in the adrenal denervated animal(4). In hypophysectomized animals, 2-DG induced catecholamine release but no increase in FFA. This confirms that some pituitary factor (or factors) is necessary for the lipolytic action of catecholamines(12). Our experiments do not reveal the nature of this pituitary factor(s), but do show that the pituitary-adrenocortical system is either of no importance or requires some additional pituitary factor. In view of earlier findings(13, 14) it seems likely that lack of thyroid hormone(s) is responsible for the altered response to 2-DG in the hypophysectomized rat.

The reason for the decreased FFA after 2-DG in the adrenalectomized, cortisone treated rat is not clear. A possible explanation would be the increased insulin release occurring after 2-DG(15); furthermore, injection of insulin has been found to reverse the effect of 2-DG on FFA in humans(11). Another alternative would be that 2-DG as such affects specific reactions involved in FFA metabolism within the adipose tissue or elsewhere and that this effect of 2-DG *in vivo* becomes evident only in the absence of the adrenal medulla.

Summary. Plasma free fatty acids (FFA) were followed in the rat after injection of 2-deoxy-D-glucose (2-DG). In the intact animal 2-DG caused elevation of FFA, probably due to release of adrenal medullary hormones. In the hypophysectomized rat there was no change in FFA after 2-DG, in spite of pronounced secretion of catecholamines and independent of cortisone substitution. In the adrenalectomized rat 2-DG caused a marked decrease of FFA, likewise independent of cortisone medication.

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Connective Tissue XII. Stimulating Effects of Estrogens on Collagen Synthesis in Rat Uterine Slices.* (29504)

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The uterotrophic effect of estrogen and other steroids(1-6) has been ascribed partly to water inhibition(1,2,7) and partly to uterine growth(3,7). While studying the nature of these changes, Harkness and Harkness (8) and Woessner(9) observed in rats and humans, respectively, that during pregnancy, total uterine collagen increased steadily.

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Harkness *et al* reported(10) that the increase in weight of the pregnant uterus was principally due to noncollagenous protein (NCP). We have reported that the uterus of the rat has a greater capacity for *in vivo* synthesis and degradation of collagen than any other tissue studied(11). Later it was shown that the rat uterine slices can synthesize collagen *in vitro*(12) and that degree of synthesis was related to the estrous cycle (18).

Kao's uterine slice technique of incubation (KUST)(12) has now been used to study the action of estrogens on *in vitro* synthesis of collagen and NCP. The present report describes the effects of female sex hormones on protein synthesis in the uteri of normal and ovariectomized rats *in vivo* and *in vitro*. The data include: (a) total amount and concentration of collagen and NCP, (b) synthesis of collagen as measured by conversion of C^{14}-lysine to C^{14}-hydroxylysine, (c) synthesis of NCP as measured by incorporation of C^{14}-lysine, and (d) effective dosage of estrogen and useful dose-sacrifice intervals.

Material and methods. Ovariectomized rats and unoperated controls, body weights between 200 and 250 g, purchased from Hormone Assay Laboratories, Inc., Chicago, Ill., were delivered to our laboratory 7 days after operation and were used in the experiments 5 to 7 days later. The rats were given Purina Chow and water *ad libitum*. For intramuscular injection, each dose of estrogen† was dissolved in 0.1 ml of sesame oil;† the progesterone,† in 0.1 ml of propylene glycol. In the *in vitro* experiments, the estradiol was dissolved in 0.1 ml of acetone and added to the incubation medium.

Experimental. Unless otherwise indicated, each experiment involved animals from a single shipment with age and weight closely matched. Normal and ovariectomized controls from corresponding groups of animals were employed. In certain instances sham injections of sesame oil were given to determine the influence, if any, of manipulation on the uterine changes. In most of the experiments 1.0 μg estradiol benzoate intramuscularly daily for 3 days was given, but larger, smaller and single injections were also used for comparison. When 3 daily doses were employed, the animals were killed with chloroform 3 hours after the last injection. With single doses of 1.0 μg they were sacrificed 3 or 16 hours later.

Four-tenths gram of uterine slices from pooled sample of each group of animals was incubated at 37°C with 1.875 μc of uniformly labeled C^{14}-lysine† (specific activity 52.7 μc/mg) in 5 ml Krebs-Henseleit medium under an atmosphere of 95% O_2 and 5% CO_2 (12). After 6 hours incubation the slices were removed, blotted and homogenized in 10 ml saline containing 0.25% lysine. The homogenate was centrifuged at 15,000 rpm for 30 min. The saline supernate was combined with the incubation medium. Collagen was extracted from the residue and hydrolyzed; its hydroxylysine and lysine were separated by column chromatography; and chemical and radioactivity levels were determined as reported previously(12). Data are not corrected for counting efficiency because this was constant at 40%.

