitrogen (N) content and percent moisture (M) were also determined.

Materials and methods. Five groups of 6 rats of the Holtzman strain weighing from 172 to 236 g at time of autopsy were used. The stages of the estrous cycle considered were: diestrus, proestrus, estrus and postestrus. One group of rats was studied for each stage. Vaginal smears were examined for 3 weeks to make certain that autopsy would coincide with the height of each particular stage. A control group of 6 animals, castrated 4 weeks prior to time of autopsy, was included. The dry weights of the tissues were obtained on a microbalance after the tissues were lyophilized for 30 hours with a VirTis freeze-dryer. Chemical procedures employed to determine OHP, Hx and N were: 1) OHP by the method of Neuman and Logan(5); 2) Hx by the Boas method(7); 3) total N by the micro-Kjeldahl method of Koch and McMeekin(8). OHP, Hx and N values were based on the mean dry weight for each group calculated in 2 ways: 1) concentration (μM/g) and 2) amount (μM) in the entire uterus or vagina. These data were further studied by making calculations in mg of: 1) C by multiplying the weight of OHP $\times$ 7.46(9); 2) N in C by multiplying C $\times$ 0.186(10); 3) N in Hx by multiplying the weight of Hx by 0.079; 4) noncollagen protein (NCP) by multiplying the weight of total N $\times$ 6.25 after correcting for the N in C and Hx. The square roots of the weights of C and NCP were determined to obtain a more equal response variable among the observed values. All data were tested for significance by the use of Student's "t" test. The mean and standard error of the mean were calculated for all groups of the data.

Results. Uterus. Cyclical changes in OHP in the intact animal (Fig. 1 and 2) were as follows: 1) concentration of OHP was lowest at estrus (195 μM/g $\pm$ 5.85). This was less than at proestrus (251 μM/g $\pm$ 19.16, $P<.05$) and at diestrus (247 μM/g $\pm$ 10.80, $P<.01$); 2) amount of OHP reached its peak during postestrus (28.2 μM $\pm$ 4.50), was slightly less at estrus (26.2 μM $\pm$ 2.16) and was lowest in diestrus (14.4 μM $\pm$ .70); 3) amounts for both postestrus and estrus were

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TABLE I. Concentration and Amount of Collagen and Noncollagen Protein in the Uterus in the Castrate and During the Estrous Cycle.

	Castrate control	Diestrus	Proestrus	Estrus	Postestrus
		Amount (mg/100g body wt)			
Collagen*	1.13 ± .02†	7.14 ± .27	10.71 ± 1.31	12.68 ± 1.13	14.65 ± 2.65
Noncollagen protein‡	1.04 ± .14	15.91 ± 1.19	22.45 ± 2.92	36.06 ± 5.13	26.57 ± 3.40
		Concentration (mg/g of tissue)			
Collagen	350.87 ± 25.26	241.00 ± 10.55	244.80 ± 18.00	191.05 ± 5.73	255.90 ± 31.23
Noncollagen protein	348.94 ± 60.73	534.15 ± 26.66	509.76 ± 30.73	534.45 ± 28.17	452.66 ± 28.09

* Corrected for nitrogen in hexosamine.
‡ Based on hydroxyproline determinations.
† Mean ± standard error.

greater than for diestrus ($P<.02$ and $P<.001$ respectively); 4) concentration and amount of C (in mg) varied as did OHP (Table I). Concentration of OHP in the castrate was significantly less but the total amount was greater ($P<.001$) than in the intact animal in all stages of the estrous cycle (Fig. 1 & 2).

The rhythmical gains and losses of Hx were as follows: 1) concentration reached a peak at estrus (33.6 μM/g ± 0.95) and remained high through postestrus (33.0 μM/g ± 0.61), with both values significantly greater than that recorded at proestrus ($P<.05$, Fig. 1); 2) during estrus the total amount of Hx was greatest and it was significantly higher than at either diestrus when it was least ($P<.001$) and at proestrus when it was at an intermediate level ($P<.02$, Fig. 2). Both concentration and total amount of Hx were significantly larger in all stages of the estrous cycle than in the control group ($P<.001$).

The variation in M content and dry weight coincided with the rhythm of the estrous cycle—at diestrus it was significantly less than at proestrus ($P<.05$) and at estrus ($P<.05$, Fig. 1). The percent M found during these stages exceeded that determined for controls ($P<.05$ to $P<.01$, Fig. 1).

