Fructose metabolism in the rat endometrium under normal and experimental conditions

D. P. Boshier and June M. Katz

Department of Anatomy, University of Auckland, Auckland, New Zealand

Summary. Fructose-1,6-diphosphate (FDP) hydrolysis, which was measured in tissue extracts from mature rat endometrium, was maximal during late dioestrus and early pro-oestrus and reflected the variations in the plasma levels of ovarian steroids. Treatment of ovariectomized animals with oestradiol or medroxyprogesterone acetate increased FDP hydrolysis when compared with control animals, although oestradiol was the more effective. FDP hydrolysis was greater in extracts of endometrial tissue from uterine horns containing a silk IUD or of decidual tissue from pseudo-pregnant rats, in which the peak was on Day 9, than in extracts of endometrium from the control contralateral horn. These findings are interpreted as linking glycogen metabolism in normal endometrium, IUD-containing horns, and decidual tissue to the role of FDP in gluconeogenesis and the promotion of glycogen storage.

Introduction

Since Walaas, Walaas & Löken (1952) showed that the treatment of isolated uterine segments with oestradiol greatly enhanced the energy-liberating glycolytic processes, the endocrine control of uterine carbohydrate metabolism has been of interest. Singhal, Valdares & Ling (1967) and Valdares, Singhal & Parelukar (1968) found that oestradiol-17 β induced significant increases in phosphofructokinase and hexokinase activities. The activity of pyruvate kinase, another glycolytic enzyme, was also elevated after the administration of a single dose of oestradiol to spayed rats (Asua, Rozen-gurt & Carminatti, 1968). Asua *et al.* (1968) considered that the elevated glycolysis in the uterine tissue was related to increased synthesis of the three 'pacemaker' enzymes involved in glycolysis—hexokinase, phosphofructokinase and pyruvate kinase. Although the utilization of glucose-6-phosphate (G6P), the product of the hexokinase reaction, has been investigated in several laboratories (see Warren & Crist, 1973), there appear to have been no studies of the metabolism in the rat uterus of FDP, the product of the irreversible phosphofructokinase reaction. It is now generally recognized that the balance between FDP formation and its hydrolysis is an important regulatory factor of glycolysis and gluconeogenesis (Atkinson & Walton, 1967), and is therefore a control point in the promotion of glycogen storage (McGilvery, 1970), which is involved in many endometrial activities.

Materials and Methods

Virgin female hooded rats, weighing approximately 150 g and with free access to Diet 86 (Westfield Freezing Works, Auckland, N.Z.), were maintained in an air-conditioned, temperature- (20–21°C) and light- (from 07.00 to 19.00 hours) controlled room. Vaginal smears were taken daily and recorded as described previously (Boshier & Holloway, 1973). Females were used only after the completion of two regular 4-day cycles. The various treatment groups and the numbers of animals investigated are shown in Table 1.

All the animals in Groups 5–9 were bilaterally ovariectomized and left for 2 weeks before treatment with hormones; vaginal smears were continued. Hormones were injected s.c. and the regimens were: Group 5, ovariectomized controls; Group 6, 0.2 ml arachis oil daily; Group 7, 0.1 μ g oestradiol-

Table 1. Hydrolysis of fructose-1,6-diphosphate (mean $\pm$ S.D.) in the endometrium of the rat

