EARLY EFFECTS OF ESTRADIOL ON NUCLEIC ACID METABOLISM IN THE RAT UTERUS*

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Previous studies on the metabolism of formate-C^{14}, glycine-2-C^{14} (1), and serine-3-C^{14} (2) in surviving rat uterine segments have revealed that the incorporation of radioactivity into the purine bases of mixed nucleic acids is a highly sensitive indicator of early estrogen action. In the present paper further attempts to characterize the estrogen-induced alterations of nucleic acid metabolism in the rat uterus are reported.

It has been demonstrated that after a single dose of estradiol the content of ribonucleic acid (RNA) per rat uterus remained constant for from 3 to 6 hours (induction period), and then accumulated rapidly during the ensuing 18 hours. In contrast, the content of deoxyribonucleic acid (DNA) per uterus remained constant throughout the 1st 24 hours. Protein accumulation followed in the wake of the RNA increase.

In parallel incorporation studies in surviving uterine segments, it was observed that the labeling of nucleic acid purines with $C^{14}O_2$ in positions other than 2 and 8 was accelerated 2- to 3-fold after 6 hours of treatment with estradiol. These results are similar to those obtained with "1-carbon" precursors in earlier studies (1, 2). In contrast, the labeling of the nucleic acid uridine was only moderately increased by treatment with estradiol. This increase was observed only during the subsequent period of RNA accumulation. This difference in the specific activities of acid-insoluble adenine and uridine was found to reflect a difference in the specific activities of the respective acid-soluble precursors. In response to 6 hours of treatment with estrogen, the uterine pool of acid-soluble uridine compounds was increased but their specific activity remained near that of the control tissue; in contrast, the specific activity of the acid-soluble purines was increased strikingly although the pool size increased only slightly.

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Methods

Female rats,[1] weighing approximately 180 gm., were subjected to ovariectomy through the dorsal approach and maintained on a diet of commercial dog chow (Purina) for at least 4 weeks before experimentation. In any single experiment all the animals were of the same age and were subjected to ovariectomy at the same time.

At zero time the ovariectomized rats were injected in the tail vein with 1 ml. of buffered saline which contained 10 γ of estradiol-17β (3); control rats received 1 ml. of buffered saline only. After various periods of treatment with estrogen, the animals were decapitated and the uterine horns removed. Each horn was incised longitudinally and cut into four equal segments. The segments from two to four animals were pooled as control and estrogen-treated tissues, respectively. Eight segments were selected randomly from the respective pools for each duplicate incubation flask. The reaction medium consisted of 2 ml. of Robinson's medium (4) which contained 44 μmoles of glucose and 5 μmoles of $NaHC^{14}O_3$ (2 μc. per μmole). In practice, a stock solution of $NaHC^{14}O_3$ was prepared by careful trapping of $C^{14}O_2$ liberated from $BaC^{14}O_3$ with acid in a solution containing equivalent amounts of alkali. After passing 100 per cent oxygen through the flasks, 0.1 ml. of the radioactive bicarbonate solution was added to each from a constriction pipette as quickly as possible. These operations were conducted at 0°. The subsequent incubations were carried out for 1 hour at 37° with constant shaking. The reaction was terminated by the addition of 0.5 ml. of 20 per cent perchloric acid and the excess of radioactive gas aerated from the acidified incubation medium.

The tissue was frozen in liquid air and pulverized in a stainless steel mortar. The extractions were carried out as previously described to yield the acid-soluble, lipide, and nucleic acid-protein fractions (3). When indicated, these residues were spread on tared aluminum plates and counted for radioactivity.

For isolation of uridine, thymine, adenine, and guanine, the nucleic acid-protein residues, weighing from 15 to 30 mg., were hydrolyzed directly with 1 ml. of 98 per cent formic acid in sealed tubes for 2 hours at 165°. After cooling, the tubes were cautiously opened by melting the tips in a flame and the acid was evaporated in a desiccator. The dried residues were extracted with 2.0 ml. of 0.01 N HCl. This supernatant fluid plus two 0.5 ml. washings were transferred to ion exchange columns composed of 40 × 7 mm. Dowex 50 (hydrogen form) layered on top of an equal amount of Dowex 1 (formate form). The first 3 ml. collected plus a 2 ml. water wash contained all the thymine, uracil, and uridine present in the hydrolysates. Where indicated, aspartic and glutamic acids were eluted

[1] Holtzman Rat Company, Madison, Wisconsin.

with 2 N HCl and isolated as the dinitrophenyl derivatives according to the method of Perrone (5). The purines were eluted from the Dowex 50 columns with 6 N HCl.

