EFFECT OF ESTRADIOL-17β ON COLLAGEN BIOSYNTHESIS, DEGRADATION AND RE-UTILIZATION IN VIVO

Dorothy H. Henneman, M.D.
Ortho Research Foundation, Raritan, N. J.

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SUMMARY. Estradiol-17β was administered to adult, female guinea pigs in which collagen had been labelled during rapid growth by the chronic administration of ^{14}C-L-proline. In skin, treatment reduced collagen biosynthesis and increased collagen degradation for de-novo biosynthesis elsewhere. In the granuloma produced by γ-carrageenan, estradiol-17β produced no change in the total amount of newly-synthesized collagen, in the amount of ^{14}C-label derived from the degradation of prelabelled collagen or in the amount derived by the re-utilization of acid-soluble collagen. In the uterus and metaphyseal bone, estradiol-17β increased both collagenous and non-collagenous de-novo protein biosynthesis. The proline for this was derived, at least in part, from the degradation of prelabelled collagen, probably from skin.

Estradiol-17β has been shown to inhibit the effects of both penicillamine and β-aminopropionitrile on skin and bone collagen (1,2). This estrogen may therefore modify the biochemical pathways concerned with collagen polymerization. Increases in tensile strength of skin following the administration of estradiol-17β lend support to this suggestion (2). Nevertheless, changes in the rate of collagen biosynthesis, degradation, or re-utilization may also be responsible for the observed changes. Estradiol-17β, administered in vivo, appears to inhibit collagen biosysthesis in skin thereby decreasing the content of soluble collagen and total skin mass (1,2). In bone, on the other hand, total bone mass, the percentage wet weight extractable with 10% EDTA, and the percentage of insoluble, non-extractable organic matrix are increased (2,3). Because of these diverse effects of estradiol-17β in different connective tissues, its effects on total body collagen metabolism was studied in adult female guinea pigs in which all collagen had been prelabelled by the chronic administration of ^{14}C-L-proline during the period of rapid growth.

MATERIAL & METHODS. Six female guinea pigs (200g) were studied using the

technique of Klein, et al for prelabelling collagen by the chronic administration of labelled proline (4,5). Vitamin C (50mg/d), Purina chow, and supplemental greens were administered throughout the study (78 days). ^{14}C- proline (UL)[1] was injected intramuscularly every day for thirty days (total dose 1000μCi/k, days 1-30) to all six animals. Estradiol-17β in cottonseed oil was injected subcutaneously every day for 36 days (10-15 μg/k/d, days 43-78) to four animals. The dose of estrogen was monitored with care so that the rate of body-weight gain was the same for the control and treated animals. Unlabelled proline (770mg/d) was administered by stomach tube to all animals on days 59-78. On day 69, γ-carrageenan[2] (4ml of a 1% solution in 0.15M NaCl) was injected subcutaneously in the abdomen of all animals for the production of newly-synthesized collagen. All animals were sacrificed on day 79.

The animals were decapitated and exsanguinated, and aliquots of skin, granuloma, uterus and metaphyseal bone taken for study. Details of all procedures used in these studies have been published (1,6). Trunk skin, exclusive of the area above the granuloma, total granuloma and uterus, and pooled metaphyseal fragments (lower femoral and upper tibial) from both hind limbs were weighed. In each of these four tissues analyses were made of (1) total ^{14}C-label per unit weight and also in the total tissue, (2) collagen, nitrogen (7), and non-collagenous protein[3] per unit weight and also in the total tissue, and (3) proline and hydroxyproline specific activity in the collagen and proline specific activity in the non-collagenous protein. Aliquots of skin and granuloma were serially extracted in neutral salt (0.15M and 0.50M NaCl) and in acid, pH 3.6 (0.50M NaCi-

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1. New England Nuclear, specific activity 205μCi/μmole proline (UL).
2. Sea Kem, #7, purified carrageen. Marine Colloids, Inc.
3. One mg of collagen contains 13.3% hydroxyproline, or approximately one μmole, and 18.6% nitrogen, or 13.3μmole. One mg of non-collagenous protein contains 11.4μmole nitrogen. Total nitrogen in a tissue expressed as μmoles (minus the 6% due to non-protein nitrogen) minus collagenous nitrogen in μmoles and divided by 11.4 equals a good approximation of the mg non-collagenous protein present in a given tissue.

trate) and the specific activity of proline and hydroxy-proline in these extracts and in the insoluble residue measured. The percentage wet weight extractable by defatting and drying, by treatment with 10% EDTA, pH 7.4, followed by defatting and drying, and the percentage of residual organic matrix were measured on the metaphyseal bone (1,2).

