Biochemical Pharmacology, Vol. 17, pp 1251–1260. Pergamon Press. 1968. Printed in Great Britain

METABOLIC CONTROL MECHANISMS IN MAMMALIAN SYSTEMS—II.

HORMONAL REGULATION OF UTERINE PHOSPHOHEXOSE ISOMERASE*

RADHEY L. SINGHAL and JOSEPH R. E. VALADARES

Department of Pharmacology, University of Ottawa, Faculty of Medicine, Ottawa, Canada

(*Received* 16 *October* 1967; *accepted* 22 *December* 1967)

Abstract—The regulation of phosphohexose isomerase activity by various estrogenic hormones, progesterone and testosterone has been studied in the uterus of the ovariectomized rat. Whereas estriol failed to exert any significant effect on uterine enzyme activity, estradiol-17β increased phosphohexose isomerase to 294 per cent, diethylstilbestrol to 170 per cent and estrone to 139 per cent of the control values. Time-course studies demonstrated that a single i.m. injection of estradiol-17β (10·0 μg/100 g) elevated phosphohexose isomerase to 153, 175, 250 and 222 per cent at 4, 8, 16 and 24 hr respectively. Actinomycin, an inhibitor of DNA-directed RNA synthesis, and ethionine, an inhibitor of protein synthesis, effectively prevented the estrogen-induced increases in uterine enzyme activity observed during the 24-hr period. In addition, 5-fluorouracil as well as cycloheximide blocked the peak increase in phosphohexose isomerase observed at 16 hr after administration of estradiol. Neither progesterone nor testosterone produced any significant effect on the basal levels of the uterine enzyme; however, both hormones were capable of suppressing the estrogen-induced enzyme increases.

In order to study the effect of chronic estrogen treatment on uterine phosphohexose isomerase, ovariectomized rats were treated by injection with estradiol-17β, 2·5 μg/100 g, twice a day for 3 days. Enzyme activity in uteri of these rats increased to 237, 325 and 398 per cent at 24, 48 and 72 hr respectively. Actinomycin given concomitantly with estradiol largely prevented the hormone-induced enzyme response. The present results complement our earlier findings on the hormonal control of uterine phosphofructokinase and lend additional support to the view that stimulation of the synthesis of certain RNA species is one of the primary actions of estrogenic hormones.

THE HORMONAL induction of phosphofructokinase (ATP: D-fructose 6-phosphate 1 phosphotransferase, EC 2.7.1.11.†) in the uterus of the ovariectomized rat has been described in an earlier communication.[1] Studies with various estrogenic hormones have revealed that estradiol-17β was the most potent inducer of phosphofructokinase activity. This estradiol-induced increase in the activity of uterine phosphofructokinase was shown to represent enzyme synthesis *de novo* in studies using inhibitors of RNA and protein synthesis.[1–4] Moreover, since sex hormones are known to regulate important metabolic processes in their target structures, it was of interest to investigate

* This investigation was supported by grants from the Medical Research Council of Canada. A preliminary account of portions of this work has appeared previously in *Proc.* 7th *Int. Congr. Biochemistry*, Tokyo, Japan (1967).

† See Report of the Commission on Enzymes. Pergamon Press, Oxford (1961).

the behaviour of another carbohydrate-metabolizing enzyme, phosphohexose isomerase (D-glucose 6-phosphate ketol-isomerase, EC 5.3.1.9*). The present work describes the hormonal regulation of phosphohexose isomerase in the uterus of the ovariectomized rat and provides evidence to support the view that the estrogen-induced rapid increases in the activity of phosphohexose isomerase are the result of new enzyme synthesis.

MATERIALS AND METHODS

Animals and experimental conditions. Young female Wistar rats weighing 180–200 g were used in these studies. Bilateral ovariectomies were performed and the animals were used 2 weeks post-operatively as described earlier.[1, 2, 5] The following experimental design was used.

Comparison of the effect of various estrogens. Groups of ovariectomized rats were given i.m. injections of estradiol-17β, estrone, estriol and diethylstilbestrol and were sacrificed 16 hr later. The hormones were administered in a single dose of 10·0 μg/100 g rat.