The pyrimidine and purine fractions were chromatographed on paper with use of the *tert*-butanol-HCl (6) and the *n*-butanol-H_2O systems (7), respectively. After elution of the components from the paper, aliquots were plated for counting and the amounts of the isolated bases were determined spectrophotometrically with use of the molar extinction coefficients of Markham and Smith (8). This procedure, which will be reported in greater detail elsewhere,[2] gives a quantitative measure of the nucleic acid bases cited as well as radiologically pure samples for determination of the specific activities. In certain instances the method of LePage was used to obtain the purines (9).

All the determinations of radioactivity in these experiments were made on solid samples plated on tared aluminum planchets and counted in gas flow proportional counters for a sufficient period to obtain 5 per cent statistical accuracy. The data are corrected for self-absorption and counting efficiency and expressed as counts per minute per mg. of dry protein-nucleic acid residues or as counts per minute per micromole of the nucleic acid components isolated.

Results

Accumulation of Nucleic Acids and Protein—The effect of a single 10 γ dose of estradiol on the level of DNA, RNA, and protein during the 1st 24 hours of estrogenic response is given in Table I. Throughout this period the DNA per uterus, expressed as thymine per flask (four uterine horns), remained constant. Accordingly, it was convenient to express all other results per micromole of thymine. The RNA, expressed as micromoles of uridine per micromole of thymine, increased rapidly after an initial lag period which lasted 6 hours in most of the experiments. Once the accumulation of RNA was initiated, the rate of accumulation tended to increase during the period of measurement (Fig. 1). In some experiments the accumulation of RNA approached an exponential curve. In such cases a shorter induction period (approximately 3 hours) was observed.

The increase in total nucleic acid adenine (Fig. 2) also reflects the accumulation of RNA after treatment with estradiol; however, in this case the increase in RNA purines was superimposed on a high base line contributed largely by the DNA purines.

In experiments in which the level of RNA was followed up to 40 hours after hormone treatment, the RNA content (measured as micromoles of acid-insoluble uridine per flask or per uterus) declined after 25 hours.

[2] Jervell, K. F., Diniz, C. R., and Mueller, G. C., submitted for publication.

Earlier studies have shown that, under the conditions employed in these experiments (*i.e.* 10 γ of estradiol in a single intravenous dose), the level

TABLE I

Effect of Estradiol on Accumulation of Protein and Nucleic Acid in Rat Uteri

Hrs. of estradiol treatment	μmoles thymine per flask	Mg. protein per μmole thymine	μmoles uridine per μmole thymine
Control	1.46	20.4	0.097
	1.43	19.5	0.102
6	1.19	20.4	0.099
	1.47	18.8	0.100
12	1.58	24.6	0.126
	1.51	23.0	0.135
18	1.45	24.6	0.167
	1.68	26.5	0.178
24	1.45	29.5	0.219
	1.48	30.1	0.222

The system was as described in the text and under "Methods." Each number represents uterine tissue from two animals. RNA measured as micromoles of uridine and expressed as the ratio of micromoles of uridine per micromole of thymine during the 1st 24 hours after hormonal treatment. The control values were the same at 6 and 18 hours.

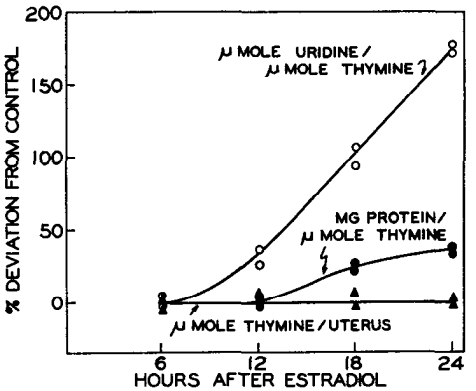

FIG. 1. Alteration in uterine composition after single dose of estradiol. 10 γ of estradiol were injected intravenously at zero time; DNA was measured as micromoles of thymine. RNA was measured as micromoles of uridine and expressed as the ratio (micromoles of uridine per micromole of thymine) during the 1st 24 hours after hormonal treatment.

of active hormone becomes limiting prior to this time (3). When animals were given a subcutaneous injection of 100 γ of estradiol in 1 ml. of corn oil and a similar injection after 24 hours, a continuous increase of RNA

could be followed up to 40 hours. No increase in DNA, when measured as thymine per flask, was observed in this period. These data agree with those of Telfer (10), who showed that new cell production as reflected by increased DNA per uterus occurs some time after 40 hours of continuous treatment with estrogen. This is also in agreement with determination of the mitotic rate by the colchicine technique as reported by Gelfant *et al.* (11).