The Hx/OHP ratio for both concentration and amount in the whole organ was largest at estrus (0.17, less during the other stages of the cycle and least for the castrate (0.07).

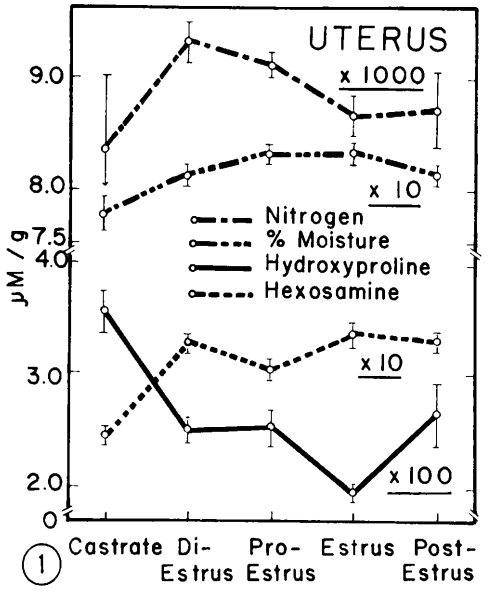
The concentration of N/g among the various stages of the estrous cycle and in the castrate was not significantly different (Fig. 1). However, the amount of N was rhythmical with the greatest quantity present during estrus (1171 μM) which was significantly more than at diestrus ($P<.001$) and at proestrus ($P<.05$, Fig. 2). The amount of N in the castrate was least of all groups (77 μM compared to normals, $P<.001$).

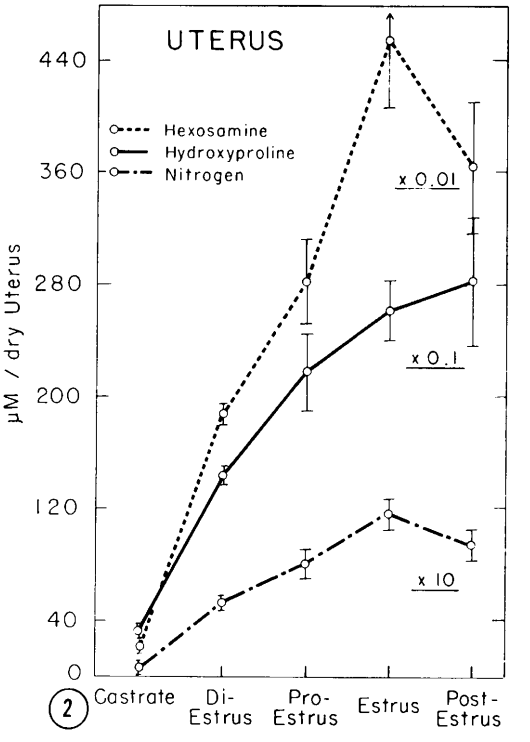
The concentration of NCP did not behave as did C: 1) it did not vary rhythmically although it was significantly less in the castrate than in the intact animal (Table I); 2) it was greater in concentration in the intact animal at estrus when estrogens are known to be in greater abundance whereas, at the same time, the concentration of C appeared to decrease. On the other hand, the total amount of NCP increased from the lowest level in the castrate, rose to an intermediate level at diestrus and reached a peak at estrus (Table I). These increases in total NCP were greater in proportion than C; thus the NCP contributed more to the total growth than did C as the uterus enlarged from diestrus to estrus.

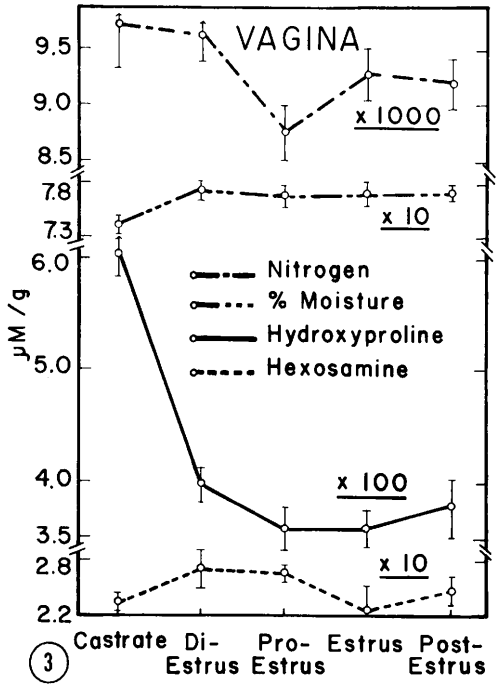
The mean uterine weight (mg/100 g body weight) was also cyclical: during diestrus it was least (157 ± 8.58) and at estrus it was greatest (397 ± 33.45). These weights were significantly different ($P<.001$).