Effects of 5-fluorouracil and cycloheximide. In order to investigate the effects of 5-fluorouracil and cycloheximide on the estradiol-induced increase in uterine phosphohexose isomerase activity, estradiol-treated rats were injected i.p. with either 5-fluorouracil (15·0 mg/100 g) or cycloheximide (100 μg/100 g) 30 min prior to administration of the hormone. All animals were killed 16 hr after hormone injection.

Time-course of actinomycin and ethionine inhibition during 24 hr. Estradiol-treated rats were given actinomycin (25 μg/100 g) or ethionine (100 mg/100 g) i.p., and groups of rats were sacrificed at 4, 8, 16 and 24 hr after administration of the hormone.

Sequential effects for 72 hr after injection of actinomycin to estradiol-treated rats. The following 2 groups of ovariectomized rats were employed: 1) estradiol-treated rats; 2) estradiol-injected animals given actinomycin. Estradiol-17β (2·5 μg/100 g) was injected i.m. twice a day for 3 days at 12-hr intervals. Actinomycin (5 μg/100 g) was administered i.p. once every morning 30 min prior to hormone injection. Groups of rats were killed at 24, 48 and 72 hr after initiation of hormone treatment.

Effect of progesterone. Groups of control rats as well as those treated with estradiol-17β were injected i.m. with progesterone (5 mg/100 g) and sacrificed after 16 hr.

Effect of testosterone. The effect of testosterone administration on uterine phosphohexose isomerase was examined in the following groups of ovariectomized rats: (a) control rats; (b) animals injected with estradiol; (c) rats injected i.m. with testosterone; d) estradiol-injected animals given testosterone in a dose of 2·5 mg/100 g; e) estradiol-treated rats injected with testosterone, 5·0 mg/100 g. All rats were sacrificed 16 hr later.

Preparation of homogenate and supernatant fluid. The preparation of uterine homogenates and supernatant fluids has been described.[1, 3, 5] Phosphohexose isomerase activity was assayed in the supernatant under linear kinetic conditions according to the method of Glock *et al.*[6] Enzyme activity was calculated as μmoles of substrate metabolized/hr/g wet wt. tissue (at 37°) times weight of the uterus, as described earlier.[1, 2, 7, 8] The results were subjected to statistical evaluation and significant

* See *Report of the Commission on Enzymes*, Pergamon Press, Oxford (1961).

differences between the means (calculated as P values) are shown. No statistical significance is indicated when the P value was $> 0{\cdot}05$.

Chemicals. Estradiol-17β, DL-ethionine and the sodium salt of glucose 6-phosphate were obtained from Sigma Chemical Co. Estrone, estriol, diethylstilbestrol, progesterone and testosterone were purchased from Nutritional Biochemicals. Generous supplies of cycloheximide, 5-fluorouracil and actinomycin D were donated, respectively, by Upjohn, Hoffman La Roche Inc., and Merck, Sharp & Dohme.

RESULTS AND DISCUSSION

Comparative effect of four estrogenic hormones on uterine phosphohexose isomerase activity. The effect of estradiol-17β, estrone, estriol and a nonsteroidal estrogen, diethylstilbestrol, on uterine enzyme activity was examined in the ovariectomized rat and the data are presented in Table 1. Estradiol-17β was found to be the most potent

TABLE 1. EFFECTS OF FOUR ESTROGENIC HORMONES ON UTERINE PHOSPHOHEXOSE ISOMERASE ACTIVITY IN OVARIECTOMIZED RATS*

Treatment	Uterine weight (g)	Enzyme activity
Control	0·14 ± 0·01 (100)	340 ± 22 (100)
Estradiol-17β	0·32 ± 0·02 (228)†	1002 ± 51 (294)†
Diethylstilbestrol	0·22 ± 0·01 (157)†	576 ± 15 (170)†
Estrone	0·19 ± 0·01 (136)	472 ± 9 (139)†
Estriol	0·14 ± 0·01 (100)	338 ± 15 (99)

* Means ± S.E. represent 3 determinations of enzyme activity. Each determination was carried out in uteri pooled from 2–3 rats. Ovariectomized rats were injected with various estrogenic hormones (10 μg/100 g) i.m., and were sacrificed after 16 hr. Data are also given in percentages (in parentheses), taking the values of control rats as 100 per cent.

