Studies on the Folate Coenzyme Metabolism in the Castrated Rat and Treated with 17β-Estradiol (36792)

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It has been demonstrated that one of the earliest effects of the estrogens on the uterus is the stimulation of the production of nuclear RNA (1–5). This could be an essential prerequisite for most metabolic and physiologic effects of estrogens and, in particular, for those promoting growth and development (6).

Folate coenzymes are directly involved in the synthesis of purine and pyrimidine nucleotides (7, 8) and prompted a study on the effects of castration and the treatment with 17β-estradiol on the metabolism of folates[1] in the liver and uterus of female adult rats. In a previous paper (9) it was shown that whereas castration caused only slight changes in the content of folate coenzymes of the liver and uterus, 17β-estradiol administration led to values higher than normal. As most of the metabolic processes in which these coenzymes are involved appear to be very depressed in castrated animals, the normal folate coenzyme content observed in the absence of the hormone might be the consequence of a lower synthesis together with a lower utilization. To verify this hypothesis we have examined the effect of castration and of 17β-estradiol administration on the synthesis *in vivo* and *in vitro* of folate coenzymes.

Materials and Methods. Animals. Female albino rats (Wistar) 20 wk old, 220–250 g in weight, divided into 4 groups, were used. Half of the animals were ovariectomized by the middorsal approach under ether anesthesia. After 3 wk one group of normal and one of ovariectomized rats were injected subcutaneously with 17β-estradiol 10 μg/100 g of body wt in 0.2 ml of 1% ethanol, 0.05 *M* phosphate buffer–saline solution (pH 7.5) every other day for 6 days. Intact and ovariectomized rats were injected with the same volume of vehicle to serve as controls.

In vivo *experiment.* To determine the capacity of conversion *in vivo* of folic acid into its activated forms, we have assayed the liver contents of folate coenzymes and the levels of their metabolites excreted in the urine. Thirty-six hours after the last injection, 8 rats of each group were injected intraperitoneally with 200 μg of folic acid/100 g of body weight, and given 5 ml of 0.005 *M* NaCl by stomach tube. The rats were placed in individual metabolism cages and urine samples were collected for 12 hr in bottles containing 100 mg of potassium ascorbate. The total folate activities in urine were assayed aseptically with *L. casei* ATCC 7469, the reduced forms with *P. cerevisiae* ATCC 8081. The rats were killed by cervical fracture 48 hr after the last injection of hormone. The livers and uteri were quickly removed and placed into ice-cold water for the folate coenzyme determination. Extraction of these compounds from the tissues and the microbiological assay were performed by the method of Bird, McGlohon and Waitkus (10).

In vitro *experiment.* The enzymic activities involved in the synthesis of folate coenzymes were determined in the liver and uterus.

The animals were killed 48 hr after the last injection of the hormone and the livers and uteri were immediately removed, then were homogenized and centrifuged. In the supernatants H_4folate dehydrogenase (EC

[1] The following abbreviations are used: H_2folate, dihydrofolate; H_4folate, tetrahydrofolate; 5,10-CH_2-H_4folate, N^5,N^{10}-methylenetetrahydrofolate; 10-HCO-H_4folate, 10-formyltetrahydrofolate; 5,10-$CH{=}H_4$ folate, N^5,N^{10}-methylidynetetrahydrofolate.

TABLE I. Effect of Castration and of 17β-Estradiol Treatment on Conversion of Folic Acid into Activated Forms: Urinary Excretion of Folate Derivatives by Rats After Injection of Folic Acid.[a]

Experimental animals	Folate derivatives excreted in urine	
	L. casei[b]	*P. cerevisiae*[c]
Normal rats	447 ± 12	15.7 ± 0.77
Normal rats + 17β-estradiol	293 ± 4[e]	16.7 ± 0.40
Castrated rats	605 ± 49[d]	25.8 ± 0.37[e]
Castrated rats + 17β-estradiol	431 ± 6	13.4 ± 0.63

[a] All values are expressed in μg of folate metabolites excreted/12 hr/rat and represent mean of eight animals ± SE of the mean.

[b] *L. casei* measures all folate forms.

[c] *P. cerevisiae* measures folate forms reduced to tetrahydro level except the 5-CH_3-H_4folate.

