Connective Tissue XI. Factors Affecting Collagen Synthesis by Rat Uterine Slices.* (28930)

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Previously, we reported that of 4 tissues studied, the uterus of the rat has the highest capacity for synthesizing collagen *in vivo*(1). Later, we found that the uterus is the best soft tissue of the rat for demonstrating appreciable synthesis of collagen *in vitro*(2). Recently, we assessed some factors effecting collagen synthesis by sponge biopsy connective tissue *in vitro*(3). The present study is designed to confirm the latter report(3) with a different tissue and also to furnish additional information concerning collagen synthesis.

Method. In all experiments, except the experiment on the effect of the estrous cycle, uterine slices from rats, Wistar strain, age 6-12 months, were so pooled as to represent an even distribution of tissue from each animal in each incubation flask. Four-tenths gram of tissue was incubated with 1.875 μc of uniformly labelled C^{14}-lysine (purchased from Nuclear Chicago Corp., Des Plaines, Ill., specific activity 52.7 μc/mg) in Krebs-Henseleit medium under conditions described previously(2). After six hours' incubation, the uterine slices were removed; collagen was extracted and hydrolyzed; its hydroxylysine and lysine were separated by column chromatography; chemical determinations and radioactivity were determined as reported previously(2). The radioactivity in the hydroxylysine was used as a measure of collagen synthesis. Data represent the mean of each 3 samples unless otherwise stated. In most instances, the mean variations did not exceed 25%.

In the experiment on the effects of the estrous cycle on uterine formation of collagen, vaginal smears were checked in each animal immediately before the experiment. Three groups were distinguished in relation to degree of vaginal cornification. Samples from individual animals were used for incubation.

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TABLE I. Collagen Synthesis in Rat Uterine Slices; Effect of Alterations in Medium.

	Specific activity (cpm/mg)*		
	Collagen	Hydroxylysine	Lysine
Complete medium†	157	1,550	3,193
" – NaCl	13	40	309
" – Na citrate	183	1,790	4,420
" – $NaHCO_3$	131	1,362	2,920
" – KH_2PO_4	67	809	1,990
" – $MgSO_4$	177	2,220	3,780
" – Glucose	137	2,218	3,633
" – $CaCl_2$	143	1,590	2,940

* Data are not corrected for counting efficiency.

† The complete Krebs-Henseleit medium contained per 5 ml, NaCl 44.5 mg, $NaHCO_3$ 12.5 mg, KH_2PO_4 5.0 mg, $MgSO_4 \cdot 7H_2O$ 3.0 mg, sodium citrate 1.5 mg, $CaCl_2$ 1.5 mg and glucose 1.0 mg.

Results. In Table I is shown the effect of separately eliminating each ingredient of the incubation medium on collagen synthesis by rat uterine slices. The most striking effect was observed when sodium chloride was not present in the medium. Collagen synthesis was reduced to 1/40 of the control level. Elimination of KH_2PO_4 from the incubation medium decreased the collagen synthesis to ½ of control level. Elimination of $NaHCO_3$ or glucose revealed a slight depression in the synthesis of collagen. The omission of other components from the incubation medium had no significant effect on collagen synthesis.

The influence of the pH of the incubation medium on collagen synthesis by rat uterine slices is shown in Table II. The highest values for collagen and its hydroxylysine and lysine were obtained at pH values lying between 7.4 and 8.4. A reasonable amount of synthesis was observed at all pH's tested between starting pH 6.4 and 9.4.

The oxygen requirement in collagen synthesis by uterine slices of rats is shown in

TABLE II. Collagen Synthesis in Rat Uterine Slices; Influence of pH.

pH		Specific activity (cpm/mg)*		
Starting†	Final	Collagen	Hydroxy-lysine	Lysine
3.4	6.2	7	0	160
4.4	6.3	20	739	407
5.4	6.6	80	1,030	1,800
6.4	7.3	155	1,620	3,270
7.4	7.7	197	1,680	3,560
8.4	7.8	202	1,740	4,080
9.4	8.0	183	1,510	3,310
10.4	8.3	68	480	1,460

* See legend, Table I.
† pH of incubation medium used.

Table III. Replacement of 95% O_2-5% CO_2 by air decreased the collagen synthesis to 1/3 of control level. Substitution of 95% O_2-5% CO_2 by N_2 completely abolished the ability of the preparation for collagen synthesis.

The influence of temperature treatment before and during incubation on collagen synthesis by uterine slices of rats is shown in Table IV. Optimum temperature for incubation was 37°C. Of the pre-experimental temperatures 0° was the best. Subjecting the tissue to a −17° temperature prior to incubation decreased collagen synthesis to 1/6 of control level. Cooling the tissue to −70° before incubation decreased collagen synthesis to 1/100 of control level. Incubating the tissue at 25° decreased collagen synthesis to 1/2 that of control level. Incubating the tissue at 0°-10° decreased collagen synthesis to 1/50 that of control level. Incubating the tissue at 45° decreased collagen synthesis to 1/20 that of control level. It is clear that the optimum temperature range for collagen synthesis is close to 37°C.