† Statistically significant difference as compared with the values of control rats ($P = < 0{\cdot}05$).

inducer among the estrogens tested. After its administration, phosphohexose isomerase activity increased to 294 per cent of control values. Estriol failed to exert any significant effect on enzyme activity, while phosphohexose isomerase was increased to 170 per cent by diethylstilbestrol and to 139 per cent by estrone. Uterine weights were increased to 228, 157 and 136 per cent by estradiol-17β, diethylstilbestrol and estrone, respectively. The parallelism between the known relative physiological potencies of these estrogenic compounds and their enzymatic responses is of interest, and is similar to the observations reported earlier for phosphofructokinase.[1]

Effect of 5-fluorouracil and cycloheximide on estradiol-induced increases in uterine phosphohexose isomerase. Previous studies suggested that the estrogenic induction of phosphohexose isomerase may involve stimulation of the synthesis of certain RNA species. This conclusion was supported by studies in which the estradiol-induced

increase in the activity of uterine phosphohexose isomerase was markedly blocked by actinomycin D, an inhibitor of messenger-RNA synthesis, and by puromycin, an inhibitor of protein synthesis acting at the level of soluble-RNA.[5] The effects of two other inhibitors, 5-fluorouracil and cycloheximide, were examined in order to understand better the mechanisms underlying these hormone-induced enzyme responses. Administration of estradiol-17β increased uterine weights to 250 per cent of the control values. When estradiol-injected rats were given either 5-fluorouracil or cycloheximide, the observed increases were markedly inhibited and uterine weights in these two groups remained at 144 and 142 per cent respectively. Fig. 1 shows that

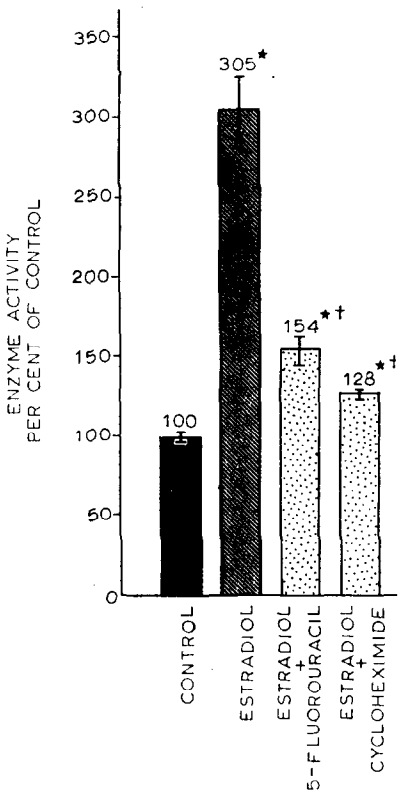

FIG. 1. Effect of 5-fluorouracil and cycloheximide on estradiol-induced increase in uterine phosphohexose isomerase. Bars represent the means and S.E. of three values, each obtained by pooling uteri from 3–4 rats. Ovariectomized rats were injected with estradiol-17β (10 μg/100 g) i.m., and were sacrificed after 16 hr. 5-Fluorouracil (15 mg/100 g) or cycloheximide (100 μg/100 g) was administered i.p. 30 min prior to estradiol injection. Data are given in percentages, taking the values of control rats as 100 per cent. * = Statistically significant difference as compared with the values of control rats ($P = < 0{\cdot}05$). † = Statistically significant difference as compared with the values of estradiol-treated animals without 5-fluorouracil or cycloheximide administration ($P = < 0{\cdot}05$).

the administration of estradiol increased phosphohexose isomerase activity to 305 per cent of the control values. However, when 5-fluorouracil or cycloheximide was given to rats injected with estradiol, the rise in enzyme activity was largely abolished and phosphohexose isomerase remained at 154 and 128 per cent respectively. These

studies as well as those reported earlier[5] on the effects of actinomycin, puromycin and ethionine support the suggestion that the increases observed in uterine phosphohexose isomerase activity after estrogen treatment represent enzyme synthesis *de novo* rather than an activation of pre-existing enzyme protein.