Vagina. OHP concentration in the castrate was significantly more than that determined for each of the 4 stages of the estrous cycle but the amount of OHP was not significantly altered by castration as compared to the OHP in the various stages of the estrous cycle. In neither the concentration nor the amount was there a difference in the mean quantities of OHP among the 4 stages of the estrous cycle (Fig. 3 & 4).

Both concentration and amount of Hx in the castrate animals were significantly smaller when compared to the estrous cycle ($P<.05$ to $P<.001$, Fig. 3 and 4) but among the various stages of the cycle there were no significant differences.







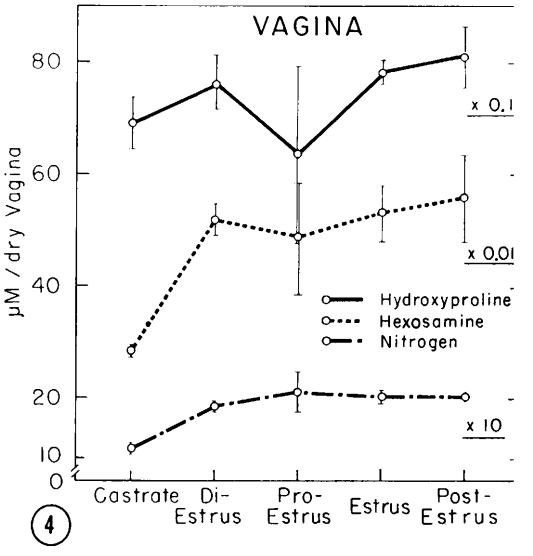


FIG. 1. Changes in concentration (µM/g) of nitrogen, % moisture, hydroxyproline and hexosamine in the uterus during the estrous cycle. Standard error of mean indicated by small vertical lines.

FIG. 2. Changes in amount (µM) of hexosamine, hydroxyproline and nitrogen per dry uterus during estrous cycle. Standard error of mean indicated by small vertical lines.

FIG. 3. Changes in concentration (µM/g) of nitrogen, % moisture, hydroxyproline and hexosamine in vagina during estrous cycle. Standard error of mean indicated by small vertical lines.

FIG. 4. Changes in amount (µM) of hydroxyproline, hexosamine and nitrogen per dry vagina during estrous cycle. Standard error of mean indicated by small vertical lines.

The concentration of N within the cycle did not fluctuate except in one instance, *i.e.*, proestrus compared to diestrus ($P<.01$), nor was there any significant difference compared to the castrate. The total N in all stages of the estrous cycle was significantly greater than in the castrate animals ($P<.05$ to $P<.001$), but none of the values among the stages were significantly different.

The M content was greater in all stages of the cycle as compared to the castrate; within the various stages, however, there was no intergroup difference (Fig. 3).

