Aust. J. Biol. Sci., 1984, **37**, 137–46

Synthesis and Degradation of Labelled Glycogen Pools in Preimplantation Mouse Embryos during Short Periods of *in vitro* Culture

W. R. Edirisinghe,[A,B] *R. G. Wales*[A] *and I. L. Pike*[A,C]

[A] School of Veterinary Studies, Murdoch University, Murdoch, W.A. 6150.
[B] Present address: Department of Obstetrics and Gynaecology, National University of Singapore, Kandang Kerbau Hospital, Singapore 0821.
[C] Present address: *In vitro* Fertilization Program, Northridge Hospital Medical Center, 18300 Roscoe Boulevard, Northridge, California 91328, U.S.A.

Abstract

The incorporation and turnover of glucose carbon by mouse embryos during short periods of *in vitro* culture were studied using [U-^{14}C]glucose as marker. Particular attention was given to the synthesis and degradation of the acid-soluble and acid-insoluble glycogen pools.

During a 5-h culture period, incorporation into all fractions isolated increased during development from the 1-cell to the early blastocyst stage. During maturation of the blastocyst, incorporation into acid-insoluble glycogen fell whilst that into acid-soluble glycogen continued to rise linearly over this 24 h period. At the late blastocyst stage over 80% of total glucose carbon accumulated was in this latter fraction. The decrease in acid-insoluble glycogen observed above was found to occur as a sudden change between 96 and 104 h post-hCG.

The turnover of glucose carbon incorporated into the glycogen pools was studied using pulse–chase culture techniques. The results indicate that the considerable amounts of glycogen accumulated at the later stages of preimplantation development cannot act as a major energy source *in vitro* except in the absence of energy substrates. Under these conditions of energy starvation, however, the breakdown of acid-insoluble glycogen is inhibited.

The findings show that the late preimplantation embryo accumulates large amounts of acid-soluble glycogen *in vitro* due to increased rate of synthesis and low rate of degradation. It is suggested that the falling levels of glycogen reported in the uterine embryo are most likely a result of environment factors other than the availability of energy substrates.

Extra keywords: mouse embryo metabolism.

Introduction

Investigations of the total glycogen content of preimplantation mouse embryos (Stern and Biggers 1968; Ozias and Stern 1973) and the incorporation of glucose into embryonic glycogen (Brinster 1969; Pike and Wales 1982*a*) have shown that the mouse embryo cultured *in vitro* has the ability to synthesize considerable amounts of glycogen, especially during the later stages of preimplantation development. It has also been shown by Pike and Wales (1982*a*) that the preimplantation mouse embryo incorporates glucose into two pools of glycogen, acid-soluble glycogen and an acid-insoluble form referred to as desmoglycogen. The former predominates at the later stages of development and the latter during the early cleavage stages.

In their studies these latter authors used a 24-h culture period to study the incorporation of labelled glucose into the various biochemical fractions. As the uterine embryo cleaves every 10 h (Bowman and McLaren 1970; Allen and McLaren 1971; Barlow *et al.* 1972) after the first cleavage division, the results obtained over a 24-h culture period represent the synthetic ability of the embryo while it is undergoing at least one cleavage division. In order to study the metabolism at more specific stages, it would seem appropriate to use shorter periods of culture to minimize the complication of cleavage changes.

0004-9417/84/030137$02.00

In contrast to the considerable synthesis of glycogen *in vitro* referred to above, the glycogen content of the freshly collected embryo decreases prior to implantation (Stern and Biggers 1968; Ozias and Stern 1973). This reduced content of glycogen observed *in vitro* during preimplantation development could be due to the increased rate of degradation of glycogen in parallel to its increased rate of synthesis. However, no extensive study of the utilization of glycogen as an energy source has yet been undertaken.

The pulse–chase technique offers a suitable method for studying the degradation of glycogen in embryos. The feasibility of utilizing the pulse–chase culture technique for the study of the metabolism of mouse embryos has been investigated (Pike *et al.* 1973) and the technique has been used to study the turnover of labelled carbon pools in preimplantation mouse embryos (Pike and Wales 1982*b*). In this latter study it was found that no significant degradation of acid-soluble glycogen occurred unless the chase medium lacked an exogenous energy source.