Time-course of actinomycin and ethionine inhibition of estradiol-induced increases in uterine phosphohexose isomerase during 24 *hr.* An analysis of the increases in phosphohexose isomerase induced by estradiol-17β indicated that the earliest detectable significant increase in enzyme activity occurred 4 hr after a single injection of the hormone.[5] It therefore seemed pertinent to investigate if this early increase was also the result of new enzyme synthesis. Accordingly, the effect of actinomycin and ethionine on the hormone-induced sequential changes produced in uterine phosphohexose isomerase activity was examined. The results are illustrated in Fig. 2. Whereas no

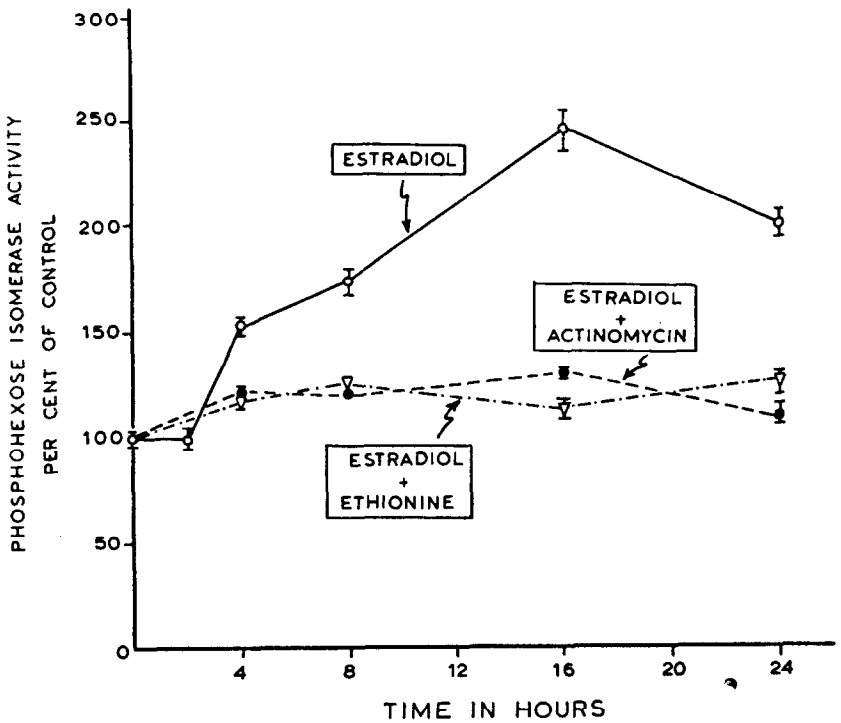

FIG. 2. Sequence of events during actinomycin and ethionine prevention of estradiol-induced increases in uterine phosphohexose isomerase during a 24-hr period. Each point represents the mean $\pm$ S.E. of 3 values, each obtained by pooling uteri from 2–3 rats. Ovariectomized rats were injected with estradiol-17β (10 μg/100 g) i.m., and were killed at the time intervals indicated. Actinomycin (25 μg/100 g) or ethionine (100 mg/100 g) was given i.p. at zero time along with estradiol. Data are given in percentages, taking the values of control rats as 100 per cent.

change in phosphohexose isomerase activity was observed 2 hr after hormone administration, enzyme activity was elevated to 153, 175, 250 and 222 per cent at 4, 8, 16 and 24 hr respectively. However, when actinomycin or ethionine was injected simultaneously with estradiol, uterine phosphohexose isomerase failed to rise and the values in these groups of animals remained near the normal range. After injection of estradiol-17β, uterine weights increased to 165, 162, 178 and 165 per cent of the

control values at 4, 8, 16 and 24 hr respectively The increases in uterine phosphohexose isomerase activity at 16 and 24 hr after hormone administration were considerably greater than those observed for the corresponding uterine wet weights.[5] Treatment with actinomycin or ethionine resulted in partial inhibition of the estradiol-induced increases in uterine weights during the entire 24-hr period. The data indicate that the early enzyme increases induced by estradiol-17β also represent new enzyme synthesis.