Localization of RNA Increase after Treatment with Estrogen—Uterine tissues were removed from control and estradiol-treated animals 24 hours after injection, and 10 per cent homogenates were prepared in 0.25 M

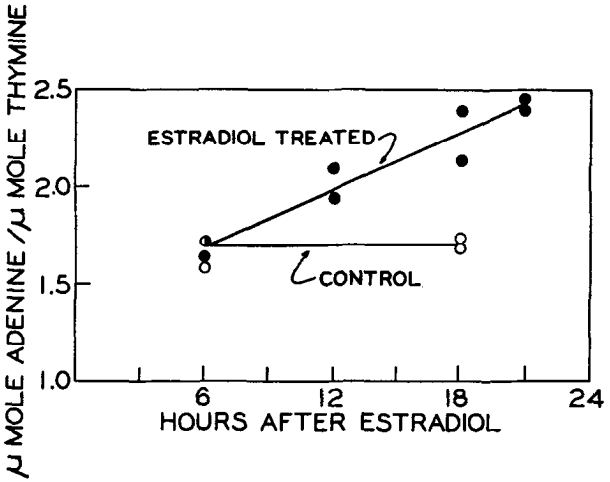

FIG. 2. The effect of estradiol on the accumulation of mixed nucleic acid adenine in the rat uterus. 10 γ of estradiol injected intravenously at zero time. Mixed nucleic acid adenine measured during the 1st 24 hours after hormonal treatment.

sucrose. After centrifugation at 8000 × *g* for 10 minutes at 0° (12), the supernatant and sedimented fractions were analyzed for acid-insoluble uridine as under "Methods." As shown in Table II, the majority of the RNA-uridine is easily sedimentable. A similar situation has been recently reported by Hendler, who showed that the cytoplasmic RNA-containing basophilia in hen oviduct tissue are readily sedimentable (600 × *g* in a few minutes) from a homogenate prepared in a salt-free sucrose medium in which more than 90 per cent of the cells was broken (13). Whether these observations reflect a higher degree of association between the RNA granules (14) and the endoplasmic reticulum (15) than for most other tissues or whether an artificial aggregation of the "microsome" material occurs cannot be decided at this time. The estrogen response was demonstrable in both fractions.

Incorporation of $C^{14}O_2$ into Nucleic Acid Purines—The stimulating effect of treatment with estrogen on the labeling of nucleic acid purines is shown

TABLE II

Localization of RNA in Homogenates from Uterine Tissue Treated with Estrogen

	Control	Plus estrogen
Homogenate	0.0935	0.223
Supernatant fraction	0.024	0.040
Homogenate		
Sedimented fraction	0.100	0.208
Supernatant "	0.011	0.035
Homogenate		
Sedimented fraction	0.079	0.214

Uterine tissue was homogenized for 8 minutes in 0.25 M sucrose to yield 10 per cent homogenates. After centrifugation for 10 minutes at 8000 × *g*, the sediment was washed once by resuspension in sucrose solution. Homogenates or fractions thereof were precipitated with perchloric acid and analyzed for uridine as described under "Methods." Data are expressed as micromoles of uridine per micromole of thymine from the original whole homogenate. The estrogen-treated animals received a single dose of 10 γ of estradiol 24 hours before analysis.

TABLE III

Effect of Estradiol on Incorporation of $C^{14}O_2$ into Mixed Nucleic Acid Purines of Surviving Rat Uteri

Cosubstrate	None		Formate	
Isolated component	Control	Estrogen-treated	Control	Estrogen-treated
Adenine	71	346	53	278
	51	295	78	285
Guanine	115	575	115	523
	117	602	132	
Protein	314	292	342	365
	280	312	310	354

The animals were killed 6 hours after administration of estradiol or control solution. Uterine segments from four horns were incubated for 1 hour as described under "Methods." Mixed nucleic acid purines were isolated according to the method of LePage (9). The remaining dry protein residues were plated and counted as described earlier. The data are expressed as counts per minute per micromole in the case of the purines and counts per minute per mg. in the case of the protein. Where indicated, the incubation medium contained 0.003 M sodium formate as cosubstrate.