In one experiment (Table V) the protein in the combined incubation medium and saline supernate, for convenience termed supernatant protein (SP), was precipitated with 5% trichloroacetic acid (TCA) and the precipitate was washed several times with 5% TCA. The amount and radioactivity of the SP and also the protein in the residue left after extraction of collagen (NCP) were determined(12).

Throughout all experiments, uterine and collagen weights refer to *in vivo* effects, while all specific activities refer to results obtained from the incubation of uterine slices *in vitro*.

Results. Experiment 1. Effect of ovariectomy on collagen synthesis by rat uterine slices (Tab. I & Fig. 1). The concentration of collagen in the uterus of the ovariecto-

† Estradiol benzoate was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio, and uniformly labeled C^{14}-lysine (specific activity 52.7 μc/mg) from Nuclear-Chicago Corp., Chicago, Ill. Estrone, estriol and progesterone were made available through the courtesy of Upjohn Co., Kalamazoo, Mich., and sesame oil through the courtesy of Parke, Davis and Co., Detroit, Mich.

TABLE I. Effect of Ovariectomy on Collagen Synthesis by Rat Uterine Slices *in vitro*.
(Exp 1)

Animal status	No. rats	Uterine wt/rat (g) (A)	No. detn.	Collagen (mg) per Uterus: Incubation‡ (B)	Collagen (mg) per Uterus: (C) = (B/.4 × A)	Specific activity (cpm/mg): Collagen (D)	Specific activity (cpm/mg): Hydroxy-lysine§	Specific activity (cpm/mg): Lysine§	Total cpm recovered: Per incubation (B × D)	Total cpm recovered: Per uterus (C × D)
Ovariex	7	.11 ± .00*	2†	22.86 ± .01	6.39	189 ± 15	593	5,520	4,320 ± 341	1,190
							547	3,750		
Normal	4	.54 ± .11	4	13.92 ± .76	18.79	922 ± 124	10,100	15,200	12,800 ±1,370	17,300
							12,200	17,700		
P		<.001		<.01		<.01			<.01	

* Mean ± S.E.(13). † Combined samples from more than 2 animals. ‡ Each incubation represents .4 g of uterine tissue. § Data in these columns represent specific activity for individual samples, *i.e.* all samples have not necessarily been chromatographed on the Dowex column.

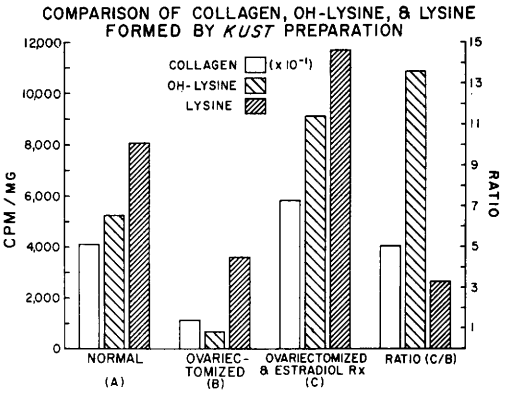

FIG. 1. Comparison of specific activities of collagen, hydroxylysine and lysine formed by uterine slices from normal, ovariectomized and ovariectomized-estradiol-benzoate-treated donor rats. Each bar represents an average of all determinations made in the 7 experiments recorded. Average specific activity = Σ (no. of determinations × specific activity)/total determinations.

mized animals was 100% higher than that of the controls, but total collagen was two-thirds less.

Incubation of uterine slices from ovariectomized rats gave collagen, hydroxylysine and lysine fractions with specific activities approximately 20%, 5% and 25%, respectively of those observed with uteri from normal rats. The total counts per minute recovered in the collagen fraction of incubated uteri of ovariectomized rats were 7% those of the normal rats.

Experiments 2 and 3. Effect of several ovarian hormones on collagen synthesis by rat uterine slices (KUST) (Tab. II & Fig. 1). In each of these experiments, analyses for normal and ovariectomized untreated animals have been simultaneously included to afford each individual study its own control. The data so derived from normal and ovariectomized rats are confirmatory of the results of Exp. 1. After ovariectomy, the concentration of uterine collagen was 55 to 60% higher than in the normal animal, while the total collagen was 67% less. Specific activities of collagen, hydroxylysine and lysine fractions were approximately 40%, 20% and 75% those found in the uterine slices from normal animals. Sesame oil had no effect on the above measurements in any of the experimental animals.