The influence of each of the various stages of the estrous cycle on collagen synthesis by rat uterine slices is shown in Table V. The uterus was at its greatest weight, with the greatest total amount of collagen present and the largest capacity for synthesizing collagen when the follicular hormone of the ovary was exerting its maximal activity. In contrast, shortly after the onset of estrus, the uterus was least able to synthesize collagen.

TABLE III. Oxygen Requirement in Collagen Synthesis by Rat Uterine Slices.

Atmosphere	pH		Specific activity (cpm/mg)*		
	Starting†	Final	Collagen	Hydroxy-lysine	Lysine
95% O_2-5% CO_2	7.4	7.4	69	378	1,940
Air	7.4	7.2	33	117	1,071
N_2	7.4	7.6	8	0	186

* See legend, Table I. † See legend, Table II.

Discussion. The data obtained here relating to the important ingredients of the incubation medium and to optimal pH for collagen synthesis were in full agreement with our previously published data on factors affecting collagen synthesis by sponge-biopsy-connective-tissue(3). In general, in both uterine and sponge biopsy connective tissue, NaCl, $NaHCO_2$ and KH_2PO_4 are the most essential environmental ingredients promoting the synthesis of collagen by fibroblasts. These salts may affect the transport of the C^{14}-amino acid through the cell membrane of the fibroblast. The effects of removing the above salts from the incubation medium were more inhibitory of sponge biopsy connective tissue than of uterine tissue. Since the former is a pure connective tissue system, it seems likely that these factors selectively act upon the fibroblast. The need for oxygen confirms not only our previous data(3) but also the data of other workers(4-6).

TABLE IV. Influence of Temperature on Collagen Synthesis by Uterine Slices of Rat.

Incubation temp	Temp before incubation	Specific activity (cpm/mg)*		
		Collagen	Hydroxy-lysine	Lysine
45°	0°	13	28	193
37°	0°	*100*	*546*	*2,235*
37°	−17°	20	87	462
37°	−70°	6	6	98
25°	0°	36	261	913
10°	0°	6	11	148
0°	0°	6	19	108

* See legend, Table I.

There have been no previous reports regarding changes in collagen content of the uterus and in its capacity for synthesizing collagen during various phases of the estrous cycle. The present findings support the conclusion that the uterus shows its greatest capacity for collagen synthesis at the height of follicular hormone activity. This conclusion

TABLE V. Influence of Estrous Cycle on Collagen Synthesis by Rat Uterine Slices.

Vaginal smear	No. of animal	Wt of uterus (g)	mg collagen / g of uterus	Total collagen (mg)	No. of sample	Specific activity (cpm/mg)* Collagen	Hydroxy-lysine	Lysine
A Epithelial cells only	3	.90 ±.08† A=B<.05‡	71 ± 1	64 ± 5 A=B<.05	6	51 ± 4 A=C<.05	416 ± 64	1790 ± 108
B Cornified cells only	4	.54 ±.08	71 ± 6	38 ± 6	4	43 ± 5	393 ± 61	1440 ± 123
C Leucocytes and epithelial cells	3	.67 ±.10	69 ± 5	48 ±10	4	39 ± 3	333 ± 50	1480 ± 123

* See legend, Table I.
† Mean ± standard error(8).
‡ P values relating to age differences, when within 5% level of confidence, are listed. Statistical calculations are made according to Bailey(8).

is emphasized by the observations of Harkness(7), that uterine collagen increases during pregnancy.

Summary. 1. Factors affecting collagen synthesis *in vitro* have been studied in uterine slice systems of the rat. 2. The results agree well with those of our previous report concerned with collagen synthesis in sponge biopsy connective tissue. Elimination of NaCl from the incubation medium was accompanied by a more striking decrease in collagen synthesis than followed the removal of any other constituent. Elimination of KH_2PO_4 from the incubation medium caused a significant decrease in collagen synthesis. Maximal collagen synthesis occurred when the starting pH was 8.4 and the gas in the incubation vessels was composed of 95% oxygen and 5% carbon dioxide. 3. Optimum temperature for keeping tissue prior to incubation was 0°. Optimum incubation temperature was 37°. 4. Collagen synthesis reached a peak in uterine slices obtained from animals at the height of follicular hormonal activity.

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A Fat Mobilizing and Anorectic Substance in the Urine of Fasting Rats.* (28931)

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Weil and Stetten(1) reported an increase in the liver lipid content of mice receiving extracts of urine obtained from fasted rabbits. Chalmers *et al*(2) reported isolation of a similar fraction from urine of fasted humans, using the same procedure. In the intact mouse, subcutaneous injections of this extract caused a transient hypoglycemia, ke-

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