in Tables III and IV and in Fig. 3. After 6 hours the purines were labeled three to four times faster than the controls. The results compare

favorably with those obtained earlier with glycine, formate (1), and serine (2) as the radioactive precursors. The inclusion of a pool of non-radioactive formate failed to alter the labeling of the purines from $C^{14}O_2$; thus

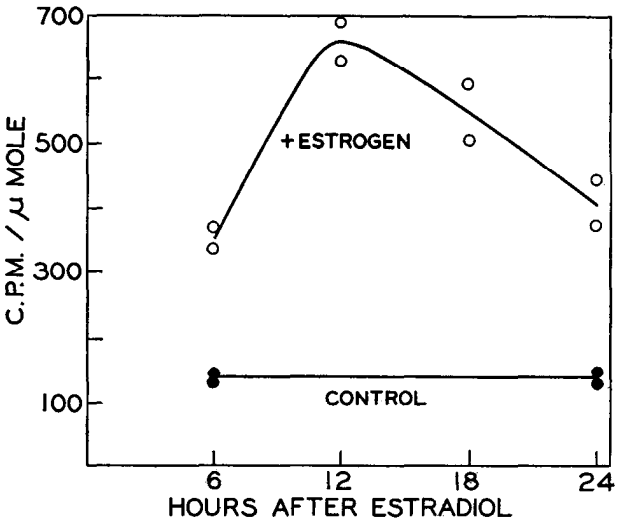

FIG. 3. The effect of estradiol treatment on the labeling of nucleic acid purines in surviving uterine segments incubated with $C^{14}O_2$. Nucleic acid purines isolated from uterine segments incubated with $C^{14}O_2$ for 1 hour at 37° as described under "Methods."

TABLE IV

Effect of Estradiol on Incorporation of $C^{14}O_2$ into Uridine, Thymine, and Mixed Nucleic Acid Adenine of Surviving Uterine Segments

Isolated component	Treatment with estradiol			
	6 hrs.		12 hrs.	
	Control	Estrogen-treated	Control	Estrogen-treated
Uridine	7500	7350	7850	8060
	7150	7250	8250	8100
Thymine	5	13	11	14
	11	14	12	16
Adenine	69	260	107	431
	66	228	96	472

Each flask contained uterine segments from two animals treated with estradiol or control solution 6 or 12 hours before sacrifice. System as under "Methods." The incubation time was 1 hour. The components were isolated after hydrolysis with formic acid as described. The data are expressed as counts per minute per micromole.

it seems most probable that this precursor labels the 6 position almost exclusively.

In this connection the similarity of the responses obtained with different purine precursors (formate-C^{14}, glycine-2-C^{14}, serine-3-C^{14}, and $C^{14}O_2$) indicates that the estrogen affects a common step or mechanism which is limiting in the attainment of the completed purine nucleotide structure.

The rate of incorporation declined rapidly after 12 hours (Fig. 3) even though the RNA was still accumulating at this time. One interpretation of this phenomenon is that the estrogen had set in motion a process in which the initial precipitating effects were wearing off or were becoming obscured by subsequent metabolic changes.

Nature of Nucleic Acid Labeling—Since mild alkaline hydrolysis yields 2′,3′-nucleoside monophosphates and nucleosides which originate from terminal residues, the nature of the estrogen-induced labeling could be tested.

Nucleic acid-protein residues from control and estrogen-pretreated uteri which had been labeled *in vitro* with $C^{14}O_2$ were hydrolyzed in 0.1 N NaOH for 18 hours at 37° (16). After the hydrolysis, 1 μmole of adenosine was added as carrier, and the neutralized mixture passed through a column of Dowex 1 (chloride form) to remove the adenine nucleotides from the eluate containing the adenosine. The latter was reisolated on Dowex 50 (hydrogen form) and purified by chromatography on paper with *tert*-butanol-HCl solvent system. The nucleotides retained initially on Dowex 1 (chloride form) were eluted with 0.5 N HCl, hydrolyzed for 45 minutes at 90°, and the purines isolated by ion exchange and paper chromatography technique as described under "Methods."

As can be seen from Table V, the increase in the specific activities of nucleic acid purines induced by treatment with estrogen was also evident in the nucleotide fraction after alkaline hydrolysis. No measurable radioactivity was recovered in the reisolated adenosine. Thus the labeled nucleotides were incorporated primarily into the nucleic acid structure through the typical phosphodiester linkages of the nucleic acid chain rather than through the labeling of the end groups.