TABLE II. Effect of Ovarian Hormones Administered to the Donor Rat upon Collagen Synthesis by Rat Uterine Slices. (Exp 2 and 3)

Animal status	No. rats	Uterine wt/rat (g) (A)	No. detn.	Insoluble collagen (mg) per Uterus: Incubation‡ (B)	Uterus (C) = (B/.4 × A)	Collagen formed *in vitro* as measured by radioactivity: Specific activity (cpm/mg) Collagen (D)	Hydroxylysine§	Lysine§	Total cpm recovered: Per incubation (B × D)	Per uterus (C × D)
2 Ovariex										
a No treatment	12	.14 ± .01*	4†	23.12 ± .45	8.09	92 ± 3	593 654	3,390 3,330	2,130 ± 57	744
b E.B.,¶ 3 × 1 μg‖	7	.32 ± .02	4	10.81 ± .17	8.65	703 ± 65	14,900 9,800	16,000 12,000	7,600 ± 307	6,080
c Progesterone, 3 × 2 mg	7	.14 ± .01	2	19.80 ± .31	6.93	150 ± 6	1,530 1,150	3,970 4,030	2,970 ± 96	1,040
2d Normal	3	.57 ± .08	4	17.70 ± .08	25.22	223 ± 39	2,540 2,880	3,810 4,440	3,950 ± 320	5,620
P		a∼b or d <.001		a∼b or d <.001 a∼c <.01		a∼b or c or d or e <.001 b∼c <.001			a∼b <.001 a∼c or d <.01	
3 Ovariex										
a Oil control	8	.12 ± .01	2	22.69 ± .90	6.81	80 ± 1	507	2,120	1,820	544
b E.B.,¶ 3 × 1 μg	4	.33 ± .03	3	13.88 ± .14	11.45	391 ± 10	6,100	7,530	5,430	4,480
c Estrone, 3 × 1 μg	9	.14 ± .01	3	22.22 ± .64	7.78	151 ± 6	925	3,870	3,360	1,170
d Estriol, 3 × 1 μg	9	.13 ± .00	3	20.05 ± .21	6.52	138 ± 5	868	3,970	2,770	900
3e Normal	4	.44 ± .07	4	15.92 ± .23	17.51	208 ± 12	2,350	3,900	3,310	3,640
P		a∼b or e <.001		a∼b or e <.001 a∼d <.05		a∼b <.001 a∼c or d or e <.01 b∼e <.001				

* † ‡ § Same as in Table I.

‖ Ovarian hormones were given every day for 3 days. Three hours after the last dose animals were sacrificed with chloroform and uterine tissues were removed for studying.

¶ E.B. = Estradiol benzoate.

Three daily injections of 1 μg of *estradiol benzoate* were associated with a 2- to 3-fold increase in weight of the uterus of the ovariectomized rat; a 50% decrease in concentration of collagen; and an unaltered or increased total collagen per uterus (Table II). After such injections, the specific activities of collagen, hydroxylysine and lysine present at the end of incubation increased 4 to 7, 12 to 20 and 4 to 5 times, respectively, as compared with amounts present in the incubated uteri of untreated ovariectomized rats. The total counts per minute recovered as collagen per incubation were increased 3 times and those per uterus were increased 8 to 9 times.

Progesterone had no effect on either weight of the uterus or amount of collagen produced by the uterus of the ovariectomized rats. In incubated uterine slices from ovariectomized animals, progesterone administration was associated with a 13% decrease in collagen concentration, while the specific activity of collagen increased by as much as 50%. Under similar conditions, the specific activity of hydroxylysine increased by 200 to 300%; and that of lysine by about 20%. The total increment in counts per minute recovered per incubation or per uterus was approximately 30%.

In the dosages used, neither *estrone* nor *estriol* administration altered the weight of the uteri of ovariectomized rats. With estriol a 10% decrease in collagen concentration occurred. The specific activity of collagen was increased 90% and 70% following injection of estrone and estriol, respectively; use of these estrogens was further accompanied by a 100% increase in specific activity of hydroxylysine and lysine; a 50% increase in total counts per minute of radioactivity recovered per incubation and a 100% increase in total counts per minute recovered per uterus.

