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ACCUMULATION OF COLLAGEN IN THE UTERUS OF THE IMMATURE RAT ADMINISTERED ESTRADIOL-17β

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Abstract—The total protein and collagen content of the uterus was determined in both the immature and adult ovariectomized rat administered 5 μg estradiol-17β for 4 or 5 consecutive days. In the immature rat, this treatment results in a 3-fold increase in the collagen content of the uterus. This increase is linear following the first three doses of estradiol-17β and appears to reach a maximum after approximately 5 days. The total protein of the uterus also increases uniformly throughout the experimental period; therefore, the per cent collagen in total protein remains essentially unchanged. These results suggest that the increase in the collagen content of the uterus of the immature rat administered estradiol-17β is due to a general stimulation of protein synthesis. The administration of either estrone, diethylstilbestrol or 17-ethynylestradiol-3-methyl ether to the immature rat also causes collagenous and non-collagenous protein to accumulate in the uterus. There is a significant loss of noncollagenous protein from the uterus of the adult rat 21 days after ovariectomy, which is only partially restored by the administration of estradiol-17β for 4 consecutive days. The collagen content of the uterus is also reduced markedly and does not increase upon administration of estradiol-17β.

WHILE THE turnover of collagen is slow in most tissues of mature animals, the relative rapidity of this process in young developing tissue and in tissue culture suggests that these may be useful systems in which to examine various aspects of collagen metabolism.[1–4] It is possible to increase the collagen content of uterine tissue of the adult rat by administering estradiol-17β, and studies *in vitro* suggest that this effect is due to a stimulation of collagen biosynthesis.[5,6] Other investigators have also reported that the collagen content of the immature uterus increases *in vivo* after several doses of estrogen.[7] Although the stimulatory effect of estradiol-17β on collagen synthesis has been demonstrated, there has been no effort to use this information to develop a system *in vivo* in which there is a rapid and reproducible increase in collagen. One of the primary objectives of these investigations was to determine if the stimulation of collagen synthesis in the uterus of the immature rat by the administration of estradiol-17β would provide a useful system in which to examine various aspects of collagen metabolism. Our results demonstrate that it is possible to elicit such a response in the uterus of the immature rat and that this response provides a useful system in which to study collagen metabolism *in vivo*.

The synthesis of noncollagenous protein by the uterus of the immature rat is exquisitely sensitive to the stimulatory action of administered estradiol-17β and there has been considerable interest in the relation of these events to early estrogen action.[8]

In studies involving collagen formation, there has been no correlation of the relationship between noncollagenous and collagenous protein synthesis *in vivo*. Such a correlation is shown in this report.

A comparison has also been made of the stimulatory effect of estradiol-17β on collagenous and noncollagenous protein synthesis in the uterus of the immature and adult ovariectomized rat.

MATERIALS AND METHODS

These experiments were conducted using either immature rats or adult ovariectomized rats of the Long–Evans strain.* The immature rats were 19 days old on the day of arrival in the laboratory and only those rats weighing 28–36 g at 19 days of age were used in these studies. For the experiments in ovariectomized rats, adults with an age variation of no more than 7 days were castrated when body weight reached 155–160 g. These animals were delivered to the laboratory 21 days later and the experiment was begun on the following day. Both sham-operated and normal rats were used as controls in these studies. An intraperitoneal dose of estrogen was administered daily in 0·2 ml for a maximum of 5 consecutive days to either immature or ovariectomized rats. Control rats were administered the same number of doses of vehicle which consisted of 5% ethanol in saline. The uterine horns and cervix of each rat were excised intact after removing all adhering tissue. Water within the lumen of the uterine horns was expressed and moisture on the exterior surfaces of the uterus was removed by blotting before determining the wet weight. (The term uterus refers to the uterine horns and cervix throughout this report.) In experiments using the immature rat, it was necessary to pool three uteri to have sufficient tissue to assay for total protein and collagen. In experiments with ovariectomized rats, each uterus was assayed individually. To determine total protein, uterine tissue was homogenized in 5% cold trichloroacetic acid in an all-glass homogenizer, the lipid was extracted and discarded using a standard procedure, and the tissue was then dried and weighed.[8] The dry residue contained approximately 5–8 per cent nucleic acids by weight. To determine the collagen content of uterine tissue, the total protein isolated by trichloroacetic acid precipitation was hydrolyzed for 16 hr at 100° in 1 ml of 6 N HCl and a portion of the hydrolysate was analyzed for hydroxyproline.[9] This method involves oxidizing hydroxyproline to pyrrole with chloramine T and heat, extraction of the pyrrole with toluene, followed by the coupling of this material with *p*-dimethylaminobenzaldehyde (Ehrlich's reagent) to form a colored complex. The optical density of this product is determined at 560 nm and compared with pyrrole standards. Hydroxyproline standards were used to determine recovery (60–70 per cent). Appropriate reagent blanks were included in each assay. The collagen content of the uterus was calculated by multiplying the hydroxyproline value by 7·23.

