tained by plotting the reciprocal of the progoitrin converted on a linear scale (1/V) against the reciprocal of the square of the progoitrin concentration on a linear scale ($1/S^2$) or of the progoitrin concentration on a logarthmic scale (log 1/S), whereas a curve is obtained if 1/V is plotted against 1/S on linear scales. The straight lines in themselves are not significant for the derivation of kinetic data, but they do suggest some degree of complexity in the reaction assayed. It is not known whether this complexity arises from the use of intact cells, the long incubation period, the possibility of two enzymatic reactions in sequence, the possibility of progoitrin serving as both activator and substrate of a single enzyme, or indeed from all or from none of these.

Summary. Myrosinase activity could be demonstrated in a variety of bacteria, particularly *Paracolobactrum,* which commonly inhabit the intestinal tract of man. Intact cells and sonicates were equally active. Ascorbate inhibited rather than potentiated this bacterial myrosinase activity.

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Connective Tissue XIII. Effect of Estradiol Benzoate upon Collagen Synthesis by Sponge Biopsy Connective Tissue.* (30182)

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Recently, we reported(1) a stimulating effect by several estrogenic substances on collagen synthesis in rat uterine tissue slices. Estradiol was the most potent. The effect was more pronounced in the hormone deficient animal than in the normal animal and was elicited only when the estrogens were given *in vivo*. Only a few studies have been made on the effects of estrogens on collagen synthesis in tissues other than uterus. Most of these reports have been concerned with changes in ground substance(2-5). An inhibitory effect of estrogen on the synthesis of DNA and protein in L-strain fibroblast tissue cultures has been recently reported by Kuchler *et al*(6). The parenteral administration of large doses of estrogen in mice has been reported to stimulate the deposition of new bone(7). This was believed to be a reaction of the primitive marrow to the injury caused by large doses of estrogen. On the other hand, a decrease in collagen in the bones of rats after estrogen administration was reported by Sobel *et al*(8). Recently, based on the application of histological techniques, Kelly has concluded that estrogen stimulates the growth of collagen in sponge connective tissue of normal rats(9).

Since any influence of estrogens can be easily demonstrated with a technique for *in vitro* synthesis of collagen(10,11), we have employed sponge connective tissue slices to determine the effect of *in vivo* administration of estradiol benzoate upon *in vitro* collagen synthesis in normal and ovariectomized rats. Our study included an estimation of 1) total amount and concentration of col-

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lagen; and 2) *in vitro* synthesis of collagen as determined by isotopic analysis.

Methods. Ovariectomized rats and normal controls were purchased from Hormone Assay Laboratories, Inc., Chicago, Ill., and shipped to us 7 days after operation. The rats were fed Purina Chow and water *ad libitum*. Ivalon sponges† 0.200 g (approximately 6 × 14 × 25 mm^3) were implanted subcutaneously on the back of the rats by the method of Boucek and Noble(12). Various amounts of estradiol benzoate‡ as shown in Table I were dissolved in 0.2 ml sesame oil and injected intramuscularly twice weekly for 4 weeks. Two-tenth ml of sesame oil was given to the control rats in order to eliminate any effect caused by the solvent. At the end of the experimental period, the sponges were removed. One gram of sponge tissue slices from each rat was incubated in 5 ml Krebs-Henseleit medium(13) with 1.875 μc C^{14}-lysine, at 37°C for 6 hours in a Dubnoff incubator. The atmosphere used was 95% oxygen and 5% CO_2. At the end of incubation period, the sponge slices were removed, blotted and homogenized with saline containing 0.25% lysine. The mixture was centrifuged at 15,000 rpm for 30 minutes and the residue washed several times with physiologic saline. Collagen was extracted from the residue by Fitch's method(14) and hydrolyzed in 6 N HCl for 16 hours in a sealed tube. Nitrogen was determined by Kjeldahl digestion and the Conway diffusion technique (15). Hydroxylysine and lysine were separated on Dowex 50-X8 column by Moore and Stein's method(16). The amount of amino acid was determined by the ninhydrin reaction(17). Radioactivity was measured in a Nuclear Chicago Low Background Counter, as reported previously(11). Data were not corrected for the counting efficiency (47%).

Results. The effect of estradiol benzoate upon collagen synthesis by sponge biopsy connective tissue in normal and ovariectomized rats is shown in Table I. The tissue weights were 10-15% lower in both normal and ovariectomized-treated rats as compared with the comparable rats which had received no treatment. The concentration of collagen was approximately 10% lower in the hormone-treated than in the untreated animals. Twenty to 25% less collagen was present in both normal and ovariectomized rats after the estradiol treatment. However, the isotopic data revealed a stimulatory effect of estrogen upon collagen synthesis. The results were significant in all experiments with tissue from ovariectomized estradiol-treated rats. The specific activities of collagen, hydroxylysine and lysine were approximately 50-100%; 200-1000% and 50-300% higher respectively in the estradiol-treated ovariectomized sponges than in the untreated sponges. The counts per minute recovered as collagen per incubation were 40-80% higher than those from untreated controls.

The minimum dose of estradiol benzoate which gave a significant increase in stimulation of collagen synthesis in sponge biopsy connective tissue was 300 μg twice weekly for 4 weeks.

In the normal animals, estradiol benzoate produced similar stimulating effects, but to a lesser extent. The effect was not statistically significant.