As compared to the normal animal, slices from estradiol-benzoate-treated ovariectomized rats showed a 2- to 3-fold increase in the specific activity of collagen; a 3 to 6 times higher specific activity of hydroxylysine; a 2 to 4 times higher specific activity of lysine; a 100% increase in total counts per minute recovered per incubation and a 20% increase in counts per minute recovered per uterus. Progesterone, estrone and estriol do stimulate collagen synthesis in the uterus of the ovariectomized rat. In the dosages employed, such stimulation was not sufficient to reestablish a normal pattern of collagen metabolism.

Experiments 4, 5 and 6: Effect of various amounts of estradiol benzoate and duration of treatment on collagen synthesis by rat uterine slices (Table III & Fig. 1). Three daily intramuscular injections of 0.01 μg estradiol benzoate in rats overiectomized 14 days earlier did not perceptibly affect the collagen-producing capacity of uterine slices. When doses of 0.1 μg estradiol benzoate were employed under similar circumstances, a modest increase in uterine weight occurred. There was also a decrease in concentration and a 2-fold increase in specific activity of collagen in such incubated tissue slices. Concurrently, the specific activity of hydroxylysine was increased 4-fold and that of lysine, 2-fold. The total counts per minute recovered were increased by 50% per incubation and by 100% per uterus.

Three daily injections of 1 μg of estradiol benzoate produced a 150% increase in uterine weight; a 40% decrease in the concentration and a 300 to 400% increase in the specific activity of collagen in KUST preparations. The specific activity of hydroxylysine was increased 1000 to 1200%, while that of lysine not more than 200 to 300%. The total recovery of radioactive material in counts per minute was increased 100 to 200% per incubation and 400 to 700% per uterus. A further increase in the dose of estradiol benzoate to 3 treatments of 10 μg or 3 treatments of 100 μg did not cause further increase in uterine weight nor in the specific activity of collagen formed in the KUST preparations. On the contrary, the specific activity of collagen was slightly lower and the concentration of collagen was higher than in uterine slices from those animals treated with 3 × 1 μg of estradiol benzoate. The specific activities of hydroxylysine and

TABLE III. Effect of Estradiol Benzoate (E.B.) Administered to Donor Rat upon Collagen Synthesis by Rat Uterine Slices. (Exp 4, 5 and 6)

Animal status	No. rats	Uterine wt/rat (g) (A)	No. detn.	Insoluble collagen (mg) per Incubation‡ (B)	Insoluble collagen (mg) per Uterus (C)= (B/.4 × A)	Collagen formed *in vitro* as measured by radioactivity — Specific activity (epm/mg) Collagen (D)	Hydroxylysine§	Lysine§	Total cpm recovered Per incubation (B × D)	Total cpm recovered Per uterus (C × D)
4 Ovariex										
a No treatment	11	.13 ± .01*	1†	20.7	6.73	137	798	3,510	2,840	922
b E.B., 3 × .01 μg‖	9	.14 ± .01	3	21.64 ± .17	7.57	97 ± 2	537 583	2,870 3,390	2,100	734
c E.B., 3 × .1 μg	7	.21 ± .01	3	15.38 ± .16	8.07	267 ± 1	3,574 2,882	6,160 5,310	4,110	2,150
d E.B., 3 × 1 μg	4	.34 ± .02	3	11.10 ± .17	9.44	555 ± 21	7,500 6,460	10,500 9,190	6,160	5,240
e E.B., 1 × 1 μg 16 hr before sacrificing	6	.25 ± .01	3	14.29 ± .35	8.93	397 ± 95	4,670 4,140	7,510 7,270	5,670	3,550
P		a∼e or d or e <.001 d∼e <.001 d∼e <.01		b∼e or d or e <.001 d∼e <.01 d∼e <.001		b∼e or d or e <.001 d∼e <.001 d∼e <.01				
5 Ovariex										
a** No treatment	12	.14 ± .01	4	23.12 ± .45	8.09	92 ± 3	593 654	3,390 3,330	2,040	710
b** E.B., 3 × 1 μg	7	.32 ± .02	4	10.81 ± .17	8.64	703 ± 65	14,900 9,800	16,000 15,500	7,600	6,070
c E.B., 3 × 10 μg	6	.32 ± .01	4	11.10 ± .30	8.86	607 ± 43	13,400 7,190	22,100 15,500	6,740	5,390
d E.B., 3 × 100 μg	6	.30 ± .01	4	11.24 ± .31	8.43	529 ± 41	6,450 5,000	8,730 8,150	5,950	4,460
P		a∼b or c or d <.001		a∼b or c or d <.001		a∼b or c or d <.001 b∼d <.01				
6 Ovariex										
a** Oil control	8	.12 ± .01	2	22.69 ± .90	6.81	80 ± 1	507	2,120	1,820	544
b E.B., 1 × 1 μg 3 hr before sacrificing	9	.12 ± .00	3	21.59 ± .07	6.48	82 ± 4	473	2,120	1,770	531
c** E.B., 3 × 1 μg	4	.33 ± .03	3	13.88 ± .14	11.45	391 ± 10	6,100	7,530	5,430	4,480
P		a∼c <.001		a∼c <.001		a∼c <.001				