RESULTS

Effect of estradiol-17β on the wet weight, total protein and collagen content of the uterus of the immature rat. Immature rats were administered either 5 μg estradiol-17β†

* Long–Evans strain rats were purchased from Blue Spruce Farms, Altamont, N.Y.

† This preparation of estradiol-17β is supplied as a chromatographic standard by CalBiochem Company.

TABLE 1. CHANGES IN WET WEIGHT, TOTAL PROTEIN AND COLLAGEN CONTENT OF THE UTERUS OF THE IMMATURE RAT ADMINISTERED ESTRADIOL-17β

Treatment*	No. of doses	Wet wt (mg)	Protein (mg)	Hydroxyproline (μg)	Collagen (mg)
Vehicle	1	18·1 ± 0·2	9·6 ± 0·6	240 ± 8	1·74 ± 0·1
Estradiol-17β	1	32·8 ± 0·6†	15·1 ± 0·6†	311 ± 33	2·25 ± 0·2
Vehicle	2	18·7 ± 0·6	11·7 ± 0·9	252 ± 16	1·82 ± 0·1
Estradiol-17β	2	48·6 ± 0·4†	23·1 ± 1·2†	452 ± 8†	3·27 ± 0·1†
Vehicle	3	20·0 ± 0·2	13·1 ± 1·2	300 ± 25	2·17 ± 0·2
Estradiol-17β	3	52·4 ± 0·2†	26·7 ± 0·6†	589 ± 36†	4·26 ± 0·3†
Vehicle	4	21·1 ± 0·4	12·0 ± 0·9	293 ± 30	2·12 ± 0·2
Estradiol-17β	4	56·6 ± 0·5†	28·7 ± 1·0†	704 ± 15†	5·09 ± 0·1†
Vehicle	5	25·6 ± 0·6	14·2 ± 0·7	373 ± 24	2·69 ± 0·2
Estradiol-17β	5	65·7 ± 0·5†	33·4 ± 0·5†	774 ± 45†	5·60 ± 0·3†

* Groups of 20-day-old rats were administered a daily intraperitoneal dose of either estradiol-17β (5 μg) or an equal volume of the vehicle used to inject estradiol-17β (5% ethanol in saline) for 1–5 days. The uteri were removed from one group of nine estradiol-treated rats and one group of twelve vehicle-treated rats 24 hr after 1, 2, 3, 4 or 5 doses; the uteri were weighed individually and then pooled in groups of three to assay for total protein and hydroxyproline. The collagen content per three uteri was calculated by multiplying the hydroxyproline value by 7·23.

† The mean value (±S.E.) for the estradiol-17β-treated group is significantly larger than that of the group administered vehicle when compared by the t-test ($P < 0·01$).

intraperitoneally or an equal volume of vehicle daily for 1–5 days starting on day 20 of age. The uteri were removed from one group of estradiol-treated and one group of vehicle-treated rats 24 hr after the administration of the final dose of estradiol-17β. The uteri were weighed individually, pooled in groups of three, and then the total protein and collagen of the pooled uteri were determined as described in Materials and Methods. The results of one experiment are shown in Table 1. There is a significant increase in the wet weight of the uterus 24 hr after a single dose of estradiol-17β. The wet weight continues to increase with each succeeding dose. There is little change in the wet weight of uteri of control rats, although some increase always occurs between day 24 and 25 of age. Data demonstrating the reproducibility of this response in two experiments are shown in Fig. 1.

There is a significant increase in total protein of the uterus 24 hr after a single dose of estradiol-17β. Total protein increases more than 200 per cent with the continued administration of estradiol-17β, while the maximum increase in the uteri of control rats was approximately 70 per cent (Fig. 2).