Sequence of events during actinomycin inhibition of estradiol-induced enzyme increases during 72 hr. In order to examine the effects of chronic estradiol treatment on uterine weights and phosphohexose isomerase activity, ovariectomized rats were injected twice a day for 3 days with smaller doses (2·5 μg/100 g) of the hormone. Table 2 shows that estradiol increased uterine weights to 166, 188 and 177 per cent at 24,

TABLE 2. EFFECT OF ACTINOMYCIN ADMINISTRATION OF UTERINE WEIGHTS DURING 72 HR OF ESTRADIOL TREATMENT*

Time (hr)		Uterine weights in estradiol-injected rats (g)	
		Without actinomycin	With actinomycin
Control	0·18 ± 0·02		
24		0·30 ± 0·04 (166)†	0·21 ± 0·01 (116)‡
48		0·34 ± 0·01 (188)†	0·27 ± 0·02 (150)‡
72		0·32 ± 0·01 (177)†	0·27 ± 0·00 (150)†‡

* Means ± S.E. represent 8–10 animals in each group. Ovariectomized rats were injected with estradiol-17β (2·5 μg/100 g) i.m. twice daily at 12-hr intervals and were sacrificed after 24, 48 and 72 hr. Actinomycin (5 μg/100 g) was administered i.p. once daily 30 min prior to the estradiol injection. Data are also given in percentages (in parentheses), taking the values of control rats as 100 per cent.

† Statistically significant difference as compared with the values of control rats ($P = < 0·05$).

‡ Statistically significant difference as compared with the corresponding values of estradiol-injected rats without actinomycin administration ($P = < 0·05$).

48 and 72 hr respectively. Pretreatment with actinomycin partially blocked these estradiol-induced increases in uterine weights. The results summarized in Table 3 indicate that estradiol treatment elevated phosphohexose isomerase activity to 237 per cent at 24 hr, 325 per cent at 48 hr and 398 per cent at 72 hr. However, the concomitant administration of actinomycin to estradiol-injected rats largely prevented these hormone-induced increases. Enzyme activity in these groups of animals increased to only 129, 175 and 170 per cent at 24, 48 and 72 hr respectively. Since actinomycin is a potent inhibitor of DNA-directed synthesis of RNA, the observed inhibition of estradiol-induced increases in uterine phosphohexose isomerase by actinomycin, lends additional support to the view that stimulation of the synthesis of certain RNA species may be one of the primary actions of estrogenic hormones.

Action of progesterone on the estrogen-induced increase in uterine phosphohexose isomerase activity. An understanding of the role of estrogenic hormones in the regulation of metabolic processes in the uterus should include a consideration of their interaction with other endogenously produced steroids. Since such interaction could

TABLE 3. EFFECT OF ACTINOMYCIN ADMINISTRATION ON UTERINE PHOSPHOHEXOSE ISOMERASE ACTIVITY DURING 72 HR OF ESTRADIOL TREATMENT*

Time (hr)		Phosphohexose isomerase activity in estradiol-injected rats	
		Without actinomycin	With actinomycin
Control	436 ± 36 (100)		
24		1035 ± 90 (237)†	576 ± 52 (129)‡
48		1421 ± 24 (325)†	764 ± 86 (175)†‡
72		1732 ± 6 (398)†	744 ± 22 (170)†‡

* Means ± S.E. represent 3 determinations of enzyme activity. Each determination was carried out in uteri pooled from 2–3 rats. Ovariectomized rats were injected with estradiol-17β (2·5 μg/100 g) i.m. twice daily at 12-hr intervals and were sactificed after 24, 48 and 72 hr. Actinomycin (5 μg/100 g) was administered i.p. once daily 30 min prior to the estradiol injection. Data are also given in percentages (in parentheses), taking the values of control rats as 100 per cent.