Group	Reproductive state	No. of animals	Hydrolysis of FDP (μ mol Pi/min/mg protein)	Statistical comparisons
1	Oestrus	4	26.05 $\pm$ 1.55	<u>*Gp 3 > Gp 4 > Gp 1 < Gp 2</u>
2	Metoestrus	4	29.66 $\pm$ 2.90	
3	Dioestrus	4	71.71 $\pm$ 4.47	
4	Pro-oestrus	4	61.16 $\pm$ 4.40	
5	Ovariectomized	3	93.69 $\pm$ 5.30	<u>*Gp 5 < Gp 6 < Gp 7 < Gp 8 < Gp 9</u>
6	Ovariectomized + oil	3	93.93 $\pm$ 4.19	
7	Ovariectomized + oestradiol + MPA	3	123.27 $\pm$ 1.14	
8	Ovariectomized + MPA	3	129.60 $\pm$ 2.64	
9	Ovariectomized + oestradiol	3	164.67 $\pm$ 2.95	
10	IUD horn, pro-oestrus	4	75.16 $\pm$ 3.09	Gp 10 versus Gp 11: $P < 0.01$
11	Contralateral horn, pro-oestrus	4	57.41 $\pm$ 3.59	
12	IUD horn, metoestrus	4	62.73 $\pm$ 1.61	Gp 12 versus Gp 13: $P < 0.01$
13	Contralateral horn, metoestrus	4	27.07 $\pm$ 2.34	
14	Pseudopregnant, Day 1	3	25.67 $\pm$ 0.88	<u>*For control horns: Gp 14 < Gp 15 > Gp 16 > Gp 17 < Gp 19 > Gp 21</u>
15	Pseudopregnant, Day 3	3	74.15 $\pm$ 7.31	
16	Pseudopregnant, Day 5	3	73.11 $\pm$ 4.39	<u>*For horn with deciduomata: Gp 18 < Gp 20 > Gp 22</u>
17	Pseudopregnant, Day 7	3	70.24 $\pm$ 2.84	
18	Pseudopregnant, deciduoma, Day 7	3	254.03 $\pm$ 4.12	Gp 17 versus Gp 18: $P < 0.01$
19	Pseudopregnant, Day 9	3	73.20 $\pm$ 1.97	
20	Pseudopregnant, deciduoma, Day 9	3	462.34 $\pm$ 10.25	Gp 19 versus Gp 20: $P < 0.01$
21	Pseudopregnant, Day 11	3	41.36 $\pm$ 10.23	
22	Pseudopregnant, deciduoma, Day 11	3	106.61 $\pm$ 3.32	Gp 21 versus Gp 22: $P < 0.01$

* Means of groups underlined are not different statistically at the 1% level.

17 β (oestradiol monobenzoate: Organon) in 0.2 ml arachis oil daily plus 2.0 mg medroxyprogesterone acetate (MPA: Upjohn) on Day 1; Group 8, 2.0 mg MPA on Day 1; Group 9, 0.1 μ g oestradiol-17 β in 0.2 ml arachis oil daily. These rats were killed by cervical dislocation 7 days after the first injection.

Intrauterine devices consisted of a 2.0 surgical silk thread inserted according to the method of Doyle & Margolis (1964). After the animals had resumed normal oestrous cycles, they were killed at pro-oestrus or metoestrus, periods of high and low uterine activity respectively (Boshier & Katz, 1975; Yochim, 1975).

Pseudopregnancy was induced by mating intact females with vasectomized males, the day on which a vaginal plug was found being designated Day 1. Deciduomata were induced by the injection of 0.2 ml arachis oil into one uterine horn at 14.00 hours on Day 5 (Finn & Keen, 1963), the contralateral horn being left as a control. Animals were killed by cervical dislocation on Days 1, 3, 5, 7, 9 and 11.

Biochemical analyses

Endometrial tissue was obtained as described previously (Boshier & Katz, 1975). Deciduomatous tissue was similarly removed and compared with the endometrial tissue taken from the contralateral horn. Tissue extract preparation followed a standard technique (Boshier & Katz, 1975), except that butanol-saturated tris-HCl was used as the homogenizing medium. The enzymatic activity of extracts prepared in this manner was double that obtained when the tissue was homogenized in tris-HCl or a detergent (Triton X-100). Protein in the tissue extracts was estimated photometrically (Lowry, Rosebrough, Farr & Randall, 1951).

Endometrial hydrolysis of FDP was measured by determining the amount of inorganic phosphate (Pi) liberated from a substrate after addition of the tissue extract (Taussky & Shorr, 1953). The optimal reaction mixture contained 0.45 ml 0.05 M-glycine/NaOH, pH 10; 0.05 ml 5×10^{-4} M-MgCl₂; and 0.01 ml 5×10^{-2} M-FDP. In most assays, 0.05 ml tissue extract was used, but when the protein concentration was high, 0.03 ml was used. The results were converted to μ mol Pi produced per min per mg protein in each ml of the total reaction mixture. One blank tube (containing boiled extract) and one experimental tube were removed after 0, 10, 20, 30 and 40 min of incubation at 37°C.