RESULTS & DISCUSSION. A. Skin. As reported earlier (1,2), total skin mass, the percentage of fat and water, and the content of collagen soluble in neutral salt and acid were markedly reduced by treatment with estradiol-17β. The relative content of insoluble collagen was increased.

The specific activity of hydroxy-proline was increased by treatment in the neutral salt- and acid-soluble collagen (Table 1) in all four treated animals. Total label in hydroxy-proline in total skin was unchanged (Table 1). Treatment had no significant effect on non-collagenous protein content but decreased the total amount per animal due to the marked reduction in skin mass. A small but consistent increase in the specific

Table 1. Specific activity of hydroxy-proline (OH-P) in neutral-salt-(0.15M + 0.50M NaCl) and acid-soluble and insoluble collagen of skin, total OH-P ^{14}C-label in trunk total skin, and proline (P) specific activity in non-collagenous protein(P-NCP) in control and estradiol-17β-treated (E_2) guinea pigs.

No. ANIMAL	COLLAGEN FRACTIONS OH-P ($dpm \times 10^3/\mu mole$) NaCl-Soluble	Acid-Soluble	Insoluble	TOTAL SKIN OH-P ($dpm \times 10^6$)	P-NCP ($dpm \times 10^3/\mu mole$)
1. Control	4.58	10.21	5.27	14.17	1.24
2. Control	4.98	6.29	5.14	12.57	1.19
3. E_2	11.13	13.99	7.24	12.13	1.32
4. E_2	7.35	10.63	5.26	10.31	1.28
5. E_2	10.02	12.53	7.53	14.83	3.40
6. E_2	8.06	10.82	5.96	9.20	1.45

activity of proline in the non-collagenous protein was observed (Table 1).

These changes suggest that estradiol-17β produced a decrease in collagen biosynthesis in skin in the presence of normal or increased collagen degradation. Degradation to constituent amino acids rather than to larger collagen fractions appeared to have taken place since treatment produced a marked reduction in acid-soluble collagen, the fraction which Klein, et al believe is the precursor for collagen re-utilization without total degradation (4,5). Since the total ^{14}C-label was unchanged, de novo biosynthesis from prelabelled collagen cannot explain the higher specific activity in the soluble collagen fractions of the treated animals.

B. Granuloma. The administration of estradiol-17β produced no change in the total weight or the amount of collagen in the granuloma. The content of neutral salt- and acid-soluble collagen, however, was reduced and the relative amount of insoluble collagen increased as already reported (6). Although, as in skin, the specific activity of proline and hydroxy-proline was increased in the neutral salt-soluble collagen, it was decreased in the acid-soluble fraction (Table 2). The amount of ^{14}C-label and the percentage of total ^{14}C-skin-label present in the granuloma were not modified by treatment (Table 2). The increased percentage of skin-label in the granuloma of animal 6 reflects the decreased OH-P label in the skin (Table 1,#6) rather than a significant increase in reutilized skin collagen in the granuloma. Estradiol-17β did not inhibit collagen biosynthesis in the granuloma despite the fact that the content of soluble collagen was reduced. This suggests that rapid polymerization of the soluble fractions to the insoluble residue may have occurred. The low specific activity as well as the low content of acid-soluble collagen suggest that this estrogen may have reduced the re-utilization of collagen from other connective tissues to the granuloma. As already indicated, Klein et al (4,5) have suggested that redistribution of acid-soluble collagen provides a pathway for in vivo collagen re-utilization.

Table 2. Specific activity of hydroxy-proline (OH-P) in acid-soluble collagen, total ^{14}C-label in the whole granuloma produced by the injection of γ-carrageenan, and the percentage of the total label in skin present in the granuloma in control and estradiol-17β-treated (E_2) guinea pigs.