[d] Significance of differences from values for normal animals: $p < .05$; [e] $p < .001$.

1.5.1.3) was determined by measuring the decrease in the absorbance at 340 mμ caused by the conversion of NADPH to NADP (11). Serine hydroxymethyltransferase (EC 2.1.2.1) was evaluated by measuring colorimetrically both the free HCHO and the bound HCHO in 5,10-CH_2-H_4folate with the acetylacetone reagent (12). 5,10-CH_2-H_4 folate dehydrogenase (EC 1.5.1.5) and 10-HCO-H_4folate synthetase (EC 6.3.4.3) were assayed by determining spectrophotometrically the 5,10-CH=H_4folate formed (13, 14). Protein was determined by the method of Lowry *et al.* (15) with crystalline bovine plasma albumin as the standard.

Results and Discussion. Urinary excretion of folate metabolites showed a marked increase of the compounds active for *L. casei* ($p < .05$) and for *P. cerevisiae* ($p < .001$) in the castrated animals compared with controls (Table I). Hormone treatment to normal rats caused a decrease in the urinary excretion of the metabolites active for *L. casei* ($p < .001$); the same treatment to castrated animals returned the quantity excreted of these compounds to normal values.

Liver folate coenzymes showed a decrease of total folate activity in castrated rats compared with normal rats ($p < .001$). An evident decrease was observed in the content of reduced forms ($p < .001$) and, in particular, of H_4folate ($p < .01$); this difference was partially due to a higher content of nonreduced forms ($p < .001$) (Table II).

Liver enzymes in castrated animals exhibited a decrease of H_4folate dehydrogenase activity ($p < .01$) and a more marked fall in serine hydroxymethyltransferase, 10-HCO-H_4folate synthetase and 5,10-CH_2-H_4folate dehydrogenase ($p < .001$). The treatment with 17β-estradiol completely normalized H_4folate dehydrogenase and, partially, the other three enzymic activities (Table III).

Following castration a significant decrease

TABLE II. Effect of Castration and of 17β-Estradiol Treatment on the Conversion of Folic Acid into Its Activated Forms: Folate Coenzymes in Rat Liver After Injection of Folic Acid.

Compounds[a]	Liver folate activity			
	Normal rats	Normal rats + 17β-estradiol	Castrated rats	Castrated rats + 17β-estradiol
All folate forms	15,016 ± 370	14,150 ± 202	13,033 ± 237[d]	13,875 ± 373[b]
All nonreduced forms	1426 ± 42	1350 ± 69	1910 ± 20[d]	1372 ± 12
All tetrahydro forms	13,590 ± 330	12,800 ± 133	11,123 ± 217[d]	12,502 ± 27
5-CH_3-H_4folate	6740 ± 223	6855 ± 488	5490 ± 171	6117 ± 60
10-HCO-H_4folate	3415 ± 257	3023 ± 139	3278 ± 89	2715 ± 221
5-HCO-H_4folate	1191 ± 30	1121 ± 56	1310 ± 72	1274 ± 50
H_4folate	2243 ± 376	1800 ± 439	616 ± 115[c]	2395 ± 55

[a] All values are expressed in ng/g tissue and represent mean of six determinations on different animals ± SE of the mean.

[b] Significance of differences from values for normal animals: $p < .05$; [c] $p < .01$; [d] $p < .001$.

TABLE III. Effect of Castration and of 17β-Estradiol Treatment on Enzymic Activities Catalyzing the Metabolism of Folate Coenzymes in Rat Liver.[a]

Experimental animals	H_4folate dehydrogenase[b]	Serine hydroxymethyltransferase[c]	10-HCO-H_4folate synthetase[d]	5,10-CH_2-H_4folate dehydrogenase[d]
Normal	5.27 ± 0.16 (5)	1206 ± 65 (5)	4037 ± 67 (5)	361 ± 11 (5)
Normal + 17β-estradiol	4.75 ± 0.68 (5)	1319 ± 82 (5)	3675 ± 266 (5)	327 ± 13 (5)
Castrated	4.29 ± 0.13 (15)[f]	482 ± 18 (13)[g]	2617 ± 86 (13)[g]	178 ± 6 (15)[g]
Castrated + 17β-estradiol	5.11 ± 0.30 (7)	889 ± 55 (11)[f]	3349 ± 236 (9)[e]	269 ± 9 (9)[e]