After terminating the reaction by the addition of 1 ml cold 25% trichloroacetic acid, the mixture was centrifuged at 1300 g for 15 min and a 1.0-ml aliquot of the supernatant was added to 1.0 ml of the reagent (Taussky & Shorr, 1953) used to detect Pi. The characteristic blue colour developed within 10 min at 20°C and the absorbance was read against the appropriate blank at 660 nm. The rate of FDP hydrolysis was directly proportional to the amount of tissue extract added, which contained 25–250 μ g protein, and the reaction was linear for the first 30 min. Absorbance was related to phosphate concentration from a standard curve using KH₂PO₄ solutions of different concentrations.

Statistical analyses

Analysis of the results involved the use of standard techniques for the analysis of variance, followed when necessary by Duncan's (1955) Multiple Range Test to determine the statistical significance of between-group differences.

Results

The results are shown in Table 1.

Oestrous cycle. Comparisons of Groups 1 to 4 showed that in intact females the rate of endometrial hydrolysis of FDP was low during oestrus and metoestrus, but was more than doubled during dioestrus and pro-oestrus ($F = 122.99$; d.f. = 3,12; $P < 0.01$).

Hormone treatments. The hydrolysis of FDP was significantly higher ($F = 138.34$; d.f. = 4,10; $P < 0.01$) in tissue obtained from animals treated with MPA (Groups 7 and 8) or oestradiol (Groups 7 and 9) than in that from rats receiving no treatment (Group 5) or arachis oil alone (Group 6). Oestradiol (Group 9) increased FDP hydrolysis more ($P < 0.01$) than did MPA (Group 8), but when oestradiol + MPA was given (Group 7), the increase was little different from that following MPA alone. In both groups of control animals in these experiments (Groups 5 and 6), substrate hydrolysis was of the same order as that found during dioestrus, although higher.

Intrauterine device. During pro-oestrus and metoestrus, the hydrolysis of FDP in endometrium from horns not containing an IUD (Groups 10 and 12) was comparable with that in the cyclic animal (Groups 4 and 2); but in the IUD-containing horn, tissue activity was significantly greater than that in control tissue ($P < 0.01$).

Pseudopregnancy. The utilization of FDP by endometrium from pseudopregnant rats on Day 1 (Group 14) was similar to that observed at oestrus (Group 1), rising on Days 3 and 5 (Groups 15 and 16) to values comparable with those at dioestrus (Group 3). In the developing decidualoma, substrate hydrolysis by Day 7 (Group 18) was three times that in the control tissue (Group 17); by Day 9 it was six times as great (Groups 19 and 20); but by Day 11 it had decreased markedly (Groups 21 and 22). In tissues from the control contralateral horns from Day 3 to Day 9, hydrolysis of FDP was comparable with that found at dioestrus, but by Day 11 it also had decreased considerably.

Discussion

The present results are similar to those of Baquer & McLean (1972), who found that oestradiol given to immature rats doubled the activity of fructose-1,6-diphosphatase (FDPase) in whole uterine homogenates. In the ovariectomized females in our study, the progesterin, alone or in combina-

tion with oestradiol, increased FDP hydrolysis by 35%, whereas oestradiol alone raised it to 75% over that present in control tissue extracts. These findings support the hypothesis of Yochim (1975) that progesterone generates a pattern of metabolism, whereas the effect of oestrogen is to increase the rate of metabolism in the pattern. Moulton (1974) observed a similar potentiating effect of oestrogen on progesterone-stimulated glucose-6-phosphate dehydrogenase activity in decidual tissue of the rat but this does not seem to occur with the endometrial esterases (Boshier & Katz, 1975).

