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Effect of Injury on Collagen Resorption in the Involuting Rat Uterus[1] (38368)

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(Introduced by N. L. Noble)

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The postpartum involution of the mammalian uterus is accompanied by extensive and extremely rapid breakdown of collagen; the half-life of collagen in this situation may be as low as 30 hr (1). It is an interesting feature of this system that one can produce a wound in the uterus as in caesarean section, that results in normal localized connective tissue proliferation and fibroplasia, while the remainder of the organ is in an extreme catabolic state. Wound healing has been studied in the postpartum uterus of the rabbit and dog by Schwarz (2) and Siegel (3), respectively. Both found that the healing processes are rapid and normal.

Morrione and Ru (4) extended these observations to a quantitative study of collagen changes in the postpartum rat uterus following a crushing injury to one horn. The loss of collagen that normally accompanies involution was retarded by the injury. Histology revealed the expected healing process and fibrosis. The total collagen content was interpreted as the resultant of the interplay of normal degradation and neoformation of collagen by the reparative fibrosis. It was also suggested that collagenolysis was decreased, although the evidence for this was not firm.

We have attempted to determine the relative contributions of synthesis and breakdown to the overall collagen metabolism of the injured uterine horn using an approach developed by Ryan and Woessner (5). In their experiments collagen was labeled in the uterus during pregnancy and breakdown was followed *post partum*. Normal collagen breakdown led to a loss of total counts and of specific activity of the collagen; estradiol treatment resulted in a slower loss of total counts and the specific activity remained close to the initial value. This points clearly to an inhibition of collagen breakdown by estradiol, since the stimulation of new collagen synthesis would have reduced the specific activity of the remaining collagen. The present paper applies this approach to the injured rat uterus and shows that the major effect of injury is to inhibit the collagen breakdown process.

Materials and Methods. Treatment of animals. Female albino rats, about 16 days pregnant, were purchased from Sprague–Dawley, Inc., Madison, WI. The rats were housed in individual cages and given Purina Lab Chow and water *ad lib.* The time of parturition was ascertained within 3–4 hr. The average weight of the rats was 290 g after parturition.

Within 6–12 hr after parturition an injury was administered to one horn of the uterus by the method of Morrione and Ru (4). The abdomen was opened under anesthesia and the horn was crushed with a wide hemostat closed firmly but not engaging the first ratchet. This clamping process was repeated along the length of the horn, avoiding injury to the major blood supply and the immediate sites of placental attachment. The contralateral horn received no injury and served as a paired control. If one horn had an abnormally low number of implantation sites (0–2), the rat was discarded. The injured horns became hemorrhagic but were not torn or broken. The abdominal wounds were sutured and the rats were returned to their young.

Four days after parturition, the rats were killed and the uterine horns were dissected free of mesentery and cervix. The separated horns were weighed and then chilled. All subsequent steps

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up to hydrolysis were carried out at 4°.

Studies of collagen metabolism. To measure the resorption of prelabeled collagen, L-[U-^{14}C] proline (New England Nuclear Corp., Boston, MA), 209 mCi/mmole, was dissolved in 0.9% NaCl and adjusted to pH 7 to give them a final concentration of 50 μCi/ml. A group of rats was given a series of three intraperitoneal injections at 20 hr intervals at approximately the 18–20th day of pregnancy for a total dose of 3 μCi/100 g body weight. Part of the group was killed at parturition to determine the starting level of labeled hydroxyproline in collagen. The remainder of the group was subjected to the crushing injury at parturition and killed after 4 days to determine the amount of labeled collagen remaining.

All uteri, whether labeled or not, were minced and ground in glass homogenizers in distilled H_2O (10 vol/g tissue). The homogenates were transferred to Visking dialysis tubing. Labeled homogenates were dialyzed against large volumes of water. Unlabeled homogenates were dialyzed against small volumes of water in order to recover the diffusible free hydroxyproline.

The nondiffusible fractions were dried on a steam table and hydrolyzed in 6 *N* HCl, 16 hr, 110°. The hydrolysates were analyzed for hydroxyproline (6) and hydroxy-[^{14}C] proline (7). Free hydroxyproline was measured in the diffusates without prior hydrolysis.

Radioactive hydroxyproline derivatives were counted in a Packard Tri-Carb Model 574 liquid scintillation spectrometer (machine efficiency 75–78%). Results were corrected for background and to 100% efficiency by the use of external standards. The scintillation fluid contained 80 g naphthalene, 7 g 2,5-diphenyloxazole, and 300 mg 1,4- bis-(4-methyl-5-phenyloxazol-2-yl)-benzene in 1 liter toluene–dioxane–ethanol (4:4:2.4 by volume).