Incorporation of $C^{14}O_2$ into RNA-Uridine—The labeling of RNA-uridine presented a contrasting situation to that of the purines (Table IV and Fig. 4). After 6 hours of treatment with estrogen, the specific activity of uridine was the same from treated and control uteri. Only when the uridine per uterus (or micromoles of uridine per micromole of thymine) began to rise was there an increase in the total amount of radioactivity incorporated into the RNA; in most of the experiments this rate reached a maximum after 12 hours of treatment with estrogen and thereafter declined in a manner similar to the purine labeling. The specific activity values of

the isolated uridines, however, were the same for both control and estrogen-treated tissues. Hydrolysis of labeled uridine showed that within the

TABLE V

Alkaline Hydrolysis of Uterine Nucleic Acids

	Control	Plus estrogen
Adenine	127	495
	177	508
Guanine	172	882
	130	911

The nucleic acid-protein residues were derived from 1 hour $C^{14}O_2$ incorporation experiments with uterine segments from control and 6 hour estrogen-treated animals. The residues were hydrolyzed directly in 0.1 N NaOH for 18 hours at 37°. The nucleotides were isolated on Dowex 1 (chloride form), eluted with 0.5 N HCl, hydrolyzed for 45 minutes at 90°, and the purines isolated by ion exchange and paper chromatography as described under "Methods." Data are expressed as counts per minute per micromole of purine.

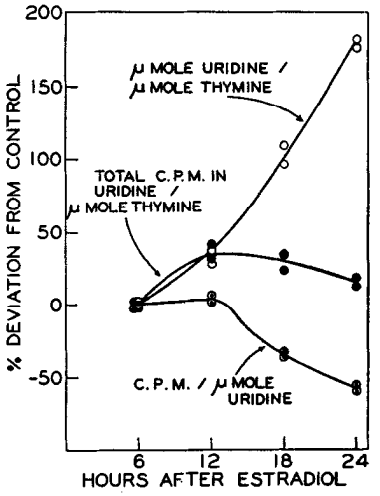

FIG. 4. The effect of estradiol on the labeling of nucleic acid uridine in surviving uterine segments incubated with $C^{14}O_2$. 10 γ of estradiol injected intravenously at zero time. Segments from four uterine horns incubated with $C^{14}O_2$ for 1 hour at 37° as described under "Methods." RNA measured as micromoles of uridine and expressed as ratio (micromoles of uridine per micromole of thymine) during the 1st 24 hours after hormonal treatment.

accuracy of the methods all the incorporated radioactivity from $C^{14}O_2$ was contained in the uracil portion of the nucleoside (Table VI). Similar

results were also obtained when uterine segments from control and estrogen-treated animals were incubated in a medium containing orotic acid-6-C^{14}.

Incorporation of $C^{14}O_2$ into DNA-Thymine—In accordance with the constancy of thymine per uterus, the labeling of thymine from $C^{14}O_2$ was very low and of questionable significance (Table IV). A similar result was obtained with orotic acid-6-C^{14}, which has been shown to be readily incorporated into DNA-thymine in tumor slices (17) and in slices isolated from regenerating liver (18). In accordance with the results of Jeener (19) and Telfer (10), the predominant products of early estrogen action lead to cellular hypertrophy rather than hyperplasia and DNA synthesis.

Effect of Estradiol Treatment on Incorporation of $C^{14}O_2$ into Acid-Soluble Nucleotides—In the attempt to explain the apparent difference in the effect

TABLE VI

Localization of Radioactivity in RNA-Uridine

Uridine	Uracil
7675	7850
7690	7800

Uridine was isolated from uterine segments incubated with $C^{14}O_2$ for 1 hour at 37° as described under "Methods." Uridine samples were hydrolyzed 3½ hours at 170° in 98 per cent formic acid. Uracil was isolated by paper chromatography with *n*-butanol-H_2O as the solvent system (7). Data are expressed as counts per minute per micromole of uridine and counts per minute per micromole of uracil.

of estrogens on the labeling of nucleic acid uridines and purines, analyses were conducted on the acid-soluble fraction to determine the activity of the pools of the respective precursor. Due to the low level of these nucleotides, it was necessary to confine these analyses to the free purine bases or uridylic acid nucleotides of the acid-soluble fraction. It is considered, however, that they represent to a certain degree the state of the precursor pool since rapid equilibration of radioactivity occurs between the constituent nucleotides (20). Table VII gives the results of a carrier experiment in which the total counts incorporated into acid-soluble guanine after 1, 3, and 6 hours of treatment with estrogen were determined. After 1 hour a slight increase was observed; however, by 6 hours the incorporated radioactivity reached five times that of the control level.