Our demonstration that the endometrial hydrolysis of FDP was maximal during dioestrus and pro-oestrus, when related to the effects of oestradiol and MPA in ovariectomized females, suggests that there is a close link between the plasma levels of oestrogen and progesterone and FDP utilization. Progesterone, concentrations of which increase early in dioestrus (Hashimoto, Henricks, Anderson & Melampy, 1968), stimulates the activity of the FDP-hydrolysing enzymes and this activity would be maintained by the high levels of oestrogen and progesterone during pro-oestrus (Hashimoto *et al.*, 1968; Brown-Grant, Exley & Naftolin, 1970). Hydrolysis of FDP during dioestrus and early pro-oestrus could therefore facilitate the deposition, through F6P, G6P and UDP-glucose, of the glycogen found during late pro-oestrus (Rubulis, Jacobs & Hughes, 1965).

The utilization of FDP in an IUD-containing horn appears to be markedly affected by the presence of the device, over-riding to some extent the endocrine status of the female, for whilst the increase in substrate hydrolysis was over 30% in the pro-oestrous animals, it was over 130% in those at metoestrus. As mentioned above, the metabolism of FDP can be related to glycogen levels. Parr (1966) demonstrated histochemically that glycogen accumulated in the epithelium of an IUD-containing horn, and a nylon IUD has been shown biochemically to produce increases in uterine weight, water, glycogen and nucleic acids (Wrenn, Wood & Bitman, 1969). The increased hydrolysis of FDP which we have demonstrated would facilitate this increase in uterine glycogen.

The increased tissue utilization of FDP during development of deciduomata can also be linked with the increasing glycogen levels in developing deciduomata of pseudopregnant rats (Cecil, Bitman, Connolly & Wrenn, 1962). The utilization of FDP and glucose in the pseudopregnant rat uterus appears to differ because increased glucose-6-phosphate and 6-phosphogluconic acid dehydrogenase activity are not required to support either the gradual differentiation that precedes the time of decidual sensitivity, or the rapid endometrial differentiation after induction of the deciduoma (Pepe & Yochim, 1971); an elevated FDP utilization is necessary for deciduomal differentiation.

We cannot be sure from the present study whether the FDP hydrolysis results solely from FDPase activity. It has been suggested that this enzyme plays a regulatory role as a key enzyme in the irreversible reaction $F-1,6-P_2 \rightarrow F-6-P + Pi$ of the gluconeogenic process (Krebs, 1964). The assay we used (Tausky & Shorr, 1953) measures Pi liberated after FDPase activity and after nonenzymatic hydrolysis of triose phosphates produced by F-1,6-P aldolase in the tissue extract (Newsholme & Randle, 1962). The significance of the specific contributions of each of these two enzymes in the endometrium is unknown. Sergeev, Maiskii & Seredonin (1973) have demonstrated that F-1,6-P₂ aldolase activity is accelerated during oestrus. In our studies, oestrus was the period of minimal production of Pi from FDP. If F-1,6-P₂ aldolase activity is maximal at this time of minimal FDP utilization, it is likely that FDPase is the enzyme of primary importance in the endometrial hydrolysis of FDP.

This work was supported by the Medical Research Council of New Zealand. We are grateful also for the advice of Associate Professor E. Moustafa, Department of Biochemistry, University of Auckland, and Dr C. J. R. Thorn, Department of Biochemistry, University of Cambridge, England.

References

ASUA, L.J. DE, ROZENGURT, E. & CARMINATTI, H. (1968) Estradiol induction of pyruvate kinase in the rat uterus. <i>Biochim. biophys. Acta</i> 170, 254–262.	ATKINSON, D.E. & WALTON, G.M. (1967) Adenosine triphosphate conservation in metabolic regulation. Rat liver citrate cleavage enzyme. <i>J. biol. Chem.</i> 242, 3239–3241.