A small increase in the collagen content of the uterus is observed 24 hr after a single dose of estradiol-17β. However, the collagen content of the uterus is not significantly higher than that in control rats until two or more doses of estradiol-17β are administered. The change in the collagen content of the uterus of control and estradiol-17β-treated rats in two experiments is shown in Fig. 3. The reproducibility of this response depends, in part, upon using rats only between day 20 and 25 of age. The increase in the collagen content of the uterus is linear during the first 3 days of estradiol-17β administration (Fig. 4). The loss of linearity is due primarily to the increase in the collagen content of the uteri of control rats. The per cent of collagen in total protein of

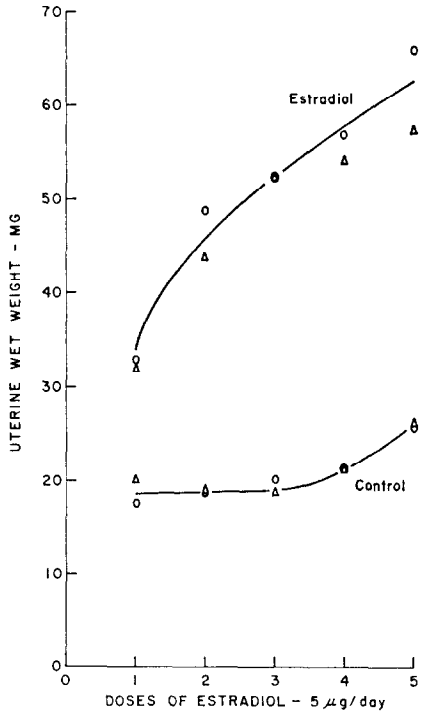

FIG. 1. Change in uterine wet weight of the immature rat administered either 5 μg estradiol-17β or vehicle (control) daily for 1, 2, 3, 4 or 5 consecutive days. Each value is the mean uterine wet weight of twelve rats treated with estradiol-17β and nine rats administered vehicle. The results of two experiments are plotted.

the uterus in these experiments and others remains between 15 and 20 per cent, indicating that estradiol-17β affects neither noncollagenous nor collagenous protein synthesis preferentially but, rather, stimulates total protein synthesis.

A comparison was made of the effect of various doses of estradiol-17β on the wet weight, total protein and collagen content of the uterus of the immature rat (Table 2). Groups of 20-day-old rats were administered either 0·01, 0·1, 1 or 5 μg estradiol-17β daily for 3 days, while control rats were administered vehicle. The uteri were removed from all groups 24 hr after the third dose, weighed and pooled in groups of three for assay of total protein and hydroxyproline. The wet weight and collagen content of the uterus increases significantly after the administration of 0·01 μg estradiol-17β daily for 3 days. A near maximum increase in wet weight and collagen occurs after the administration of 0·1 μg estradiol-17β for 3 days. There is a small increase in total protein of the uterus after the administration of 0·01 μg estradiol-17β. A significant increase in total protein occurs after the administration of 0·1 μg (62 per cent), 1 μg (90 per cent) or 5 μg (118 per cent).

A comparison was made of the effect of administering several natural and synthetic estrogens on wet weight, total protein and collagen content of the uterus of the immature rat (Table 3). Groups of rats were administered 0·1 μg of each of the estrogens

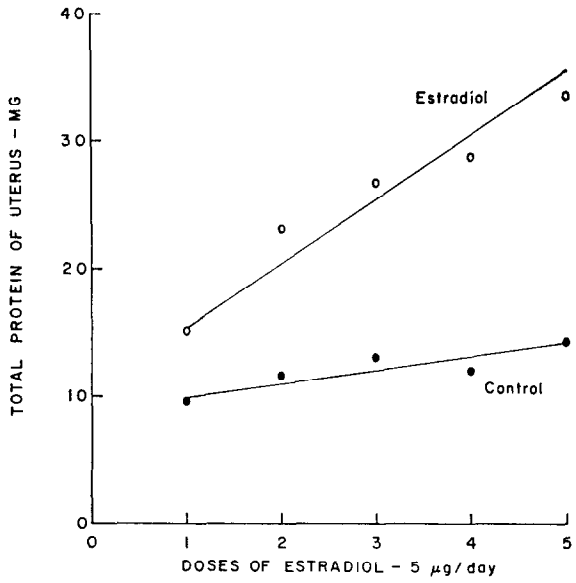

FIG. 2. Change in the total protein content of the uterus of the immature rat administered either 5 μg estradiol-17β or vehicle (control) daily for 1, 2, 3, 4 or 5 consecutive days. Each value is the mean protein content of the pooled uteri (three per assay) from twelve rats treated with estradiol-17β and nine rats administered vehicle. These data are from one of the experiments shown in Figs. 1 and 3.