The present experiments were undertaken to study:

(1) the incorporation of glucose into various biochemical fractions, especially the glycogen pools of the preimplantation mouse embryo during a short period (5 h) of culture, particularly around the time of first differentiation when there appears to be a switch from the synthesis of acid-insoluble to acid-soluble glycogen;

(2) the synthesis and turnover of acid-soluble glycogen *in vitro* during preimplantation development especially at the later stages when increased accumulation of acid-soluble glycogen occurs in embryos cultured *in vitro* compared with those developing *in utero*.

Materials and Methods

General

Random-bred albino mice of the Q.S. strain were superovulated and embryos were collected by flushing the reproductive tracts. The methods and media used to flush, collect and wash embryos have been described previously (Pike and Wales 1982*a*). Incorporation of glucose was studied by incubation in culture medium supplemented with 25 mM lactate, 0·25 mM pyruvate plus 0·28 mM [U-^{14}C]glucose (The Radiochemical Centre, Amersham, England) at a concentration of 0·28 mM and a specific activity of 30 μCi/μmol (1·1 MBq). All incubations were carried out in droplets of medium under oil at 37°C in a humidified atmosphere of 5% CO_2 : 95% air. Following treatment, the embryos were washed through two changes of non-radioactive medium (2 ml per wash), collected in a very small volume (<5 μl) and stored at −70°C until extraction into various biochemical fractions.

Incorporation Studies

In the first experiment embryos at the one-cell, two-cell, eight-cell, morula–early blastocyst and late blastocyst stages were collected approximately 24, 48, 72, 96 and 120 h post-hCG and incubated for 5 h in radioactive medium as described above. After culture the embryos were washed and stored at −70°C until extracted.

The incorporation of glucose carbon over the later stages of development was studied further in the second experiment. Embryos were collected 96, 104 and 112 h after injection of hCG, washed and cultured in medium containing radioactive glucose for 5 h. After culture the embryos were washed and stored as described above.

Turnover Studies

The turnover of acid-soluble and acid-insoluble glycogen at the later stages of development was also studied. Morula and blastocyst-stage embryos, collected 96, 104, 112 and 120 h post-hCG were pulse-labelled for 1 h in radioactive glucose. Approximately one-third of the pulse-labelled embryos were selected at random for extraction to determine the incorporation of labelled glucose carbon into biochemical fractions during the pulse. The remaining embryos were returned to culture in basic medium with or without the addition of unlabelled glucose (0·28 mM) for a 5 h chase period. At the completion of chase culture embryos which appeared normal were recovered for fractionation.

In the next experiment the effect of chase culture in the absence of simple energy substrates on the turnover of glycogen by morulae–early blastocysts was studied. Embryos were collected 96 h after hCG and pulse-labelled for 1 h as above. After collecting a sample of these embryos for extraction, the remaining pulse-labelled embryos were randomly allocated to three different chase culture media in approximately equal numbers. These media were: (1) the basic culture medium containing 25 mM lactate and 0·25 mM pyruvate, (2) the medium above supplemented with 0·28 mM glucose, and (3) medium similar in composition to the above but lacking the energy substrates glucose, lactate or pyruvate. Following 5 h chase, the embryos were recovered and stored as in previous experiments.

Fractionation of Embryos

The labelled embryos were fractionated into different biochemical fractions using a technique based on those used by Pike and Wales (1982*a*, 1982*b*) with certain modifications. Complete disruption of the embryos was ensured by the addition of 0·1 ml of distilled water and taking the samples through two freeze–thaw cycles. Fresh mouse liver homogenate was added as carrier and the acid-insoluble compounds were precipitated by the addition of perchloric acid (PCA) to the final concentration of 5% (v/v). The acid-soluble fraction was separated by centrifugation and cold ethanol added to precipitate acid-soluble glycogen.