In the same experiment the total counts incorporated into acid-soluble uridine 5′-phosphate (UMP) were also determined (Table VIII). In contrast to lack of effect of estradiol pretreatment on the labeling of the nucleic acid uridine, 6 hours of treatment with estradiol increased the total

count in uridine 5′-phosphate to nearly three times that of the control level.

A summary of three experiments in which the amounts and specific

TABLE VII

Effect of Estradiol on Labeling of Guanine in Acid-Soluble Fraction

Treatment *in vivo*	Control	Plus estrogen
hrs.		
1	95	105
	84	100
3		263
		136
6	75	359
	78	368

10 γ of estradiol were injected intravenously at zero time. Segments from three uterine horns, 1 hour incubation with $C^{14}O_2$. 0.58 μmole of guanosine 5′-phosphate carrier was added to an aliquot of acid-soluble fraction. Hydrolysis, 25 minutes at 90° in 4 per cent perchloric acid. Guanine was isolated as described under "Methods." Data are expressed as total counts per minute in guanine per micromole of thymine.

TABLE VIII

Effect of Estradiol on Labeling of Uridine 5′-Phosphate in Acid-Soluble Fraction

Treatment *in vivo*	Control	Plus estrogen
hrs.		
1	8,970	11,950
	9,550	9,100
3		12,500
		7,550
6	8,500	29,500
	10,400	27,400

The system was as in Table VII. 0.83 μmole of UMP was added as carrier; after hydrolysis, UMP was reisolated by ion exchange (Dowex 1, chloride form) and paper chromatography with *tert*-butanol-HCl as solvent system (6). Data are expressed as total counts per minute in UMP per micromole of thymine.

activity of adenine and uridine 5′-phosphate were determined is presented in Table IX. It is clear from the consideration of these data that size of the adenine pool increases only slightly with estrogen treatment, whereas the UMP pool increased strikingly. In Experiment III (twelve rats per group) it was possible to determine the specific activity of the UMP; it is interesting that this specific activity was similar for both the control and the treated tissues. Thus the increase in total radioactivity incorporated

TABLE IX

Effect of Estrogen on Amount and Labeling of Acid-Soluble Nucleotides

Experiment I		Experiment II		Experiment III	
Adenine	UMP	Adenine	UMP	Adenine	UMP
Control rats, μmole isolated					
0.470 0.490	0.0060			0.238	0.0037
Estrogen-treated rats, μmole isolated					
0.565 0.540	0.011			0.282	0.024
Control rats, specific activity, c.p.m. per μmole					
		2465		2270	30,600
Estrogen-treated rats, specific activity, c.p.m. per μmole					
		6465		9630	26,700
Control rats, total c.p.m. incorporated					
		1080 945			141
Estrogen-treated rats, total c.p.m. incorporated					
		4260 4560			783

The system was as described under "Methods." Uterine segments were incubated with $NaHC^{14}O_3$ (except Experiment I). Selective hydrolysis of purine nucleotides, separation, and isolation by ion exchange and paper chromatographic techniques were as described. Where indicated, 1 μmole each of adenosine 5′-phosphate and of UMP were added as carriers. The data are expressed as micromoles of adenine or UMP per micromole of thymine, counts per minute per micromole of adenine or UMP, and total counts per minute incorporated per micromole of thymine. 6 hours of estrogen treatment: Experiment I, the data are obtained from six control and six estrogen-treated rats. No radioactivity measurements; Experiment II, data obtained from twelve control and twelve estrogen-treated rats. Endogenous nucleotides from 0.9 aliquots. Carriers added to 0.1 aliquots and reisolated. No quantitative recoveries of endogenous nucleotides from this experiment; Experiment III, same as in Experiment II.

into acid-soluble uridine 5′-phosphate was nearly matched by an enlargement of the diluting pool. In the case of adenine the estrogen accelerated the incorporation of radioactivity into a pool size similar to the control level, thus resulting in a higher specific activity of the precursor pool of nucleotides used in the synthesis of the nucleic acids.