† Statistically significant difference as compared with the values of control rats ($P = < 0{\cdot}05$).

‡ Statistically significant difference as compared with the corresponding values of estradiol-injected rets without actinomycin administration ($P = < 0{\cdot}05$).

conceivably alter estradiol-induced biochemical responses, the effects of administration of progesterone and testosterone on phosphohexose isomerase activity were studied. Table 4 shows that progesterone alone failed to exert any appreciable influence on either uterine weight or the activity of phosphohexose isomerase. In this

TABLE 4. EFFECT OF PROGESTERONE ON ESTRADIOL-INDUCED INCREASE IN UTERINE PHOSPHOHEXOSE ISOMERASE ACTIVITY IN OVARIECTOMIZED RATS*

Treatment	Uterine weight (g)	Enzyme activity
Control	0·18 ± 0·01 (100)	436 ± 36 (100)
Progesterone	0·17 ± 0·01 (93)	414 ± 16 (95)
Estradiol-17β	0·29 ± 0·01 (164)†	1015 ± 17 (233)†
Estradiol-17β + progesterone	0·20 ± 0·01 (112)‡	470 ± 10 (108)‡

* Means ± S.E. represent 4 determinations of enzyme activity. Each determination was carried out in uteri pooled from 2–3 rats. Ovariectomized rats were injected with estradiol-17β (10 μg/100 g) i.m., and were sacrificed after 16 hr. Progesterone (5 mg/100 g) was given i.m. 30 min prior to estradiol administration. Data are also given in percentages (in parentheses), taking the values of control rats as 100 per cent.

† Statistically significant difference as compared with the values of control rats ($P = < 0{\cdot}05$).

‡ Statistically significant difference as compared with the values of estradiol-injected rats without progesterone administration ($P = < 0{\cdot}05$).

series of experiments, estradiol-17β increased uterine weight to 164 per cent and phosphohexose isomerase to 233 per cent of the control values. When estradiol-treated rats were pretreated with progesterone, the observed estrogen-induced increases in uterine weight and phosphohexose isomerase activity were prevented and the values remained near control levels. A similar suppresion by progesterone of the estrogen-induced increase in the activity of another uterine enzyme, phosphofructokinase, has been described.[1]

Effect of testosterone on the estradiol-induced increase in uterine phosphohexose isomerase. The known mutual antagonism between the actions of estrogens and androgens[9, 10] led us to investigate the effect of testosterone administration on estradiol-induced increases in uterine phosphohexose isomerase activity. Testosterone alone failed to exert any significant influence on uterine weights. However, the estradiol-induced increases in organ weights were largely inhibited by testosterone and remained at 144 per cent of the control values. The results illustrated in Fig. 3

