Effect of parenteral administration of oestrogen and progesterone on the glycogen metabolism of mouse morulae–early blastocysts *in vivo**

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Summary. Morulae–early blastocysts were pulse-labelled with radioactive glucose and subsequently transferred for 24 h to ovariectomized recipients maintained on different hormone regimens. Embryos transferred to recipients treated with progesterone utilized more glycogen than did those incubated in untreated controls. Treatment with oestrogen alone had no significant effect on glycogen turnover of transferred embryos and, when given in combination with progesterone, antagonized the effect of progesterone. Priming doses of oestrogen given >4 days before progesterone had no significant effect on the response to progesterone.

The results indicate that the high levels of progesterone circulating in the mother before implantation cause glycogen degradation in the embryo and account for the low glycogen content of uterine embryos compared to those cultured *in vitro* in the presence of glucose.

Introduction

Late preimplantation mouse embryos utilize the majority of preformed glycogen when transferred to the reproductive tract of synchronized pseudopregnant recipients whereas those cultured *in vitro* conserve most of their intracellular glycogen stores (Edirisinghe, Wales & Pike, 1984a). It would therefore appear that the uterine environment can exert an influence upon the metabolic pathways operative in the embryo at this time.

Ovarian steroids may be involved in this effect of the uterus on the embryo because they are of primary importance in bringing about the synchronization of growth and development of the reproductive tract with that of the early, free-living embryo. Progesterone dominates during most of the preimplantation period but in murids a peak of oestrogen occurs at Day 4 of pregnancy and is required for the initiation of implantation (Shaikh, 1971; McCormack & Greenwald, 1974). Investigations using exogenous steroids in ovariectomized mice have indicated that an initial peak of oestrogen (priming oestrogen) is important in sensitizing the endometrium for the subsequent action of progesterone and oestrogen (nidatory hormones) to give maximum deciduoma formation (Finn & Martin, 1970).

The endometrium shows structural (Finn, 1977) and biochemical (Yochim, 1975) changes in response to the changing patterns of steroid secretion and there are changes in the volume and composition of luminal fluids as a result of the hormonal environment (Restall, 1966; Restall & Wales, 1966, 1968; Iritani, Gomes & VanDemark, 1969; Iritani, Nishikawa, Gomes & Van Demark, 1971; Wales & Restall, 1971; Tantayaporn, Mallikarjuneswara, de Carlo & Clemetson, 1974; Beier, 1974; Surani, 1975; Fishel, 1979). Control of the metabolism of the embryo by ovarian

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hormones has also been demonstrated. For example, diapausing mouse embryos show a low rate of incorporation of [^{35}S]methionine (Weitlauf & Greenwald, 1968) and a low rate of production of CO_2 (Menke & McLaren, 1970). Both of these metabolic processes increase after administration of oestrogen and progesterone (Weitlauf, 1974). Ozias & Weitlauf (1971) have studied the effect of ovarian hormones on the total glycogen content of blastocysts collected from ovariectomized pregnant mice treated with oestrogen and progesterone: oestrogen increased and progesterone decreased the amount of glycogen in the embryos.

In the present experiments, the control of the metabolism of embryonic glycogen by ovarian hormones at the late stages of preimplantation development has been investigated.

Materials and Methods

General. Female mice were induced to superovulate and morula-early blastocyst-stage embryos were collected 96 h after the hCG injection, as described previously (Pike & Wales, 1982). For collection, washing and culture of embryos the basic culture medium used was a modified Krebs-Ringer bicarbonate solution supplemented with 25 mM-lactate and 0.25 mM-pyruvate, as used in previous studies (Pike & Wales, 1982). All in-vitro incubations were carried out in droplets of medium under paraffin oil at 37°C in a humidified atmosphere of 5% CO_2 :95% air.

Pulse labelling of embryos. The morula-early blastocyst embryos were pulse labelled by culturing for 2 h in a medium containing 0.28 mM-[$\text{U-}^{14}\text{C}$]glucose with a specific activity of 1.11 MBq/ μmol . After labelling, the embryos were recovered and washed through two changes (2 ml/wash) of medium identical to that used in culture but containing non-radioactive glucose. Some of the pulse-labelled embryos were collected and stored at -70°C for fractionation to study the incorporation of label during the 2-h pulse period.