Discussion. The effect of estrogen upon uterine growth is a well known phenomenon (19-24). The role of protein synthesis in early estrogen action has been discussed by Mueller *et al*(25). Recently Morgan(26), Smith(27), Fainstat(28) and workers in our laboratory(1) have reported that estrogens are effective agents for stimulating collagen synthesis in the rat uterus. Therefore, a stimulating effect of estrogen upon collagen synthesis in the rat uterus is well established.

A stimulating effect of estrogen upon collagen synthesis in tissues other than the uterus has been seriously questioned, due to the conflicting results previously reported (2-9). Stimulation of bone formation has been reported in mice but was not observed in other animals(7,29-31). Our chemical analyses have shown a slight decrease in both total amount and concentration of collagen in the sponge in estradiol-treated animals. This is in agreement with the results of others, who have found a decrease in bone

† Clay Adams, New York.

‡ Purchased from Nutritional Biochemicals Corp., Cleveland, Ohio.

TABLE I. The Effect of Estradiol Benzoate upon Collagen Synthesis by Sponge Biopsy Connective Tissues in Normal and Ovariectomized Rats.

Animal status	No. of animals	Wt of sponges (g)	Collagen/g of sponge (mg)	Total collagen /sponge (mg)	Collagen formed *in vitro* as measured by radioactivity: Specific activity (cpm/mg) Collagen	OH-Lysine	Lysine	cpm recovered /incubation
Exp 1								
Ovariectomized								
a. Oil only	6	2.19 ± .06†	21.53 ± 1.33	47.40 ± 3.20	79 ± 7	700‡ 559	777‡ 939	1,660 ± 102
b. E.B.* treated 1 μg	7	1.99 ± .03	20.32 ± .81	40.60 ± 1.60	84 ± 6	1,110 1,280	1,350 1,640	1,670 ± 88
c. " " 30 μg	6	1.95 ± .10	20.13 ± 1.06	40.10 ± 1.00	88 ± 8	604 1,270	963 1,550	1,770 ± 210
d. " " 300 μg	6	1.92 ± .06	20.00 ± 1.31	37.60 ± 2.88	119 ± 5	1,330 1,500	1,850 1,910	2,380 ± 205
P		a∼b <.02 a∼d <.01		a∼d <.05 a∼e <.05	a∼d <.01			a∼d <.001
Normal								
e. Oil only	6	2.02 ± .13	18.68 ± .58	37.80 ± 2.01	101 ± 8	1,210 1,160	1,560 1,610	1,900 ± 169
f. E.B. treated 300 μg	6	1.70 ± .06	17.09 ± .61	28.80 ± .71	124 ± 9	1,410 1,560	1,650 1,820	2,090 ± 95
P		e∼f <.05		e∼f <.01				
Exp 2								
Ovariectomized								
g. Oil only	4	2.10 ± .04	21.19 ± .40	44.50 ± .84	35 ± 2	66 48	467 360	747 ± 40
h. E.B. treated 300 μg	6	1.98 ± .03	18.25 ± .08	36.05 ± .75	69 ± 4	424 411	936 887	1,260 ± 54
P			g∼h <.01	g∼h <.001	g∼h <.001			g∼h <.001
Exp 3								
Ovariectomized								
i. Oil only	11	1.87 ± .04	20.97 ± .68	39.38 ± 1.88	48 ± 9	348 358	476 1,074	1,020 ± 189
j. E.B. treated 300 μg	11	1.78 ± .03	21.21 ± .53	37.79 ± 1.16	89 ±11	1,498 1,200	1,890 1,520	1,850 ± 200
P					i∼j <.01			i∼j <.01

* Estradiol benzoate treated, 1, 30 or 300 μg twice weekly for 4 wk. † Mean ± standard error(18). ‡ Two samples of each group were used for separation of hydroxylysine and lysine.

collagen(8) and an inhibition of growth of fibroblasts(6) following estrogen treatment. However, our isotopic data definitely demonstrate greater specific activities of collagen, hydroxylysine and lysine, and recovered radioactivity per incubation in the sponge tissue of the estradiol-treated than in the corresponding tissue of the untreated animal. From these data, it is asumed that estradiol benzoate induces not only an active synthesis, but also an active catabolism of collagen. This deduction is further supported by the observed decrease in amount and concentration of collagen in this tissue in the hormone-treated animal. The stimulating effect of estradiol benzoate on collagen synthesis in sponge biopsy connective tissue is different from that in uterus. In the latter, the stimulation of synthesis of collagen was much greater than the catabolism of collagen, thus resulting in an increase in the amount of collagen(1).

Summary. 1. The effect of estradiol benzoate upon collagen synthesis in sponge biopsy connective tissue has been studied in normal and ovariectomized rats. 2. A dose of 300 μg, twice weekly for 4 weeks, produced a decrease in sponge weight, collagen concentration and total amount of collagen. However, the radioisotopic data demonstrated a stimulating effect of estrogen on collagen synthesis, as there was an increase in the specific activity of collagen, hydroxylysine and lysine and the total counts per minute recovered per incubation. These results lead to the conclusion that estradiol stimulates the metabolism of collagen. 3. The stimulatory effect of estradiol on collagen synthesis in sponge biopsy connective tissue is more readily demonstrated in the ovariectomized than in the intact rat.

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