daily for 3 days as described previously. As expected, estradiol-17α had no effect on the uterus, while estradiol-17β was the most potent of the three naturally occurring estrogens. Estriol administration caused a significant increase in the wet weight and collagen content of the uterus, and a small increase in total protein that was not statistically significant. There was no change in these parameters after the daily administration of 0·1 μg estrone, but significant changes did occur when the dose of estrone was increased to 1 μg/day. However, these changes were still less than those observed with 0·1 μg/day of estradiol-17β. The effect of 0·1 μg 17-ethynylestradiol-3-methyl ether (mestranol) on the uterus was approximately the same as that of an equivalent dose of estradiol-17β. The uterine changes caused by diethylstilbestrol exceeded those caused by the other estrogens.

Effect of estradiol-17β on the wet weight, total protein and collagen content of the uterus of the adult ovariectomized rat. The daily administration of 5 μg estradiol-17β was begun on day 22 after ovariectomy and was continued for 4 consecutive days. The quantitation procedures for total protein and hydroxyproline were the same as those used for the immature rat, except that each uterus was large enough to be assayed separately. The uteri from a group of sham-operated rats were removed for assay each day that uteri from ovariectomized animals were removed during the experimental period. In a second experiment, uteri obtained from a group of normal rats on the first and last day of the experimental period were assayed for wet weight, total protein and collagen. As shown in Table 4, there is a marked fall in the wet weight, total protein and collagen of the uterus 21 days after ovariectomy. These reductions remained

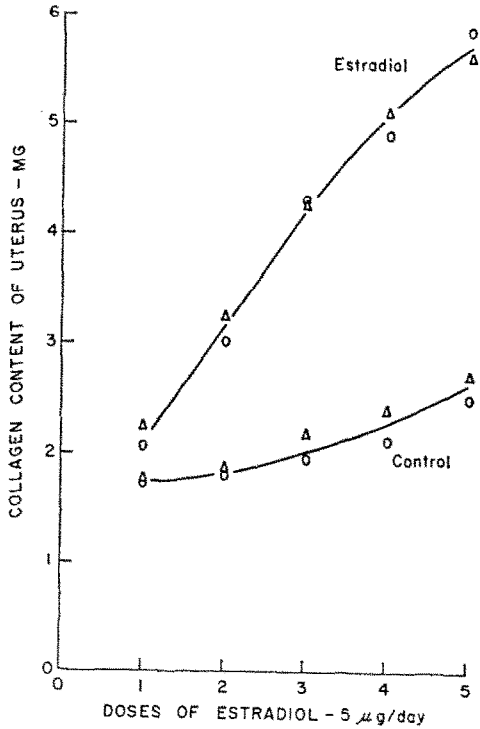

FIG. 3. Change in the collagen content of the uterus of the immature rat administered either 5 μg estradiol-17β or vehicle (control) daily for 1, 2, 3, 4 or 5 consecutive days. Each value is the mean collagen content of the pooled uteri (three per assay) from twelve rats treated with estradiol-17β and nine rats administered vehicle. The results of two experiments are plotted. The mean daily increase in the collagen content of the uterus is plotted in Fig. 4. These data are from the experiments shown in Fig. 1.

relatively constant throughout the experimental period. The mean reductions in wet weight, total protein and collagen for all untreated ovariectomized rats (Ovx + V) were 76, 70 and 59 per cent, respectively, for the experiment shown in Table 4, and 77, 75 and 57 per cent for a second experiment. The per cent collagen in the total protein of the uterus increased from 19 in the sham-operated rat to 32 in the ovariectomized animal, because the protein content of the uterus fell more than the collagen content. Similar results were obtained in a second experiment. A maximum increase in wet weight and total protein occurred in the uterus of the ovariectomized rat after three doses of estradiol-17β, while the collagen content did not change. In a second experiment, the collagen content of the uterus was unchanged after either one, two, or four doses of estradiol-17β, but a significant increase (20 per cent) was observed in the group administered three doses of the hormone. The mean percentage increase in uterine wet weight and total protein in all ovariectomized rats administered estradiol-17β was 82 and 39 per cent, respectively, in one experiment, and 100 and 41 per cent in another experiment. These parameters remained, then, significantly below those found in either sham-operated or normal rats. The mean body weight ranged from 206 to 240 g during the experimental period and there was no significant change in the body weight of rats administered estradiol-17β.