Chase in vitro. Approximately 30-40 of the pulse-labelled embryos were placed in droplets of medium containing 0.28 mM-non-radioactive glucose and chase-cultured for 24 h under standard culture conditions. After culture the embryos were washed through two changes (2 ml/wash) of medium identical to that used during chase and stored for fractionation.

Chase in vivo. In Exp. 1 pulse-labelled embryos were transferred into the uteri of ovariectomized mice that had been treated with ovarian hormones. Hormone treatments of the ovariectomized animals began 10 days after surgery. The ovariectomized mice (3-4 in each group) received the following hormonal treatments by s.c. injections in 0.1 ml arachis oil every 24 h for 3 days: Group 1, arachis oil only (control); Group 2, 25 ng oestradiol benzoate; Group 3, 2.0 mg progesterone; and Group 4, 25 ng oestradiol benzoate + 2.0 mg progesterone.

Transfer of embryos to the uteri of treated animals was carried out on the 3rd day of hormone treatment. Approximately 20 morulae-early blastocysts, pulse-labelled *in vitro* for 2 h, were transferred into each uterine horn of mice in each treatment group. The embryos were recovered 24 h after transfer by flushing the uteri with culture medium. The percentage recovery of embryos from each treatment group was 16, 9, 14 and 7 from Groups 1 to 4 respectively. The embryos were washed twice (2 ml/wash) through medium containing 0.28 mM-non-radioactive glucose and stored at -70°C before fractionation.

In Exp. 2 the effect of oestrogen priming on the utilization of glycogen by transferred embryos was studied. Embryos were transferred to ovariectomized mice that had been given a priming dose of oestrogen before the nidatory hormone and the results were compared with those after transfer to unprimed recipients. The time interval and the doses of oestrogen used for priming in this experiment have been shown to cause maximal responsiveness of the uterus to subsequent treatment with nidatory hormones (Finn & Martin, 1972). The ovariectomized mice were allotted to two groups.

Group A mice were treated daily with arachis oil only (0.1 ml) for 3 days. Group B mice were given priming injections of 100 ng oestradiol benzoate in 0.1 ml arachis oil daily for these 3 days. Four days after the last priming injection, the mice given arachis oil only were further divided into two sub-groups making a total of 3 groups (2 unprimed, 1 primed, with 3–4 mice in each group) and the following hormone regimens were administered daily for 3 days: Group A1, unprimed + arachis oil (control); Group A2, unprimed + 2.0 mg progesterone; and Group B, primed + 2.0 mg progesterone. Embryos previously pulse-labelled *in vitro* for 2 h were transferred (~10 embryos/uterine horn) on the 3rd day of the nidatory hormone treatment. The embryos were recovered 24 h after transfer, washed and stored for fractionation. The percentage recovery of embryos from Groups A1, A2 and B was 30, 29 and 22 respectively.

Fractionation of embryos. Embryos obtained from different treatment groups were fractionated into major biochemical fractions using the extraction procedure described previously (Edirisinghe *et al.*, 1984b). The radioactivity in these fractions was then determined using scintillation spectrometry and the glucose carbon incorporated (in pg atoms/embryo) was calculated.

Results

Experiment 1

As shown in Table 1 embryos transferred to ovariectomized mice treated with progesterone had a much lower accumulation of glucose carbon in the acid-soluble glycogen fraction than did those

Table 1. Glucose carbon accumulated in various biochemical fractions of morulae–early blastocysts cultured for 2 h in a medium containing 0.28 mM-[U-¹⁴C]glucose followed by 24-h chase in ovariectomized recipients

Group	Treatment of Recipient	N†	Accumulation of glucose carbon (pg atoms/embryo) in:		
			Acid-soluble fraction		Acid-insoluble fraction
			Glycogen	Non-glycogen	
1	No hormone	14	6.54 ± 0.82	1.29 ± 0.62	3.60 ± 0.32
2	Oestrogen	11	5.30 ± 0.43	0.79 ± 0.13	1.89 ± 0.59
3	Progesterone	14	2.91 ± 1.41	2.33 ± 0.80	2.52 ± 0.72
4	Oestrogen + progesterone	7	7.57 ± 1.23	2.05 ± 1.28	2.02 ± 0.40

Values are mean ± s.e.m. for 3 replicates.

† Mean no. of embryos/replicate.