	Acid-Soluble Collagen ($dpmx10^3/\mu m$ OH-P)	Total Counts ($dpmx10^6$)	% of Label in Skin present in granuloma %
1. Control	1.80	2.919	9.8
2. Control	0.75	2.475	9.2
3. E_2	0.31	2.560	10.2
4. E_2	0.19	2.147	9.7
5. E_2	0.37	3.364	10.2
6. E_2	0.16	2.906	14.9

Table 3. Specific activity of proline (P) and hydroxy-proline (OH-P) in uterine collagen, the total proline label in non-collagenous protein (P-NCP), and the total ^{14}C-label in the collagen of the whole uterus in control and estradiol-17β-treated (E_2) guinea pigs.

No. ANIMAL	COLLAGEN ($dpmx10^3/\mu mole$) P	OH-P	P-NCP ($dpmx10^3$)	TOTAL ^{14}C-LABEL COLLAGEN ($dpmx10^3$)
1. Control	4.13	10.16	969	476
2. Control	3.33	8.33	693	378
3. E_2	1.22	3.33	1912	705
4. E_2	1.99	4.25	2657	760
5. E_2	2.55	5.73	2118	843
6. E_2	3.06	7.85	1889	1015

C. Uterus. Treatment with estradiol-17β increased, as expected (8), total uterine weight, collagen, and non-collagenous protein. The content of these

per unit dry weight were also increased. The specific activity of proline and hydroxy-proline in collagen and of proline in non-collagenous protein were decreased (Table 3), but the total label in these proteins was markedly increased (Table 3).

Estradiol-17β treatment appeared therefore to have increased uterine collagen and non-collagenous protein derived, at least in part, from the degradation of prelabelled collagen from other connective tissues such as skin. Since soluble collagen was not extracted, the possibility of the re-utilization of acid-soluble collagen as a source cannot be ruled out.

D. Bone. The administration of estradiol-17β has been shown to increase the wet weight and the dried, defatted, EDTA-extracted (10%, pH 7.4) weight of metaphyseal bone (1,2). The percentage of the wet weight remaining as organic matrix is also increased. Comparable changes were observed in the treated animals in this study (Table 4). The specific activity of hydroxy-

Table 4. Composition and weight of metaphyseal bone from both hind limbs of control and estradiol-17β-treated (E_2) guinea pigs.

No.	WET WT	WEIGHT of DRIED DEFATTED, EDTA-EXTRACTED BONE	% EXTRACTABLE with 10% EDTA	% ORGANIC MATRIX
	mg	mg	%	%
1. Control	523.9	81.2	36.80	15.15
2. Control	439.4	65.0	32.25	14.80
3. E_2	522.4	84.3	37.16	16.14
4. E_2	695.3	122.6	41.66	21.56
5. E_2	530.1	92.2	42.81	17.40
6. E_2	637.4	112.4	42.88	17.64

proline in collagen and of proline in the non-collagenous protein was increased by estradiol-17β (Table 5). The content per unit dry weight and the total amount in the pooled bone of non-collagenous protein were both increased (Table 5

Table 5. Specific activity of hydroxy-proline (OH-P) in collagen and of proline in non-collagenous protein (P-NCP), and total non-collagenous protein in metaphyseal bone (mg NCP in dried, defatted, EDTA-extracted pooled metaphyses of both hind limbs) in control and estradiol-17β-treated (E_2) guinea pigs.

No. ANIMAL	COLLAGEN ($dpmx10^3/\mu mole$ OH-P)	NCP ($dpmx10^3/\mu mole$ P)	Total Bone NCP (mg)
1. Control	0.676	0.084	5.328
2. Control	0.893	0.045	7.068
3. E_2	1.534	0.108	15.866
4. E_2	1.764	0.215	12.063
5. E_2	1.735	0.386	18.110
6. E_2	1.358	0.260	18.952

These changes suggest that estradiol-17β produced an increase in the biosynthesis of both collagen and non-collagenous protein in metaphyseal bone and, as in the uterus, enhanced the utilization of proline from degraded prelabelled collagen of other connective tissues (probably from skin) for this de novo biosynthesis. The possibility that acid-soluble collagen derived from the prelabelled collagen might have been re-utilized for part of this biosynthesis cannot be ruled out since soluble collagen was not extracted from the bone.

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