Discussion. Robertson(11,12) earlier reported that C was metabolically inert. Wolbach and Bessey(13) showed that neither concentration nor amount of C decreased during acute or chronic ascorbic acid deficiency in guinea pigs even though ascorbic acid was necessary for formation of C. Subsequently it has been shown that C content will change under different metabolic conditions, particularly under the influence of estrogens(4). Harkness and Harkness(1,14) have reported a rapid increase of C in the uterus of the pregnant rat and a rapid loss of C(2) in the involution of the total uterus. Harkness, Harkness and Moralee(3), however, were unable to demonstrate a change in the amount of C in the estrous cycle. Our study, however, has demonstrated a rhythmic change in OHP and its C equivalents during the estrous cycle. In general, the concentration decreased from diestrus to estrus whereas the entire amount/uterus increased. Each unit (g) contained less C but in the total uterus the increased amount of C coincided with the greater uterine weight. This would suggest a catabolism and/or a reduction in formation of C/unit weight in the transition from diestrus to estrus. The concentration of NCP, however, continued to be maintained in this same transition period and, for the total uterus, it accounted for more of its growth than did C. The concentration and total amount of NCP in the castrate were considerably less than in the intact animal even at diestrus ($P<.001$ and $P<.02$ respectively) and, thus, NCP may be sensitive to lower levels of the natural hormones than is C. The variations in the concentrations of Hx and NCP seemed to be related. This may indicate that Hx containing compounds link with NCP rather than C; especially since Muir(15) has shown that the protein associated with the MPS does not contain OHP. In summary, the concentration of C appeared to be reduced by the natural hormones whereas the concentration of NCP appeared to be stimulated. The rapidity of the cyclical changes with time are most significant. Averages for the durations of each stage were as follows: diestrus, 57 hours; proestrus, 12 hours; estrus, 12 hours; and postestrus (includes metestrus), 21 hours. The speed of the metabolic changes necessary to cause such large fluctuations in C, Hx, N and NCP in accordance with the particular stage of the estrous cycle makes these changes all the more significant.

In the present study the Hx content of the uterus displayed a rhythm, decreasing during diestrus and increasing during estrus, perhaps proportional to natural hormonal levels.

It was not possible to show significant cyclical changes in concentration of OHP or Hx in the vagina. The significant changes induced by castration as compared to the intact animal were: 1) a decrease in concentration of Hx of vaginal tissue; 2) an increase in OHP concentration.

It should be noted that cortisone caused a decrease in the Hx/C ratio in the skin of rats (16). This was reported to be related to a breakdown in ground substance and to a metabolically inert C content. In our study, however, in μM/g there was an increase in the Hx/OHP ratio during the transition from diestrus to estrus due to a gain in Hx and a loss of OHP.

Summary. 1) In the rat uterus it has been shown that the content of C, as measured by OHP, changed rhythmically during the estrous cycle per unit weight (g) and for the total organ. From diestrus to estrus the concentration of OHP decreased but total content increased. 2) In the uterus there was a cyclical change in the Hx content during the estrous cycle both in μM/g and in the total organ. In transition from diestrus to estrus the values became greater and were followed by a rapid loss through postestrus to that

found at diestrus. 3) The Hx/OHP ratio in the uterus was also cyclical, less during estrus than during diestrus. 4) Concentration and total amount of NCP were greater in the intact animal than in the castrate. 5) Per unit weight (g) and in the total vagina neither OHP nor Hx showed significant alterations during the estrous cycle. 6) The cyclical changes listed above may be related to fluctuations in levels of the natural hormones. 7) Studies of OHP, Hx, total N and NCP were made of the uterus and vagina of the castrate and comparisons were made to the intact animal.

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Protein Binding of Estriol Conjugates.* (28143)

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Data are presented here concerning dialysis of estriol sulfate and glucosiduronate from deproteinized plasma and solutions. Previous experiments in this laboratory suggested that estriol conjugates were bound to plasma proteins. Most reports of protein binding of steroids have been limited to studies of the free compounds primarily because of the unavailability of pure conjugates(1,2). Recently, Puche and Ness described the binding of dehydroepiandrosterone sulfate to serum albumin(3).

Methods. Human serum albumin Fraction V (HSA-5) (Pentex Corp., Kankakee, Ill.), obtained in crystalline form, was made up as 2.5 and 5.0% solutions.

Estriol glucosiduronate was obtained from: Sigma Chemical Co. (mixture), Dr. Philip Katzman (monoglucosiduronate), and Ayerst, McKenna and Harrison Ltd., and estriol sulfate was kindly provided by H. Fex, A. B. Leo, Halsinborg, Sweden.

Heparinized blood was centrifuged within 20 minutes after withdrawal and the plasma separated. Free estrogens were removed by extracting the plasma 3 times with equal volumes of ether leaving the conjugates in solution. Urines preserved with chloroform were kept in an ice box.

Plasma proteins were precipitated by addition of 4 volumes of acetone. The precipitate was centrifuged, washed 3 times with acetone, and the combined acetone supernatant was evaporated *in vacuo*. Dialysis was then carried out on the aqueous residue. The method consisted of 3 steps: I. *Dialyses* were

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