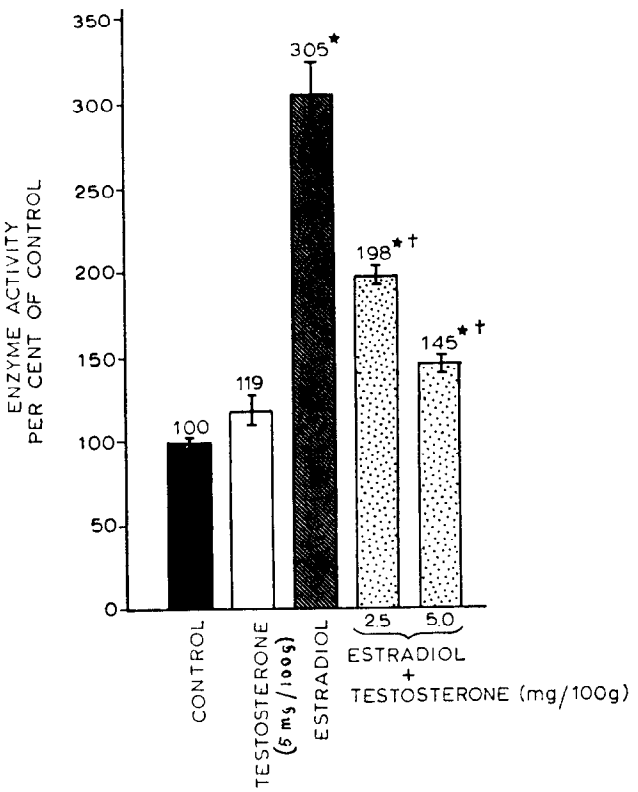

FIG. 3. Effect of testosterone administration on estradiol-induced increase in uterine phosphohexose isomerase activity in ovariectomized rats. Bars represent the mean and S.E. of 3 values, each obtained by pooling uteri from 3–4 rats. Ovariectomized rats were treated by injection with estradiol-17β (10 μg/100 g) i.m., and were killed after 16 hr. Testosterone (2·5 or 5·0 mg/100 g) was also given i.m. 30 min prior to estradiol administration. Data are given in percentages, taking the values of control rats as 100 per cent. * = Statistically significant difference as compared with the values of control rats ($P = < 0{\cdot}05$). † = Statistically significant difference as compared with the values of estradiol-treated rats without testosterone administration ($P = < 0{\cdot}05$).

show that testosterone, by itself, did not affect the basal levels of uterine phosphohexose isomerase to any significant degree. Whereas estradiol administration increased phosphohexose isomerase activity to 305 per cent of the control values, this increase was largely abolished by testosterone at the dose level of 5·0 mg/100 g. It is of interest that testosterone, when given at a lower dosage (2·5 mg/100 g) produced only partial suppression of this increase in enzyme activity. The possibility that estrogen may act as inducer, and progesterone and testosterone as suppressors of uterine enzyme biosynthesis requires further study, since these actions may be important in the various homeostatic mechanisms involved in uterine intermediary metabolism.[1]

Evidence available to date implicates regulation of protein synthesis as a basic mechanism of action of estrogenic hormones. The extensive studies of Mueller *et al.*[11–13] have demonstrated that estrogen stimulates *in vivo* the incorporation of various precursors into uterine RNA and protein. Doses of puromycin and cycloheximide known to inhibit protein synthesis and amounts of actinomycin which block the synthesis of messenger-RNA were all shown to prevent effectively estrogenic acceleration of these responses.[12, 14, 15] Underlying the hormone stimulation of RNA synthesis is an increase in the activity of DNA-dependent RNA-polymerase, as has been shown by Gorski *et al.*[14–16] Barker and Warren also have demonstrated that administration of estradiol-17β caused an increase in template activity of uterine chromatin for RNA synthesis.[17] The present observations describing the blocking of estradiol-induced increases in uterine phosphohexose isomerase by a variety of inhibitors of RNA and protein synthesis complement our previous studies on uterine phosphofructokinase[1, 3] and lend support to the view that estrogenic hormones accelerate genetic expression in the uterus.[13, 18]

Weber *et al.* have suggested that certain hormones may act as co-ordinators of metabolic events by influencing whole genome units governing the biosynthesis of functionally related key, rate-limiting enzymes.[19–22] Based on the regulatory behaviour of glucokinase, phosphofructokinase and pyruvate kinase under various hormonal and nutritional influences, these investigators have suggested that the key enzymes of hepatic glycolysis are produced on the same genic unit.[23, 24] In order to test the applicability of the "functional genome unit" concept to estrogen action in uterine tissue, a study of the hormonal regulation of key glycolytic enzymes, all three of which should be induced or suppressed in a synchronous manner, is necessary. Smith and Gorski[25] have reported that uterine tissue, unlike liver, contains little or no glucokinase (high K_m enzyme) and that glucose is phosphorylated in this tissue by the low K_m hexokinase. Their data indicated that estradiol is capable of increasing uterine hexokinase activity in the immature rat. Recent work from our laboratory on the uterus of the ovariectomized rat confirms these findings and indicates further that the observed estradiol-induced increases represent enzyme synthesis *de novo*.[2, 6] Since estrogenic hormones are capable of inducing increases in hexokinase, phosphohexose isomerase and phosphofructokinase, it appears that the enhanced glycolysis in uterine muscle after estradiol treatment[27] may be related to an increased rate of synthesis of these carbohydrate-metabolizing enzymes.

