RNA with a higher *S* value at the phenol-water interface cannot be excluded, particularly since MHV3 is thermolabile.

Thus, after MHV3 infection, an actinomycin D–insensitive 16*S* RNA is extractable at 60°C from the phenol residue obtained at 0°C. This RNA may be related to the MHV3 virus, and may in fact be viral RNA.

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17β-Estradiol: Inducer of Uterine Hexokinase

Abstract. *Administration of estradiol to ovariectomized rats induced new synthesis of uterine hexokinase which was prevented by actinomycin D, 5-fluorouracil, cycloheximide, or ethionine. The estradiol-induced increase in uterine hexokinase activity was detectable as early as 4 hours. The increase in enzyme activity was dependent upon the dose of the hormone. The evidence indicates that the increased hexokinase activity induced by 17β-estradiol may entail an acceleration of the synthesis of certain RNA species.*

An understanding of the mechanisms underlying hormone action at the molecular level is facilitated by the concept that certain hormones act on whole "functional genic units" resulting in a synchronized increase or decrease in the biosynthesis of functionally related, rate-limiting enzymes governing one-way reactions (*1*). Earlier investigations supported this concept and provided evidence for the action of glucocorticoids as inducer and of insulin as both inducer and suppressor on well-defined functional genic units in hepatic carbohydrate metabolism (*2*). The three key enzymes of hepatic glycolysis—glucokinase, phosphofructokinase, and pyruvate kinase—exhibit relatively low activities and catalyze one-way reactions. It was suggested that this triad of key glycolytic enzymes in liver might occupy the same functional genome unit because of their strategic metabolic role and similarity in response to nutritional and hormonal influences (*2*, *3*). In accordance with the "functional-genome-unit" concept of hormone action, all three of these glycolytic enzymes were expected to be induced or suppressed in a synchronous manner by male and female sex hormones in their respective target organs.

Uterine phosphofructokinase decreased on castration and returned to the normal range by treatment with estradiol (*4*). Because the estradiol-induced rise in phosphofructokinase activity was blocked by a variety of inhibitors of protein biosynthesis, it was suggested that the observed increases in uterine phosphofructokinase activity represent enzyme synthesis *de novo*. Smith and Gorski (*5*) reported that uterine tissue, unlike liver, contains little or no glucokinase [enzyme with high Michaelis constant (K_m)] and that, in the uterus, glucose is phosphorylated by the hexokinase with low K_m. We now confirm their observations and show that estradiol induces new synthesis of hexokinase in the uterus; this synthesis can be prevented by injection of actinomycin, 5-fluorouracil, cycloheximide, or ethionine.

Table 1. Effect of varying doses of 17β-estradiol on uterine hexokinase. Means ± S.E. represent three determinations of enzyme activity. Each determination was carried out in uteri pooled from two to three rats. Groups of eight to ten ovariectomized rats were given 17β-estradiol and killed after 16 hours. Percentage of control represents the statistically significant difference as compared to the values of control rats ($P < .05$).

17β-Estradiol (μg/100 g)	Hexokinase activity	Percentage of control
Control	5.6 ± 0.6	100
1	10.4 ± 0.9	186
2	11.7 ± 0.6	209
5	12.8 ± 1.4	218
10	15.3 ± 1.2	273
20	13.5 ± 0.7	241
30	13.6 ± 1.1	241

Young female rats of the Wistar strain, weighing approximately 150 g, were used 2 weeks after bilateral ovariectomy. Uteri were quickly excised, and homogenates and supernatants were prepared (*4*). Hexokinase activity was assayed in the supernatant under linear kinetic conditions by a modification (*2*) of the method of DiPietro and Weinhouse (*6*). The rate of formation of reduced nicotinamide-adenine dinucleotide phosphate was measured at 340 nm (37°C) in a constant recording Unicam spectrophotometer, model SP-800. Enzyme activity is expressed in micromoles of substrate metabolized per hour per gram (fresh weight) of uterus times the weight of the organ (*4*, *7*). The data were evaluated statistically, and the significant differences between the means were calculated as *P* values.

We were not able to show, in supernatants from uteri of either castrated or normal rats, any significant amounts of glucokinase activity with high K_m. Our observations agree with those of Smith and Gorski (*5*) who showed that the uterus of the immature rat contains little or no glucokinase with high K_m. The absence of glucokinase and the presence of high activities of hexokinases in rat intestine, heart, and skeletal muscle have been reported by Katzen and Shimke (*8*).

To detect the earliest increase in uterine hexokinase activity after administration of estradiol, we followed the sequence of events for 24 hours. Groups of ovariectomized rats were injected intramuscularly with doses of 10 μg of estradiol per 100 g of body weight and killed after 2, 4, 8, 16, and 24 hours. Figure 1 shows that the earliest detectable increase (127 percent) in uterine enzyme activity was observed 4 hours after administration of the hormone. Hexokinase activity was further increased to 154, 294, and 280 percent of the control values at 8, 16, and 24 hours, respectively. The time course for induction of uterine hexokinase by estradiol appears to be qualitatively similar to that reported for phosphofructokinase and phosphohexose isomerase (*4*).