Incorporation of $C^{14}O_2$ into Proteins—In view of the increased ability of estrogen-treated uterine segments to incorporate radioactive serine, glycine, lysine, and tryptophan, the rate of $C^{14}O_2$ incorporation into proteins was tested in stimulated and control tissue. It is evident from the data in Table III that treatment with estrogen for 6 hours had no effect on the

TABLE X

Effect of Estradiol on Incorporation of $C^{14}O_2$ into Aspartic and Glutamic Acids of Rat Uterine Protein

Isolated component	Treatment with estradiol			
	6 hrs.		12 hrs.	
	Control	Estrogen-treated	Control	Estrogen-treated
Aspartate	217	190	191	273
	175	216	166	335
Glutamate	38	39	37	57
	40	59	36	58

Aspartate and glutamate obtained in the 2 N HCl eluates from the ion exchange-treated formic acid hydrolysates (see under "Methods") were isolated as the dinitrophenyl derivatives according to the method of Perrone (5). The data are expressed as counts per minute per micromole of dinitrophenyl-amino acid. Each flask contained uterine segments from two animals treated with estradiol or control solution 6 or 12 hours before being killed.

$C^{14}O_2$ incorporation into the proteins. In a similar experiment (Table X), the proteins were hydrolyzed and aspartic and glutamic acids were isolated as the dinitrophenyl derivatives. Only after 12 hours of treatment with estradiol was there a significant stimulation of incorporation of CO_2 into these amino acids. It is possible that a higher rate of incorporation at 6 hours might have been masked by a greater dilution which resulted from larger pools of non-radioactive endogenous intermediates in estrogen-treated uteri. This phenomenon was suggested previously as an explanation for the lack of estrogen stimulation of incorporation of exogenous alanine *in vitro* (3). However, recent experiments from this laboratory[3] have demonstrated that the enzymes involved in the activation of a number

[3] McCorquodale, D. J., and Mueller, G. C., unpublished experiments.

of so called non-essential amino acids are only slightly increased in activity during the 1st 12 hours of estrogen treatment.

DISCUSSION

These experiments, as well as earlier studies (1, 2, 10), demonstrate that the nucleic acid metabolism of the rat uterus is highly sensitive to the action of estradiol. Although the effect of the hormone is revealed through a stimulation of the incorporation of radioactive precursors into both the acid-soluble nucleotide fraction and into the nucleic acid polymers, the primary action appears to involve the precursor nucleotides. Both the size of the pools and the amount of labeling are increased rapidly, owing to the action of the hormone. It is of interest that the estrogen-induced expansions of both the adenine and uridine nucleotide pools are of a similar magnitude, whereas originally the pool of adenine nucleotides in the control tissues is approximately 60 times as large as that of the uridine nucleotides. This is compatible with preliminary data which support a partial breakdown or reshuffling of existing RNA during the first few hours of estrogen treatment.

In order to characterize the role of the hormone in accelerating the labeling of nucleotides with glycine-2-C^{14}, formate-C^{14}, serine-3-C^{14}, and $C^{14}O_2$, it appears necessary now to study the individual enzymatic steps in these synthetic sequences. The observed results could be accounted for by alterations in the sizes of the intermediate pools, in enzyme activities, or both. However, in view of the observed increases of serine aldolase (21) and of seven amino acid-activating enzymes[3] in association with the early estrogenic stimulation of "1-carbon" and protein metabolism, respectively, it is anticipated that the observed changes in nucleotide metabolism will also be attended by increases in enzymes catalyzing certain key reactions. Thus the mechanism of action of the estrogen may involve its role in the activation of preexisting enzyme proteins or in the induction of enzyme formation.

SUMMARY

1. Estradiol treatment induces a rapid accumulation of ribonucleic acid in rat uteri within a 24 hour period; deoxyribonucleic acid content of the uterus remains constant during this period.

2. In the period from 1 to 6 hours after hormonal treatment *in vivo*, before ribonucleic acid accumulation, the early action of estradiol nucleotide metabolism is revealed as a stimulation of the incorporation *in vitro* of $C^{14}O_2$ into adenine, guanine, and uridine of the acid-soluble nucleotides from surviving uterine segments.

3. The effect of estradiol on the labeling of nucleic acid purines and uridine with $C^{14}O_2$ in surviving uterine segments reflects primarily the

effect of the hormone on the size and specific activity of the acid-soluble pool of precursor nucleotides.

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