Summary of the analyses of variance:

Source of variation	d.f.	Variance ratios (logarithmic transformation)		
		Acid-soluble fraction		Acid-insoluble fraction
		Glycogen	Non-glycogen	
Effect of hormones	(3)			
Effect of oestrogen	1	4.74	0.65	4.44
Effect of progesterone	1	2.48	1.68	0.35
Interaction	1	9.59*	0.02	1.69
Between replicates	2	2.40	0.61	2.81
Replicate interactions (error variance)	6	0.0266	0.1159	0.0293

* $P < 0.05$.

transferred to untreated ovariectomized mice or those treated with oestrogen. Oestrogen antagonized the effect of progesterone and embryos transferred to mice simultaneously treated with oestrogen and progesterone had an accumulation of glucose carbon in this fraction similar to that in embryos transferred to untreated or oestrogen-treated animals. This effect showed as a significant interaction between the two hormones in the statistical analysis. Accumulation in the other fractions of these embryos varied considerably between replicates and none of the changes as a result of hormone treatments of the recipients tested was statistically significant.

Experiment 2

The label in the acid-soluble glycogen fraction decreased significantly during chase (Table 2). However, the degradation of this pool of glycogen was significantly higher ($P < 0.01$) after chase *in vivo* than after chase *in vitro* (means of 2.85 and 1.04 pg atoms/embryo respectively). Furthermore, treatment with progesterone resulted in a significantly greater degradation of acid-soluble glycogen when compared to the ovariectomized control group ($P < 0.05$). Oestrogen priming of recipients

Table 2. Accumulation of glucose carbon in the biochemical fractions of pulse-labelled mouse morulae-early blastocysts following chase *in vitro* or after transfer to oestrogen-primed or unprimed ovariectomized recipients treated with progesterone

Treatment	N†	Accumulation of glucose carbon (pg atoms/embryo) in:			
		Acid-soluble fraction		Acid-insoluble fraction	
		Glycogen	Non-glycogen	Glycogen	Non-glycogen
Pulse, 2 h	28	6.65 ± 0.59	4.30 ± 0.42	1.99 ± 0.15	3.57 ± 0.75
Chase, 24 h					
<i>In vitro</i>	33	5.61 ± 0.80	0.67 ± 0.09	0.42 ± 0.08	1.70 ± 0.08
<i>In vivo</i>					
No hormone (Group A1)	13	4.55 ± 0.59	0.60 ± 0.05	0.48 ± 0.13	1.87 ± 0.38
Unprimed + progesterone (Group A2)	15	3.07 ± 0.32	1.14 ± 0.14	0.63 ± 0.24	3.10 ± 0.53
Oestrogen primed + progesterone (Group B)	11	3.79 ± 0.14	1.09 ± 0.11	0.60 ± 0.20	3.25 ± 1.24

Values are mean ± s.e.m. for 3 replicates.

† Mean no. of embryos/replicate.

Summary of the analyses of variance:

Source of Variation	d.f.	Variance ratios (logarithmic transformation)			
		Acid-soluble fraction		Acid-insoluble fraction	
		Glycogen	Non-glycogen	Glycogen	Non-glycogen
Treatments	(4)				
Pulse versus chase	1	30.93**	176.49**	47.67**	2.62
In-vitro versus in-vivo chase	1	20.29**	6.01*	1.24	1.87
Within in-vivo chase					
Effect of progesterone	1	9.25*	20.99**	0.84	2.60
Effect of priming	1	4.56	0.09	0.00	0.08
Between replicates	2	6.05*	0.80	7.31*	3.44
Replicate interactions (error variance)	8	0.0033	0.0068	0.0193	0.0308

* $P < 0.05$; ** $P < 0.01$.

before the progesterone treatment had no significant effect on the accumulation of label in this fraction or on accumulation into any other biochemical fraction isolated.

A marked utilization of the glucose carbon accumulated in the non-glycogen acid-soluble component was observed during chase, both *in vitro* and *in vivo* (Table 2). Utilization of the label from this fraction was, overall, significantly higher after chase *in vitro* than *in vivo* because embryos transferred to ovariectomized mice treated with progesterone (Groups A2 and B) contained significantly more label in this component after chase than did embryos transferred to ovariectomized mice untreated with progesterone (Group A1).