* † ‡ § Same as in Table I. ‖ Same as in Table II, except Exp 4-e, 1 dose of 1 μg was given 16 hr before sacrificing animal, and Exp 6-b, 1 dose of 1 μg was given 3 hr before sacrificing animal. ** For Exp 5, data in these 2 lines are the same as those in Exp 2; for Exp 6, same as in Exp 3 (Table II).

lysine from 3 × 100 μg estradiol treated rats were both lower than in the uterine slices from those treated with 3 × 1 μg estradiol benzoate. The total counts per minute recovered from the incubation process were lower for those uterine slice perparations made from animals treated with 100 μg × 3 of estradiol benzoate than for those from animals receiving 1 μg × 3 of the hormone.

When the rats received one injection of 1 μg estradiol benzoate 3 hours before being sacrificed, no effect on uterine tissue was observed by the methods employed. However, when such a dose was administered 16 hours before sacrificing, the stimulatory effects were comparable to those observed in animals which were given 3 daily injections of 0.1 μg of estradiol benzoate each.

Experiment 7A: In vivo and in vitro effect of estradiol benzoate on collagen synthesis in normal and ovariectomized rats (Tab. IV & Fig. 1). No significant effects on collagen production by uterine slices from either ovariectomized or normal animals were observed when estradiol benzoate was added directly to the incubation medium. *In vivo* treatment with estradiol benzoate was associated with a moderate increase in collagen concentration in KUST preparations from normal rats. When donor ovariectomized rats were injected with estradiol, highly significant effects were observed in the KUST preparations, similar to those recorded in the several preceding experiments (Tables I-III).

Experiment 7B: Influence of estradiol benzoate injected into donor animals, upon production of collagen NCP and SP by KUST preparations (Table V). These data were obtained from the rats used in Exp. 7A. Uterine proteins other than collagen were measured in normal, ovariectomized and ovariectomized-estrogen treated donor animals. In Table V is recorded the amount of collagen and of NCP in the tissue slices and the amount of protein in the combined incubation medium and saline supernate (SP). In respect to these data (Tab. V), it should be emphasized that a small amount of soluble collagen may be present in the SP, but none in the NCP fraction derived after collagen extraction. When estradiol benzoate was added directly to the incubation medium the results throughout this series of experiments mirrored the activity of KUST preparations from untreated normal and ovariectomized animals, *i.e.* there was no demonstrable estrogenic effect.

When estradiol was injected into the ovariectomized donor, 1 μg daily for 3 days, KUST preparations yielded results for collagen similar in kind and degree to those obtained in the preceding studies. Since SP and NCP fractions have generally varied directly under a wide variety of circumstances and because the amount of soluble collagen in SP is at best relatively small, comment can be made on these two fractions simultaneously. Two-thirds of the total amount of these combined fractions was derived from the tissue residue remaining after collagen extraction. This ratio appeared to hold in all experiments and at all levels of activity. The total amount of NCP plus SP per uterus was decreased approximately 65% as a result of ovariectomy. There was no decrease in the treated ovariectomized animals.

The specific activities of the NCP and SP fractions were approximately equal in amount in preparations from any given animal, and showed similar values in normal intact and ovariectomized rats. This is in sharp contrast to the behavior of collagen (Tab. IV & V) In the ovariectomized-estradiol-treated animal, the increment in the specific activity of NCP plus SP was not more than 35%, while the corresponding change for collagen was 450%.

When estradiol was given to the normal rat, the concentration and total amount of collagen increased approximately 20 and NCP plus SP 15%. Specific activities were unchanged; therefore, the total counts per minute recovered were increased.

Discussion. In several species of anmials, studies of the growth of uterine connective tissue and the mechanisms controlling it have yielded conflicting results(8-12,14-17).