Depending on the requirement of the experiment the PCA-insoluble pellet was either dissolved in NaOH and assayed for radioactivity or the acid-insoluble glycogen was extracted before dissolution in alkali and assay. When the acid-insoluble pellet was further fractionated, the PCA remaining in the pellet was removed by washing with 0·5 ml ethanol. The precipitate was then suspended in 0·8 ml of 0·1 M acetate buffer (pH 4·4) and 0·1 ml of amyloglucosidase–amylase enzyme mixture added (0·1 mg of each enzyme per 1 ml of 0·1 M acetate buffer, pH 4·4) to remove the glucose moieties from the protein-bound glycogen. After the addition of enzymes the suspension was incubated at 40°C for 30 min. The acid-insoluble macromolecules were reprecipitated by the addition of 0·1 ml of 50% (v/v) PCA and the tubes were held in an ice-bath for 15 min prior to centrifugation. The supernatant was recovered and the pellet washed twice with 0·5 ml of 5% (v/v) PCA. The supernatant and washes were pooled and assayed for radioactivity. The acid-insoluble pellet was solubilized by the addition of 0·5 ml of 0·8 M NaOH and incubation overnight at 37°C. The solubilized material was neutralized with 0·5 ml of 0·8 M HCl before it was assayed for radioactivity.

All samples were assayed for radioactivity as described previously (Pike and Wales 1982*a*). The glucose carbon accumulated (as pg atoms per embryo) in each of the fractions was calculated from the disintegration per minute detected in the samples and the specific activity of the [U-^{14}C]glucose.

In the first experiment the acid-soluble ethanol precipitate was subjected to ion-exchange chromatography to estimate charged contaminants. This fraction was found to contain 80, 71, 24, 8 and 6% of charged compounds at one-cell, two-cell, eight-cell, morula and late blastocyst stages respectively and incorporation into acid-soluble glycogen was adjusted to take into account this contamination. As the level of contamination was found to be constant at each developmental stage, data obtained for the incorporation into the acid-soluble glycogen fraction in subsequent experiments were corrected for charged contaminants using the percentages given above.

Statistical Analysis

The data obtained for each experiment was transformed to logarithms prior to analysis in order to reduce the heterogeneity of variance. The significance of results was assessed by standard analyses of variance and *t*-tests.

Results

The data obtained for the incorporation of glucose carbon into various biochemical fractions during *in vitro* culture of preimplantation mouse embryos are given in Table 1. The incorporation of glucose carbon into all fractions increased with the development of the embryo from the one-cell stage to the morula–early blastocyst stage. Further increases were also observed in all the fractions up to the late blastocyst stage except in the case of the acid-insoluble glycogen which decreased during maturation of the blastocyst. Between 50 and 75% of the total glucose carbon incorporated at the early cleavage stages was found

in the acid-insoluble fraction. This proportion changed when the embryo developed to the morula and blastocyst stages when 74 to 84% of the total glucose carbon accumulated in the acid-soluble fraction.

Table 1. Incorporation of glucose carbon into biochemical fractions of mouse embryos during 5 h *in vitro*

Culture medium contained 0·28 mM [U-^{14}C]glucose, 25·0 mM lactate and 0·25 mM pyruvate. Values are the means ± s.e.m.

Stage of development	Time post-hCG (h)	No. of replicates	No. of embryos per replicate	Glucose carbon (pg-atoms/5 h) incorporated per embryo: Acid-soluble fraction		Acid-insoluble fraction	
				Glycogen	Non-glycogen	Glycogen	Non-glycogen
1-cell	24	3	671	0·01±0·00	0·62±0·26	0·23±0·05	0·39±0·04
2-cell	48	3	250	0·05±0·01	0·67±0·08	1·45±0·23	1·28±0·31
8-cell	72	3	284	0·59±0·20	2·70±0·58	2·38±0·39	1·97±0·38
Morula–early blastocyst	96	5	67	27·60±3·81	11·73±0·89	4·76±0·45	9·14±1·02
Late blastocyst	120	4	65	49·15±5·47	21·52±5·99	1·47±0·13	11·91±1·38

Although the incorporation of glucose into acid-soluble glycogen increased significantly between all the stages, one- and two-cell embryos incorporated a very small amount of glucose carbon into this fraction compared to that of morula and blastocyst stages. The incorporation of glucose carbon into non-glycogen acid-soluble compounds increased with the development of the embryo with significant increases from the two-cell to the blastocyst stage. A seven-fold increase in the synthesis of acid-insoluble glycogen was observed between one- and two-cell stages and the levels continued to increase up to the morula–early blastocyst stage after which there was a significant decrease during the formation of the late blastocyst ($t_8 = 8·66$, $P<0·01$). The incorporation of glucose carbon into the non-glycogen acid-insoluble compounds increased from one-cell up to the late blastocyst stage with a marked increase between eight-cell and morula-early blastocyst stage embryos.