- BAQUER, N.J. & McLEAN, P. (1972) The effect of oestradiol on the profile of constant and specific proportion groups of enzymes in rat uterus. *Biochem. biophys. Res. Commun.* **48**, 729-734.
- BOSHIER, D.P. & HOLLOWAY, H. (1973) Effects of ovarian steroid hormones on histochemically demonstrable lipids in the rat uterine epithelium. *J. Endocr.* **56**, 59-67.
- BOSHIER, D.P. & KATZ, J.M. (1975) Effects of ovarian steroid hormones on esterases in the rat endometrium. *J. Endocr.* **64**, 465-474.
- BROWN-GRANT, K., EXLEY, D. & NAFTOLIN, F. (1970) Peripheral plasma oestradiol and luteinizing hormone concentrations during the oestrous cycle of the rat. *J. Endocr.* **48**, 295-296.
- CECIL, H.C., BITMAN, J., CONNOLLY, M.R. & WRENN, T.R. (1962) Glycogen in the decidual tissue of the rat uterus. *J. Endocr.* **25**, 69-76.
- DOYLE, L.L. & MARGOLIS, A.J. (1964) Intrauterine foreign body. I. Effect on reproductive processes in the rat. *Fert. Steril.* **15**, 597-606.
- DUNCAN, D.B. (1955) Multiple range and multiple F tests. *Biometrics* **11**, 1-42.
- FINN, C.A. & KEEN, P.M. (1963) The induction of deciduomata in the rat. *J. Embryol. exp. Morph.* **11**, 673-682.
- HASHIMOTO, I., HENRICKS, D.M., ANDERSON, L.L. & MELAMPY, R.M. (1968) Progesterone and pregn-4-en-20-ol-3-one in ovarian venous blood during various reproductive states in the rat. *Endocrinology* **82**, 333-341.
- KREBS, H.A. (1964) Gluconeogenesis. *Proc. R. Soc. B* **159**, 545-564.
- LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265-275.
- MCGILVER, R.W. (1970) *Biochemistry, a Functional Approach*. Saunders, Philadelphia.
- MOULTON, B.C. (1974) Progesterone and estrogen control of uterine glucose-6-phosphate dehydrogenase activity during decidual growth. *Biol. Reprod.* **10**, 526-532.
- NEWSHOLME, E.A. & RANDLE, P.J. (1962) Regulation of glucose uptake by muscle. 6. Fructose-1,6-phosphatase activity of rat heart and rat diaphragm. *Biochem. J.* **83**, 387-392.
- PARR, E. (1966) Glycogen accumulation in the rat uterus containing an intrauterine contraceptive device. *Fert. Steril.* **17**, 797-803.
- PEPE, G.J. & YOCHIM, J.M. (1971) Glucose-6-phosphate dehydrogenase activity in the endometrium and myometrium of the rat uterus during the estrous cycle and progestation. *Biol. Reprod.* **5**, 161-171.
- RUBULIS, A., JACOBS, R.D. & HUGHES, E.C. (1965) Glycogen synthetase in mammalian uterus. *Biochim. biophys. Acta* **99**, 584-586.
- SERGEEV, P.V., MAISKII, A.F. & SEREDONIN, S.B. (1973) Mechanism of the regulation of enzymic activity in rat uterus by estradiol. *Probl. Endokrinol.* **19**, 83-86.
- SINGHAL, R.L., VALDARES, J.R.E. & LING, G.M. (1967) Metabolic control mechanisms in mammalian systems. I. Hormonal induction of phosphofructokinase in the rat uterus. *J. biol. Chem.* **242**, 2593-2598.
- TAUSSKY, H.H. & SHORR, E. (1953) A microcolorimetric method for the determination of inorganic phosphate. *J. biol. Chem.* **202**, 675-685.
- VALDARES, J.R.E., SINGHAL, R.L. & PARELUKAR, M.R. (1968) 17β -Estradiol: inducer of uterine hexokinase. *Science, N.Y.* **159**, 990-991.
- WALAAS, O., WALAAS, E. & LÖKEN, F. (1952) Effect of oestradiol monobenzoate on the metabolism of rat uterine muscle. *Acta endocr., Copenh.* **10**, 201-211.
- WARREN, J.C. & CRIST, R.D. (1973) Effects of ovarian steroids on uterine metabolism. *Handbook of Physiology, Section 7: Endocrinology*, Vol. II, Pt. 2. Chap. 32, pp. 49-67. Ed. R. O. Greep. Am. Physiol. Soc., Washington.
- WRENN, T.R., WOOD, J.R. & BITMAN, J. (1969) Alteration in the uterine environment produced by IUDs. *J. Reprod. Fert.* **19**, 511-518.
- YOCHIM, J.M. (1975) Development of the progestational uterus: metabolic aspects. *Biol. Reprod.* **12**, 106-133.

Received 4 November 1975