One purpose of the present experiments

TABLE IV. Effect of Estradiol Benzoate (E.B.) *in vitro* and *in vivo* on Collagen Synthesis.¶
(Exp 7A)

Animal status	No. rats	Uterine wt/rat (g) (A)	No. detn.	Insoluble collagen (mg) per Incubation‡ (B)	Insoluble collagen (mg) per Uterus (C)= (B/.4 × A)	Collagen formed *in vitro* as measured by radioactivity — Specific activity (epm/mg) Collagen (D)	Hydroxylysine§	Lysine§	Total epm recovered Per incubation (B × D)	Total epm recovered Per uterus (C × D)
Ovariex										
a No treatment	20	.13 ± .00*	3†	24.40 ±1.05	7.93	113 ± 7	656	3,490	2,760	896
							1,010	3,720		
b E.B., 1 μg *in vitro*			2	24.58 ± .57	7.99	86 ± 0	419	2,140	2,110	687
							453	2,750		
c E.B., 3 × 1 μg *in vivo*‖	7	.31 ± .01	5	12.48 ± .23	9.67	617 ± 26	11,800	15,800	7,700	5,970
							7,180	11,000		
P		c∼a + b <.001		c∼a or b <.001		c∼a or b <.001				
Normal										
a No treatment	8	.44 ± .02	3	14.27 ± .30	15.70	244 ± 39	3,460	5,490	3,480	3,830
							3,240	6,140		
b E.B., 1 μg *in vitro*			3	14.24 ± .82	15.66	219 ± 15	2,530	5,100	3,120	3,430
							2,620	6,370		
c E.B., 3 × 1 μg *in vivo*	4	.40 ± .03	3	17.31 ± .29	17.31	213 ± 6	2,760	6,490	3,690	3,690
							2,290	5,190		
P				c∼a <.01 c∼b <.05						

* † ‡ § Same as in Table I.
‖ Same as in Table II.
¶ This study was concerned with collagen synthesis in KUST preparations from normal and ovariectomized animals to which estradiol benzoate had been added directly and from similar groups of animals to each of which injections of estrogen had been given at appropriate intervals prior to sacrificing.

TABLE V. Effect of Estradiol Benzoate (E.B.) upon Various Proteins Synthesized by Rat Uterine Slices.§
(Exp 7B)

Animal status	No. detn.	mg/incubation‡ Residue¶ Collagen	NCP**	SP**	mg/uterus Residue Collagen	NCP	SP
Ovariex							
a No treatment	3†	24.40 ±1.05*	14.90 ± .79	6.96 ± .71	7.93	4.9	2.3
b E.B., 1 μg *in vitro*	2	24.58 ± .57	14.00 ± .15	6.98 ± .20	7.99	4.6	2.3
c E.B., 3 × 1 μg *in vivo*‖	5	12.48 ± .23	16.75 ± .10	7.94 ± .22	9.67	12.9	6.2
P		c∼a or b <.001		c∼a <.01			
Normal							
d No treatment	3	14.27 ± .30	12.67 ± .36	5.60 ± .08	15.70	14.2	6.2
e E.B., 1 μg *in vitro*	3	14.24 ± .82	12.37 ± .33	5.79 ± .30	15.66	13.6	6.4
f E.B., 3 × 1 μg *in vivo*	3	17.31 ± .29	13.65 ± .23	7.84 ± .49	17.31	13.6	7.8
P		f∼d <.01 f∼e <.05 a∼d <.001	f∼e <.05 f∼d <.01 a∼d <.05	f∼d <.01 f∼e <.05			

Animal status	No. detn.	Proteins formed *in vitro* as measured by radioactivity: Specific activity (cpm/mg) Residue Collagen	NCP	SP	Total cpm recovered per Incubation Residue Collagen	NCP	SP	Total cpm recovered per Uterus Residue Collagen	NCP	SP
Ovariex										
a No treatment	3†	113 ± 7	10,500 ± 901	10,700 ± 757	2,760	156,000	74,500	896	50,900	24,200
b E.B., 1 μg *in vitro*	2	86 ± 0	6,870 ± 64	6,640 ± 220	2,100	96,200	46,300	687	31,300	15,100
c E.B., 3 × 1 μg *in vivo*‖	5	617 ± 26	15,600 ± 630	13,300 ± 314	7,700	261,000	106,000	5,970	201,000	81,800
P		c∼a or b <.001	c∼a <.01 c∼b <.001	c∼a <.01 c∼b <.001						
Normal										
d No treatment	3	244 ± 39	10,200 ± 344	12,400 ± 654	3,480	129,000	69,400	3,830	145,000	76,400
e E.B., 1 μg *in vitro*	3	219 ± 15	8,150 ± 282	8,610 ± 630	3,120	101,000	49,900	3,430	111,000	54,800
f E.B., 3 × 1 μg *in vivo*	3	213 ± 6	11,100 ± 326	11,400 ± 348	3,690	152,000	89,400	3,690	151,000	89,400
P		a∼d <.05	e∼d or f <.01							

* † ‡ Same as in Table I. § For No. of rats and uterine weights, see Table IV. ‖ Same as in Table II. ¶ Tissue slice residue after homogenized with saline. ** See text for definitions of NCP and SP.

was to test the usefulness of the uterine slice technique (KUST) for assaying the influence of various agents on formation of connective tissue. Another objective was the gathering of data on the influence of sex-linked steroids upon connective tissue metabolism in the uterus.