Table 2. Incorporation of glucose carbon into the acid-insoluble glycogen of embryos collected 96 and 104 h post-hCG and cultured *in vitro* for 5 h

Medium contained 0·28 mM [U-^{14}C]glucose, 25·0 mM lactate and 0·25 mM pyruvate. Values are the means ± s.e.m. (three replicates)

Stage of development	Time post-hCG (h)	No. of embryos per replicate	Glucose carbon (pg-atoms/5 h) incorporated per embryo: Totally	In acid-insoluble glycogen
Morula–early blastocyst	96	37	44·51±4·95	3·37±0·54
Early blastocyst	104	43	49·82±5·25	1·30±0·12

An investigation of the incorporation and turnover of glucose carbon into various biochemical fractions, especially the two glycogen pools of the embryo developing from morula–early blastocyst to the late blastocyst stage *in vitro* was carried out over 8-h intervals. In these experiments the developmental stage of the embryo has been designated in terms of hours after the injection of hCG.

The incorporation of glucose carbon into acid-insoluble glycogen by these embryos is given in Table 2. It is clear from these data that the marked decrease in the synthesis of acid-insoluble glycogen observed previously between morula–early blastocyst and late

blastocyst stages (Table 1) occurs during embryonic development between 96 and 104 h post-hCG.

Incorporation of glucose carbon into acid-soluble glycogen by embryos between 96 and 120 h post-hCG is given in Table 3. The values for embryos at 96 and 120 h post-hCG have been extracted from Table 1 for comparison of the synthesis over the full range of stages of preimplantation development from morula to late blastocyst. The incorporation of glucose carbon into the acid-soluble glycogen increased linearly over this period.

Table 3. Incorporation of glucose carbon into the acid-soluble glycogen of embryos collected 96, 104, 112 and 120 h post-hCG and cultured *in vitro* for 5 h

Medium contained 0·28 mM [U-^{14}C]glucose, 25·0 mM lactate and 0·25 mM pyruvate. Values are the means ± s.e.m.

Stage of development	Time post-hCG (h)	No. of replicates	No. of embryos per replicate	Glucose carbon (pg-atoms/5 h) incorporated per embryo: Totally	In acid-soluble glycogen
Morula–early blastocyst	96	5	67	53·23±3·57	27·60±3·81
Early blastocyst	104	3	78	59·94±5·63	34·36±5·50
Blastocyst	112	4	39	71·35±5·21	45·32±3·72
Late blastocyst	120	4	65	84·05±9·31	49·15±5·47

Table 4. Glucose carbon accumulated within the acid-soluble fractions of mouse embryos following 1 h pulse culture and after 5 h chase culture with and without unlabelled glucose

Medium for pulse culture contained 0·28 mM [U-^{14}C]glucose, 25·0 mM lactate and 0·25 mM pyruvate. Values are the means ± s.e.m. for three replicates

Stage of development	Time post-hCG (h)	Incubation[A]	No. of embryos per replicate	Glucose carbon (pg-atoms) incorporated per embryo: Totally	In acid-soluble fraction: Glycogen	Non-glycogen
Morula–early blastocyst	96	P	26	12·3±1·5	7·0±1·1	2·7±0·5
		C+G	42	9·9±1·0	6·2±0·6	1·8±0·2
		C−G	38	9·4±2·6	4·0±1·2	2·2±0·7
Early blastocyst	104	P	49	13·5±0·5	7·0±0·9	4·0±0·8
		C+G	104	9·9±0·5	6·4±0·6	1·5±0·2
		C−G	61	11·2±1·6	6·3±1·4	2·1±0·2
Blastocyst	112	P	40	17·4±0·4	8·1±0·5	6·4±0·4
		C+G	51	14·4±0·8	10·1±0·6	2·2±0·3
		C−G	40	16·4±1·4	8·0±1·2	5·0±1·7
Late blastocyst	120	P	35	24·0±1·6	14·2±1·6	7·3±0·3
		C+G	39	19·2±0·6	12·9±1·0	3·2±0·5
		C−G	36	18·0±1·3	10·1±1·5	3·9±0·5

[A] P, pulse culture; C+G, chase culture with unlabelled glucose; C−G, chase culture without unlabelled glucose.