We performed studies to establish whether smaller doses of the hormone were also capable of inducing significant increases in the activity of this uterine enzyme. Groups of ovariecto-

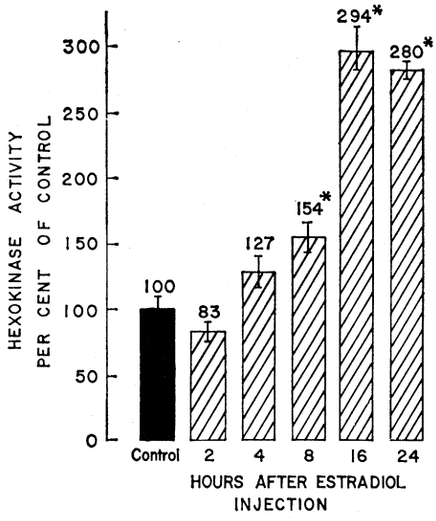

Fig. 1. Time course of estradiol-induced increase in uterine hexokinase activity during a 24-hour period. Bars represent the means and S.E. of three determinations of enzyme activity. Each enzyme assay was carried out in uteri pooled from two to three rats. 17β-Estradiol (10 μg/100 g), dissolved in a solution of ethyl alcohol and 0.9 percent NaCl, was injected intramuscularly, and groups of ovariectomized rats were killed at 2, 4, 8, 16, and 24 hours. Control rats received an equal volume of the vehicle solution alone. Data are given in percentages, with the values of control rats as 100 percent. The asterisks denote statistically significant alterations as compared with the values of control rats ($P < .05$).

Table 2. Effect of inhibitors of RNA and protein synthesis on estrogen-induced increases in uterine hexokinase. Means ± S.E. represent three to four determinations of enzyme activity. Each enzyme assay was carried out in uteri pooled from two to three rats. Ovariectomized rats were injected intramuscularly with 17β-estradiol (10 μg/100 g); at the same time, actinomycin (25 μg/100 g), 5-fluorouracil (15 mg/100 g), and cycloheximide (70 μg/100 g) were administered intraperitoneally along with estradiol. Ethionine (100 mg/100 g) was given intraperitoneally in two equally divided doses at 8-hour intervals. The rats were killed 16 hours later.

Treatment	Hexokinase activity	Percentage of control
Control	5.9 ± 0.3	100
17β-Estradiol	16.5 ± 0.9	280*
17β-Estradiol + actinomycin	7.5 ± 0.5	127†
17β-Estradiol + 5-fluorouracil	8.0 ± 0.1	135‡
17β-Estradiol + cycloheximide	5.7 ± 0.6	97†
17β-Estradiol + ethionine	8.2 ± 0.4	139†

* Statistically significant difference as compared to the values of control rats ($P < .05$).
† Statistically significant difference as compared to the values of estradiol-treated rats without administration of any inhibitor ($P < .05$).
‡ Statistically significant difference as compared to values of both control and estradiol-treated rats (with no inhibitor).

mized rats were given single intramuscular injections of 17β-estradiol in doses varying from 1.0 to 30.0 μg/100 g and killed after 16 hours. Results are given in Table 1.

Our earlier investigations suggested that the estrogenic induction of uterine phosphofructokinase and phosphohexose isomerase may represent enzyme synthesis *de novo* involving stimulation of the synthesis of certain RNA species (*4*). The nature of the estrogen-induced increases in hexokinase activity was studied with the use of various compounds known to inhibit RNA and protein synthesis. We used the following groups, each consisting of 10 to 12 ovariectomized rats: control, those treated with estradiol alone, and those treated with estradiol and then given actinomycin D, 5-fluorouracil, cycloheximide, or ethionine. Rats from all groups were killed 16 hours after administration of the hormone. Cycloheximide, which inhibits protein synthesis by blocking the transfer of amino acids from soluble RNA to protein (*9*), was most effective (Table 2) in preventing the observed estradiol-induced increase in uterine hexokinase; the enzyme values in this group of rats were in the normal range. The results suggest that the estrogen-induced increases in the activity of uterine hexokinase may represent new enzyme formation involving enhanced synthesis of RNA.

The precise mechanism or mechanisms of estrogen action still remain to be elucidated, but the evidence implicates regulation of RNA and protein synthesis as an important action of estrogenic hormones. Mueller *et al.* (*10*) demonstrated that estradiol accelerates synthetic reactions which result in a rapid accumulation of uterine RNA, protein, and phospholipid. Puromycin, cycloheximide, and actinomycin D effectively prevented these hormonal responses (*10*, *11*). Means and Hamilton (*12*) showed that 17β-estradiol enhanced the synthesis of nuclear RNA as well as the uptake of RNA precursor by the uterus within 2 minutes of hormone injection. Underlying the hormonal stimulation of RNA synthesis is a rapid increase in the activity of RNA polymerase which was completely prevented by puromycin (*11*, *13*). In addition, Barker and Warren (*14*) demonstrated that administration of 17β-estradiol caused an increase in the template activity of uterine chromatin for RNA synthesis. Our observations on the inhibition of estrogen induction of uterine hexokinase by inhibitors of RNA synthesis suggest that stimulation of RNA synthesis is an early biochemical response elicited by estrogenic hormones. These observations lend support to the work of Nicolette and Mueller (*10*, *15*), who suggested that estrogens might act at specific sites in the genome and that one of the primary actions of these hormones is to accelerate genetic expression in the uterus. Furthermore, it is quite conceivable that, analogous to the action of insulin and glucocorticoids on liver carbohydrate metabolism (*1*, *2*), estrogens might attack receptor sites at the source of enzyme production to switch on the biosynthetic action of the whole "functional genic unit" which governs the synthesis of key, rate-limiting glycolytic enzymes in the uterus.

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