Acknowledgements—The authors express their indebtedness to Dr. George M. Ling for his continued interest and encouragement. The technical assistance of Mr. M. R. Parulekar and the skilled assistance of Miss Diane Breure in the preparation of illustrations are gratefully acknowledged. Thanks are also

due to Dr. George Boxer of Merck, Sharp & Dohme, Dr. E. Mason of Upjohn and Dr. W. E. Scott of Hoffman La Roche for generous gifts of actinomycin, cycloheximide and 5-fluorouracil respectively.

REFERENCES

1. R. L. SINGHAL, J. R. E. VALADARES and G. M. LING, *J. biol. Chem.* **242**, 2593 (1967).
2. R. L. SINGHAL and J. R. E. VALADARES, *Steroids* **9**, 367 (1967).
3. R. L. SINGHAL and J. R. E. VALADARES, *Life Sci.* **5**, 1299 (1966).
4. R. L. SINGHAL and G. M. LING, *J. cell. Biol.* **31**, 109A (1966).
5. R. L. SINGHAL, J. R. E. VALADARES and G. M. LING, *Metabolism* **16**, 271 (1967).
6. G. E. GLOCK, P. MCLEAN and J. K. WHITEHEAD, *Biochem. J.* **63**, 520 (1956).
7. R. L. SINGHAL, *Life Sci.* **6**, 405 (1967).
8. R. L. SINGHAL, *J. Geront.* **22**, 343 (1967).
9. C. HUGGINS, *Yale J. biol. Med.* **19**, 319 (1947).
10. A. T. BEVER, *Ann. N.Y. Acad. Sci.* **75**, 472 (1959).
11. G. C. MUELLER, J. GORSKI and Y. AIZAWA, *Proc. natn. Acad. Sci. U.S.A.* **47**, 164 (1961).
12. H. UI and G. C. MUELLER, *Proc. natn. Acad. Sci. U.S.A.* **50**, 256 (1963).
13. J. A. NICOLETTE and G. C. MUELLER, *Endocrinology* **79**, 1162 (1966).
14. J. GORSKI and W. D. NOTEBOOM, *J. cell Comp. Physiol.* **66**, 91 (1965).
15. J. GORSKI and M. C. AXMAN, *Archs Biochem. Biophys.* **105**, 517 (1964).
16. W. D. NOTEBOOM and J. GORSKI, *Proc. natn. Acad. Sci. U.S.A.* **50**, 250 (1963).
17. K. L. BARKER and J. C. WARREN, *Proc. nain. Acad. Sci. U.S.A.* **56**, 1298 (1966).
18. J. A. NICOLETTE and G. C. MUELLER, *Biochem. Biophys. Res. Commun.* **24**, 851 (1966).
19. G. WEBER, R. L. SINGHAL and S. K. SRIVASTAVA, *Adv. Enzyme Regulat.* **3**, 41 (1965).
20. G. WEBER, R. L. SINGHAL and S. K. SRIVASTAVA, *Proc. natn. Acad. Sci. U.S.A.* **53**, 96 (1965).
21. G. WEBER and R. L. SINGHAL, *Biochem. Pharmac.* **13**, 1173 (1964).
22. G. WEBER, R. L. SINGHAL, N. B. STAMM and S. K. SRIVASTAVA, *Fedn Proc.* **24**, 745 (1965).
23. G. WEBER and R. L. SINGHAL, *Life Sci.* **4**, 1993 (1965).
24. G. WEBER, R. L. SINGHAL, N. B. STAMM, M. A. LEA and E. A. FISHER, *Adv. Enzyme Regulat.* **4**, 59 (1966).
25. D. E. SMITH and J. GORSKI, *Life Sci.* **6**, 1263 (1967).
26. J. R. E. VALADARES, R. L. SINGHAL and M. R. PARULEKAR, *Science* **159**, 990 (1968).
27. O. WALLAS, E. WALLAS and F. LOKEN, *Acta endocr., Copenh.* **10**, 201 (1952).