Results. The results are presented in Table I. First, we repeated the experiment of Morrione and Ru (4) with large numbers of animals to see if we could reproduce their results. The uteri were removed at 4 days after parturition, since the process of involution is normally almost complete by this time. It can be seen from the table that the crushed horn is much larger (heavier) than the control horn at 4 days *post partum*. An even greater effect is seen on the collagen content (total hydroxyproline) of the crushed horn, which is more than twice as high

TABLE I. Effect of Crushing Injury on the Involution of the Rat Uterus.[a]

Treatment	Days *post partum*	Wet weight mg	Total hypro[b] μg/horn	Free hypro μg/g wet weight	[^{14}C] Hypro dpm/horn	Specific activity dpm/μg hypro
Control	0	1370 ± 230 (23)	4330 ± 580 (23)		16250 ± 9850 (23)	3.90 ± 2.50 (23)
Crushed horn	4	493 + 143 (26)	1050 ± 530 (26)	39.6 + 10.1 (15)	4660 ± 1770 (11)	3.81 ± 1.99 (11)
Control horn	4	259 ± 69 (26)	451 ± 183 (26)	38.6 ± 10.5 (15)	998 ± 406 (11)	1.78 ± 0.72 (11)
P[c]		0.001	0.001	—	0.001	0.01

[a]One horn was crushed following parturition and the contralateral horn served as control. Both horns were removed at 4 days *post partum* and analyzed as described under Methods.

[b]Hypro is an abbreviation for hydroxyproline.

[c]The *P* value refers to the difference of means of paired observations at 4 days.

as in the control horn. Free hydroxyproline is the same in both horns.

A series of animals was labeled with [^{14}C] proline at the end of pregnancy so that both horns started involution with the same level and specific activity of labeled hydroxyproline. By 4 days *post partum* the loss of label from the control horn has greatly exceeded that from the crushed horn; in the crushed horn the specific activity has declined only slightly. This indicates that the crushing injury has inhibited collagen breakdown.

Discussion. The results confirm the findings of Morrione and Ru (4) that a crushing injury to the involuting uterine horn results in an apparent retardation of involution, so that the weight and the collagen content of the horn do not reach the low levels normally found by 4 days *post partum*. The 493 mg wet weight and 1050 μg hydroxyproline of the crushed horn at 4 days may be compared to previously published values (5) for the normally-involuting horn at 3 days *post partum*; viz. 310 mg wet weight and 1500 μg hydroxyproline. This shows that the crushing injury has retarded collagen breakdown by almost one day (*i.e.*, these horns are closer to the 3-day than to the 4-day controls). In fact, the wet weight of the crushed horns is even larger than the control value found at 2 days (440 mg, Ref. 5). It is obvious that the crushing injury does not merely retard all phases of involution, but has a differential effect which is more pronounced on the wet weight loss. The weight and collagen changes produced by crushing are very similar to those reported by Morrione and Ru (4); their hydroxyproline values are higher in both groups (possibly related to the method of analysis) but the ratio of crushed to uncrushed is the same.

The question then is what mechanism is responsible for the effects seen with crushing injury–does the repair response stimulate the production of new tissue and collagen or does it just inhibit the normal course of involution? Is collagen elevated in the crushed horns because of inhibited degradation or is new synthesis superimposed on normal degradation? Two points shed light on these questions. The levels of free hydroxyproline are the same in control and crushed horns at 4 days. It was shown earlier that free hydroxyproline on the third day of involution is almost double the level found at the fourth day (70 μg cf. to 37 μg, Ref. 8); that is, the more rapidly collagen is breaking down, the higher the level of free hydroxyproline. Since the crushed horn has the same level of free hydroxyproline as the control horn, and we know that breakdown is close to completion in the control horn, it must be assumed that breakdown is slow in the crushed horn.

More convincing evidence is found in the labeling experiment. At parturition each horn contained about 16,000 dpm of hydroxyproline with a specific activity of 3.90. By the fourth day *post partum*, these values for control horns have fallen to 1000 and 1.78, respectively. As collagen is broken down, the specific activity also declines. This indicates either a selective retention of the older unlabeled collagen or a gradual dilution by new synthesis of collagen toward the end of involution. The crushed horns retain more than 4 times as much radioactive hydroxyproline as the controls and the specific activity (3.8) is very close to the starting value (3.9). These results indicate that the injury has resulted chiefly in an inhibition of collagen breakdown. Had there been a stimulation of new collagen synthesis superimposed on normal breakdown, losses of labeled collagen should have been closer to the control values and the specific activity should have been reduced even more than control values due to dilution with new unlabeled collagen.

It appears, then, that superimposing a repair process on uterine involution acts to retard collagen breakdown. On the other hand, wet weight losses are retarded to a much greater extent and it seems likely that this effect is due to the superimposition of an inflammatory response as described by Morrione and Ru (4) which would contribute to the dry mass and especially the water content of the tissue. The present studies do not rule out a later synthesis of new collagen in response to the injury, indeed this is quite likely; but they do show that there is not a significant synthesis of repair collagen during the period of rapid collagen degradation.

Summary. A mechanical crushing injury was applied to one horn of the rat uterus immediately following parturition. This injury retarded involution in that horn, but not in the contralateral horn, as measured by wet weight loss and collagen breakdown. Uterine collagen was labeled with [^{14}C] hydroxyproline near the end of pregnancy. Injured horns lost this label more slowly than the uninjured horns and there was also a slower diminution of specific activity. The in-

jury induces a repair process, but it is concluded that the fibrotic phase of repair does not occur during the period of maximum collagen breakdown accompanying involution.

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