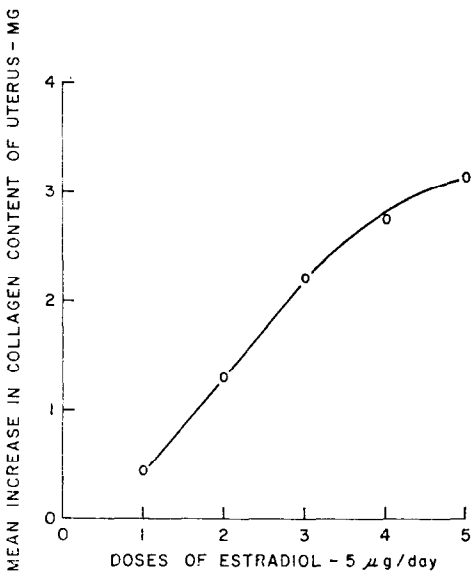

FIG. 4. Mean daily increase in total collagen determined from the data in Table 3.

TABLE 2. DOSE–RESPONSE CHANGES IN WET WEIGHT, TOTAL PROTEIN AND COLLAGEN CONTENT OF THE UTERUS OF THE IMMATURE RAT ADMINISTERED ESTRADIOL-17β

Daily dose of estradiol-17β* (μg)	Wet wt (mg)	Protein (mg)	Hydroxyproline (μg)	Collagen (mg)
0 (vehicle)	19·4 ± 0·1	12·9 ± 0·2	245 ± 3	1·78 ± 0·02
0·01	30·3 ± 0·4†	14·0 ± 0·4	315 ± 4†	2·28 ± 0·02†
0·1	49·2 ± 0·7†	20·9 ± 0·2†	410 ± 7†	2·96 ± 0·05†
1·0	52·5 ± 2·0†	24·5 ± 0·7†	418 ± 7†	3·02 ± 0·05†
5·0	55·1 ± 1·0†	28·1 ± 0·9†	433 ± 1†	3·13 ± 0·01†

* Groups of 20-day-old rats were administered a daily intraperitoneal dose of either estradiol-17β (0·01, 0·1, 1 or 5 μg) or an equal volume of the vehicle used to inject estradiol-17β (5% ethanol in saline) for 3 consecutive days. The uteri from each group of rats were removed 24 hr after the last dose, weighed individually and then pooled in groups of three to assay for total protein and hydroxyproline. There were 12 rats in each group administered estradiol-17β and 24 rats in the group administered vehicle.

† The mean value (±S.E.) for the estradiol-17β-treated group is significantly larger than that of the group administered vehicle when compared by the t-test ($P < 0·01$).

TABLE 3. COMPARISON OF THE EFFECT OF VARIOUS ESTROGENS ON WET WEIGHT, TOTAL PROTEIN AND COLLAGEN CONTENT OF THE UTERUS OF THE IMMATURE RAT

Treatment*	Wet wt (mg)	Total protein (mg)	Collagen (mg)
Vehicle	20·6 ± 1	15·8 ± 1·2	3·36 ± 0·13
Estradiol-17α	21·6 ± 1	14·9 ± 0·5	3·25 ± 0·13
Estradiol-17β	47·9 ± 1†	21·8 ± 0·9†	5·27 ± 0·25†
Estriol	32·3 ± 0·5†	18·0 ± 0·5	4·40 ± 0·08†
Estrone	22·4 ± 0·3	14·9 ± 0·7	3·86 ± 0·25
Diethylstilbestrol	86·9 ± 1†	38·8 ± 0·9†	7·84 ± 0·22†

* Groups of 20-day-old rats (12 rats/group) were administered 0·1 μg of these compounds intraperitoneally daily for 3 consecutive days while control rats were administered an equal volume of the injection vehicle. The uteri were removed 24 hr after the last dose, weighed and pooled in groups of three to assay for total protein and hydroxyproline. The results are expressed as the group mean ±S.E. for four assays.

† The group mean is significantly larger than that of the control (vehicle) group ($P < 0·01$).