While some of the data are not in agreement with results recorded by others, it must be remembered that species differences(19) and many technical variants may be responsible. As far as we know, this represents the first attempt to apply isotopic techniques directly to observations on the behavior of connective tissue moieties of the uterus under the influence of sex-linked steroid hormones. This technique has furnished a useful check against our own results which were obtained by chemical analyses as well as against the results reported by others. For instance, in the normally cycling nonpregnant rat, Morgan(14) demonstrated a decrease in collagen concentration and an increase in total collagen as the animal passed from diestrus to estrus. Smith(15) recorded an increase in both concentration and total amount of uterine collagen following ovulation. These data agree well with our recent isotopic work(18), in that collagen synthesis in both the intact and the isolated uterus is at its peak when follicular hormone is exerting its greatest effects.

Fainstat(16) found the presence of estrogen was necessary for the deposition and maintenance of a dense collagen stroma in the rat endometrium. Morgan(17) induced growth in the uterus and vagina by treatment of ovariectomized rats with estrogen. This was accompanied in both organs by an increase in total amount and a decrease in concentration of collagen. Simultaneously, there was an increase in both concentration and total amount of NCP. On the other hand, Smith *et al*(15) found that, while estrogen administered to ovariectomized animals caused a fall in the concentration of collagen in the uteri, it did not influence the total amount present. Our results are much more closely in accord with those reported by Morgan(17) than with those reported by Smith *et al*(15).

From all of the evidence presently available, it appears that uterine-oriented effects of estrogen include (a) an increase in weight of uterus and total amount of collagen and a decrease in collagen concentration in the uterus of the ovariectomized rat; (b) an increase in both concentration and total amount of NCP in the residue of uteri and of SP in uterine slice incubation media of ovariectomized animals; and (c) an increase in concentration and total amount of both collagen and NCP in the uteri of normal rats.

Harkness *et al*(10) considered the effect of estrogen on collagen formation slight as compared with its influence on overall growth and the deposition of NCP. The present data, derived from isotopic procedures, do not appear to support such a conclusion. In the estradiol-treated-ovarectomized rats, it was found that the specific activity of collagen was 4 to 7 times that of the untreated animal, while the specific activity of NCP was increased by not more than 50% under such circumstances. The total counts per minute recovered in the collagen of incubated uterine slices from treated-overiectomized animals increased 300%, while those in the NCP by no more than 60%. Expressed in terms of total counts per minute recovered for the entire uterus of each animal, collagen activity was 7-fold and NCP activity 2- to 3-fold greater in uteri of ovariectomized-treated than in untreated animals. To us, this indicates a specific action of estrogen on collagen formation.

In the normal animal, modest changes were observed following treatment with estradiol. Both cencentration and total amount of collagen, NCP and SP were significantly increased. The specific activities determined in uterine slices were not appreciably altered. In any event, it is clear that estrogen, as here employed, exerted a positive effect on the normal rat, albeit much less in degree than that seen in the hormone-deficient subject.

While injection of 0.1 μg estradiol benzoate daily for 3 days caused a significant increase in collagen synthesis in the uterus of the ovariectomized rat, one μg doses, similarly administered, still further augmented

its production. When dosage was further increased an inhibitory effect appeared. We have no explanation for this phenomenon, unless it be a secondary action mediated through the pituitary gland.

The specific point or points at which estrogen exerts its action upon the production of connective tissue by the uterus is unknown. KUST preparations from normal animals incorporated 3 times as much lysine into collagen in a 6 hour period as did uteri from ovariectomized rats (Table I). However, during the same period, hydroxylysine in slices from the normal animal was 20-fold that of the ovariectomized animal. Administration of estrogen to the ovariectomized animal restores the above values to normal. Therefore, it appears that active enzymatic hydroxylation is stimulated during collagen synthesis.