The acid-insoluble glycogen fraction decreased significantly during chase both *in vitro* and *in vivo* (from 1.99 pg atoms/embryo after pulse to a mean of 0.53 pg atoms/embryo after chase, $P < 0.01$). However, within in-vivo chase, treatment of recipients with hormones had no significant effect on accumulation into this fraction. Furthermore, incorporation into non-glycogen acid-insoluble compounds varied considerably. No significant change in the glucose carbon accumulated in this fraction was observed following chase *in vitro* or *in vivo*, even though the label remaining in this fraction after chase in progesterone-treated mice (Groups A2 and B) was almost double that in the other treatments.

Discussion

The present experiments clearly demonstrate that the progesterone-dominated uterine environment influences the turnover of glycogen in the developing embryo and that oestrogen administration at the time of transfer had no effect on glycogen metabolism. In fact, simultaneous administration of small amounts of oestrogen together with progesterone inhibited the effect of this second hormone on glycogen turnover.

Except for the effect of progesterone on glycogen metabolism, the administration of hormones to ovariectomized recipients had relatively little effect on the metabolism of the embryo. Oestrogen administration had no effect on any of the biochemical characteristics measured. On the other hand, progesterone did tend to maintain higher levels of label in the non-glycogen acid-soluble fraction during in-vivo chase. This effect could be a direct result of the stimulation to glycogen turnover in these circumstances leading to the accumulation of labelled metabolic intermediates in this pool. From inspection of the results of these experiments there was some indication that progesterone also increased the labelling of the non-glycogen acid-insoluble macromolecules. This effect was very variable and so did not appear as significant in the statistical analysis. However, stimulation of entry of isotope into this pool would not be unexpected given the evidence above that a stimulation of glycogen breakdown is associated with increased isotope levels in the pool of metabolic intermediates (non-glycogen acid-soluble pool) which could act as a source of labelled carbon for macromolecular synthesis. In other words, progesterone treatment of the recipient may have caused the channelling of substrate carbon from the storage compound, glycogen, through the metabolic intermediates in the acid-soluble fraction to the synthesis of macromolecules.

The label retained in the acid-soluble glycogen of morulae-early blastocysts after 24 h chase in ovariectomized mice treated with progesterone is similar to that observed when embryos at this stage were transferred to pseudopregnant mice for the same period of chase (Edirisinghe *et al.*, 1984a). It would therefore appear that the high levels of progesterone in the mother before implantation cause increased degradation of glycogen in embryos and account for the low glycogen content of uterine embryos (Stern & Biggers, 1968; Ozias & Stern, 1973) in comparison to those cultured *in vitro* (Ozias & Stern, 1973).

Most other evidence on the effects of hormones on the metabolism of uterine embryos has been obtained using embryos exhibiting implantational delay. Ozias & Weitlauf (1971) found that treatment of mice with progesterone during implantational delay decreased the glycogen content of the embryos below that of controls. Considering the present findings that progesterone treatment

increases glycogen turnover, the result reported for delayed embryos is not unexpected. The role of oestrogen is less clear. In embryos exhibiting delayed implantation, oestrogen has been shown to activate metabolism by increasing incorporation of precursors into RNA (Prasad, Dass & Mohla, 1968; Dass, Mohla & Prasad, 1969) and protein (Weitlauf & Greenwald, 1968; Prasad *et al.*, 1968; Dass *et al.*, 1969) and by increasing the production of CO₂ (Weitlauf, 1974; Torbit & Weitlauf, 1974) and glycogen (Ozias & Weitlauf, 1971). In the present experiment oestrogen caused no change in the metabolism of the preimplantation embryo *in utero*; its only effect was to reverse the effects of progesterone on embryonic metabolism.

The mechanism of action of progesterone on the metabolism of the developing embryo *in vivo* is not yet clearly understood. There are two possible ways that this hormone could influence the embryo: (1) it may have a direct effect on the embryo, or (2) it may act indirectly by changing the uterine milieu. The findings of Snyder, Weitlauf & Nelson (1971) that the accumulation of glycogen in blastocysts retained in the oviducts of intact as well as hypophysectomized mice was greater than that in the uterus of intact animals indicate that a direct effect of progesterone on embryos *in vivo* is unlikely. These authors suggested that any systemic hormonal influences are mediated via the local environment. The results of the present Exp. 2 suggest that the unstimulated uterus accelerates glycogen breakdown above that occurring *in vitro*. The finding that this breakdown is further accelerated by the administration of progesterone suggests that progesterone may act indirectly by stimulating greater production of some, as yet unidentified, factor or factors responsible for the control of glycogen degradation.

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