TABLE 4. EFFECT OF ESTRADIOL-17β ON WET WEIGHT, TOTAL PROTEIN AND COLLAGEN CONTENT OF THE UTERUS OF THE OVARIECTOMIZED RAT

Treatment*	No. of doses	Wet wt (mg)	Total protein (mg)	Collagen (mg)
Sham-op. + V	1	338·8 ± 30	79·0 ± 6	18·8 ± 1
Ovx + V	1	85·4 ± 3	24·2 ± 1	8·2 ± 1
Ovx + E	1	131·4 ± 9†	25·5 ± 2	8·7 ± 1
Sham-op. + V	2	353·9 ± 27	79·6 ± 5	16·7 ± 1
Ovx + V	2	84·7 ± 9	21·0 ± 1	7·1 ± 0·2
Ovx + E	2	140·4 ± 6†	28·8 ± 2†	7·5 ± 0·5
Sham-op. + V	3	390·9 ± 51	121·1 ± 5	18·6 ± 0·6
Ovx + V	3	84·1 ± 4	24·3 ± 2	7·7 ± 0·4
Ovx + E	3	165·1 ± 9†	38·4 ± 3†	6·8 ± 0·5
Sham-op. + V	4	403·9 ± 29	118·0 ± 7	17·7 ± 1
Ovx + V	4	82·5 ± 6	28·3 ± 3	7·6 ± 0·4
Ovx + E	4	174·5 ± 10†	42·8 ± 2†	8·0 ± 0·3

* Groups of seven ovariectomized (Ovx) or sham-operated rats were administered a daily dose of either 5 μg estradiol-17β (E) or an equal volume of injection vehicle (V) intraperitoneally. The uteri were removed 24 hr after one, two, three or four consecutive doses, weighed and assayed individually for total protein and hydroxyproline content. Results are expressed as the group mean ±S.E.

† The group mean of the Ovx + E group is significantly larger than that of the Ovx + V group.

DISCUSSION

The results presented in this report demonstrate that it is possible to stimulate the formation of collagenous as well as noncollagenous protein synthesis *in vivo* in the uterus of the immature rat administered estradiol-17β. The collagen content of the uterus increases approximately 3-fold when estradiol-17β is administered daily for 5

consecutive days starting with day 20 of age. This response is rapid in onset, follows a linear course, and is quite reproducible under the described experimental conditions. It appears, therefore, to be a useful system *in vivo* to investigate various aspects of collagen metabolism. Current efforts are directed at determining the appearance and magnitude of activity of proline and lysine hydroxylase enzymes in relation to the formation of collagen in the uterus of the immature and ovariectomized rat administered estradiol-17β. Our unpublished findings indicate that the activity of both enzymes increases markedly in the uterus of the immature or ovariectomized rat administered estradiol-17β.

The accumulation of collagen in the uterus of the immature rat administered estradiol-17β appears to be due to a nonspecific stimulation of protein synthesis, since the per cent collagen in total uterine protein does not change significantly throughout 5 days of estradiol-17β administration. It is possible that the accumulation of collagen is due, in part, to an inhibition of collagenase or other degradative enzymes by estradiol-17β. It is more likely, however, that a low concentration of active collagenase in the uterus would, in effect, allow an accumulation of this protein.[10]

Published reports vary regarding the direction of change of the concentration and total uterine content of collagen following estradiol-17β administration to the ovariectomized rat.[5,11,12] These differences may be due in part to variation in the duration of ovariectomy, the dosage schedule of estrogen, and the method used in quantitating collagen formation. Under our experimental conditions, the per cent collagen in total uterine protein increases 21 days after ovariectomy, but this increase is due simply to a relatively larger fall of noncollagenous protein than of collagen. Both collagenous and noncollagenous protein of the uterus fall markedly during the 21-day period after ovariectomy. In contrast to results obtained in the immature rat, the administration of estradiol-17β to the adult ovariectomized rat causes little, if any, accumulation of collagen in the uterus, while increasing the noncollagenous protein content significantly. The reason for this is not known. It would, however, be of interest to determine if the absence of accumulation of collagen in the uterus of the ovariectomized rat administered estradiol-17β is due to an increase in the turnover of this protein.

There is evidence that collagen synthesis lags behind general protein synthesis in certain tissues of the rat, such as the uterus, symphysis pubis and regenerating liver.[7,13,14] It has been suggested that this delay is related to the formation of collagen precursor material.[7] Our own results in the uterus of the immature rat administered estradiol-17β appear to be in accord with these findings, since there is a significant increase in the noncollagenous protein content of the uterus 24 hr after a single dose of estradiol-17β, while a significant increase in the collagen content of the uterus is first observed 24 hr after the second dose of the hormone. In tissue culture, this lag appears to be correlated with the appearance of the active form of proline hydroxylase.[3]

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