[a] All values represent mean ± SE of the mean; in parentheses number of rats.
[b] Nanomoles H_2folate reduced/min/mg protein.
[c] Nanomoles of HCHO utilized/20 min/mg protein.
[d] Nanomoles of 5,10-CH=H_4folate formed/20 min/mg protein.
[e] The significance of differences: $p < .05$; [f] $p < .01$; [g] $p < .001$.

of uterine 10-HCO-H_4folate synthetase was noted ($p < .001$); this activity was partially normalized by the hormone treatment. Because of the low activity of H_4folate dehydrogenase in the uterus no value has been given (Table IV). 17β-estradiol administration to normal rats did not cause significant changes in either liver or uterine enzymic activities.

From the results of the *in vivo* and *in vitro* experiments it is quite evident that 17β-estradiol can affect the metabolism of folic acid and, in particular, the synthesis of its coenzymic derivatives. The castrated rats excrete in urine more folate metabolites active for *L. casei*, which measures the total folate activity, than do normal rats suggesting the elimination of almost all folic acid injected. This may indicate a lower capacity, in castrated rats, for utilizing the vitamin for the synthesis of its coenzymes. The data relating to the liver coenzyme distribution are supporting. In fact, in castrated rats there is a significant fall in all reduced forms, particularly H_4folate, whereas there is an increase in nonreduced forms. The lower capacity of castrated rats for synthesizing folate coenzymes must be considered the consequence of severe alterations of enzymic activities catalyzing the principal steps of the biosynthetic process such as H_4folate dehydrogenase and 10-HCO-H_4folate synthetase. In fact, hormone administration to the castrated rats, restoring their enzymic activities, returns also the coenzyme concentration in the tissues and the urinary excretion of folate derivatives to normal values. Therefore, the present data support our previous hypothesis (9) according to which the lower synthesis of folate coenzymes in castrated rats may be masked by the lower need for these compounds. The higher excretion of reduced forms, active for *P. cerevisiae*, is a further affirmation. 17β-Estradiol, being able to regulate the synthesis and storage of folate coenzymes, may influence the anabolic processes in which these compounds are involved, such as nucleic acid synthesis.

Summary. The effect of 17β-estradiol on folate coenzyme metabolism was studied in normal and castrated female rats. The capacity of conversion *in vivo* of folic acid into activated forms was investigated by measuring the quantity of folate derivatives in the urine and the liver folate coenzyme contents after injection of folic acid. An increase in the urinary excretion of folate metabolites and a decrease in the liver content of reduced folate forms in castrated rats was seen compared with normal rats. Treatment of castrated animals with 17β-estradiol were corrective. The enzymic activities involved in the folate coenzyme metabolism were assayed *in vitro* both in the liver and in the uterus. Severe alterations were observed in the enzymes of castrated animals and here, too, 17β-estradiol administration restored the biochemical lesions almost completely. The re-

TABLE IV. Effect of Castration and of 17β-Estradiol Treatment on Enzymic Activities Catalyzing the Metabolism of Folate Coenzymes in Rat Uterus.[a]

Experimental animals	Serine hydroxymethyltransferase[b]	10-HCO H_4folate synthetase[c]	5,10-CH_2-H_4folate dehydrogenase[c]
Normal	66 ± 2.0 (10)	287 ± 10 (5)	26 ± 2.2 (10)
Normal + 17β-estradiol	53 ± 1.5 (10)	243 ± 15 (5)	31 ± 2.6 (7)
Castrated	63 ± 2.0 (25)	145 ± 5 (19)[e]	24 ± 1.4 (27)
Castrated + 17β-estradiol	55 ± 3.6 (16)	208 ± 18 (13)[d]	30 ± 3.3 (16)

[a] All values represent mean ± SE of the mean; in parentheses, number of rats.
[b] Nanomoles of HCHO utilized/20 min/mg protein.
[c] Nanomoles of 5,10-CH=H_4folate formed/20 min/mg protein.
[d] The significance of differences: $p < .01$; [e] $p < .001$.

sults show a possible influence on folate coenzyme synthesis by 17β-estradiol.

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