The above data may indicate one of 3 things: (a) in formation of uterine collagen, estrogen influences directly the enzymatic steps involved in conversion of lysine to hydroxylysine rather than the incorporation of lysine itself; (b) estrogen merely accelerates processes which go on at a reduced level in its absence; and (c) actions (a) and (b) are combined. The first explanation seems most likely and is supported by the experimental data (Tab. II). Under the experimental conditions used here, progesterone, estrone and estriol all exerted a stimulatory effect on collagen synthesis. Such action was demonstrated not only by chemical analyses, but also by the significantly increased radioactivity in the collagen of the hormone treated animals. However, in all instances this stimulatory effect was less than that observed after the use of estradiol. In Table II it will be noted that the stimulation produced by estrone and estriol was proportionately the same for collagen, hydroxylysine and lysine fractions. Progesterone had a slightly greater effect than estrone and estriol on the hydroxylation of lysine, while such hydroxylation appeared to be a major point of estradiol activity. While the present investigation yielded no data directly bearing upon enzymic processes taking place in uterine tissue, it is interesting that Pincus and co-workers(19), using intact mature rats, found that the concentration of carbonic anhydrase varied inversely as uterine weight, estradiol dosage and total enzyme units per uterus. In our studies, we found collagen formation and uterine weight similarly sensitive to variations in amount of estradiol administered. From both their study and ours, it may be concluded that estradiol plays a specific role in collagen formation within the uterus. It would appear that this role is at least partially mediated through the enzymic hydroxylation of lysine to hydroxylysine. Similar investigation of the proline-hydroxyproline reaction should be made.

Summary. 1. The effect of estradiol, estrone, estriol and progesterone on collagen and noncollagenous protein (NCP) synthesis was studied in ovariectomized and normal rats both *in vivo* and *in vitro* with the Kao uterine slice technique. Both chemical and C^{14}-lysine determinations were made.

2. Chemical studies revealed that: (a) Of the agents used, estradiol was most effective in stimulating the synthesis of collagen and NCP in the uteri of ovariectomized rats. Estrone, estriol and progesterone were similarly effective, but to a lesser degree. Estradiol also increased the collagen and NCP of normal rats. In the dosages employed, the uteri of estradiol-treated ovariectomized rats showed a greater capacity for synthesizing collagen than did the uteri of normal control rats. (b) After ovariectomy, there was a decrease in uterine weight, total collagen and NCP and an increase in concentration of collagen and NCP. (c) Administration of estrogen to the ovariectomized rat was associated with an increase in uterine weight, and in total amount per uterus of collagen, NCP and supernatant protein (SP). Simultaneously, the concentration of collagen decreased, while that of NCP and SP increased slightly.

3. Considered in conjunction with the chemical analyses, the radioisotopic studies with C^{14}-lysine revealed that the major effects on uterine connective tissue of estrogen injections into ovariectomized animals as determined in KUST preparations were: (a) A 400%-700% increase in specific activity of collagen and a 50% increase in specific ac-

tivity of NCP; (b) A 100% increase in total counts per minute recovered in collagen and a 50-60% increase in those of NCP per incubation; (c) a 700% increase in counts per minute in collagen and 200-300% increase in NCP per uterus; (d) A much higher ratio of specific activity of hydroxlysine from the treated rat to that from the untreated rat as compared with the ratio of specific activities of lysine from the same 2 groups of animals; (e) An effective dose of estradiol for stimulation of collagen synthesis is 1 μg with a dose-response time of 16 hours.

4. It is suggested that one of the important, if not the most important, action of estrogen in uterine connective tissue is concerned with the hydroxylation of lysine.

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Absorption of Short Chain Fatty Acids in Man.* (29505)

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Recently Barry and Smyth(1) investigated the absorption of short chain fatty acids from the gut of the rat and presented evidence to suggest that this was accelerated by an active transport system. Another explanation seemed possible(2) for diffusion of a compound across the gut wall besides being controlled by the compound's size and charge, is also modified by its lipid solubility(3,4).

We have, therefore, studied the relative rates of absorption of 7 short chain fatty acids from the human intestine and compared these with their partition into chloroform from an aqueous solution.

Patients studied and methods. The technique was similar to that used for studying sugar(5) and cortisol(6) absorption in man. The evening before the study the patients swallowed a double lumen polyvinyl tube ('Portex' M.L.T./B. external diameter 4.2 mm) to which had been attached a mercury bag. Next morning its position was checked fluoroscopically and the tube withdrawn slightly if necessary so that the mercury bag was approximately 40 cm past the duodenojejunal flexure. Fasting jejunal contents were collected from 7 patients. A solution contain-

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