In order to study the turnover of labelled carbon pools, especially the acid-soluble and acid-insoluble glycogen pools over the last stages of preimplantation development, experiments using pulse–chase culture techniques were carried out. Embryos were collected at 8-h intervals from the morula–early blastocyst to the late blastocyst stage. The accumulation of glucose carbon into the acid-soluble glycogen of these embryos during a 1 h pulse in labelled glucose and following 5 h chase in medium with or without unlabelled glucose is given in Table 4. Overall the total glucose carbon accumulated and that

accumulated in the two acid-soluble fractions isolated increased with the stage of development of the embryo. Substantial increases in both incorporation during pulse and residual label after chase were observed as the embryo developed from morula–early blastocyst to late blastocyst stage. Overall, there was no significant change in the glucose carbon retained in the acid-soluble glycogen fraction during chase culture when glucose was present in the chase medium (mean pulse value = 9·07 pg-atom per embryo; mean chase + glucose value = 8·89 pg-atoms per embryo). In the absence of glucose, chase resulted in a significant decrease in the amount of glucose carbon remaining in this fraction (mean value = 7·09 pg-atoms per embryo). However, this effect was variable between the stages of development studied and at 112 h post-hCG, no such effect was demonstrated.

The glucose carbon in non-glycogen, acid-soluble compounds decreased significantly during chase and the presence of glucose in the chase medium further decreased the label retained in this fraction following chase culture. An average of 57 and 35% of the glucose carbon in the non-glycogen, acid-soluble fraction was lost during chase in the presence and the absence of glucose respectively.

In a second experiment the incorporation and turnover of glucose carbon in the acid-insoluble glycogen fraction was studied and the data obtained from embryos collected 96 and 104 h post-hCG are given in Table 5. Glucose carbon accumulated in acid-insoluble glycogen at both stages of development decreased significantly during chase ($P<0·01$).

Table 5. Glucose carbon accumulated within the acid-insoluble fractions of mouse embryos following 1 h pulse culture and after 5 h chase culture with or without unlabelled glucose

Medium for pulse culture contained 0·28 mM [U-^{14}C]glucose, 25·0 mM lactate and 0·25 mM pyruvate. Values are the means ± s.e.m. for three replicates

Stage of development	Time post-hCG (h)	Incubation[A]	No. of embryos per replicate	Glucose carbon (pg-atoms) incorporated per embryo: Totally	In acid-insoluble fraction: Glycogen	Non-glycogen
Morula–early blastocyst	96	P	30	8·4±0·9	0·93±0·21	1·6±0·2
		C+G	38	7·1±1·3	0·38±0·05	1·6±0·1
		C−G	37	8·3±0·2	0·61±0·15	2·2±0·2
Early blastocyst	104	P	50	11·3±1·5	0·46±0·09	1·2±0·1
		C+G	54	8·5±1·7	0·15±0·03	1·2±0·1
		C−G	43	10·2±1·5	0·31±0·05	1·8±0·1

[A] P, pulse culture; C+G, chase culture with unlabelled glucose; C−G, chase culture without unlabelled glucose.

Further, more label was lost from this fraction on chase in the presence of glucose than in its absence ($P<0·05$). Overall there was a significant increase in the glucose carbon in the non-glycogen, acid-insoluble compounds during chase culture when the chase occurred in the absence of glucose ($P<0·01$). When glucose was present during chase, accumulation of label in this fraction was unaffected by chase culture.

The effect of the presence or the absence of simple energy substrates in the chase culture medium on the accumulation of glucose carbon in various biochemical fractions of morulae–early blastocysts was also studied. The data obtained for three replicates are given in Table 6. Significant amounts of glucose carbon were lost from the acid-soluble glycogen fraction when the embryos were chase-cultured in the absence of simple energy substrates ($P<0·05$). No breakdown of acid-soluble glycogen was observed in this study when chase occurred in energy-supplemented media either in the presence or the absence of glucose. The glucose carbon in non-glycogen, acid-soluble compounds decreased during chase ($P<0·01$) and the label retained in this fraction was significantly higher when the medium contained no energy substrate ($P<0·01$). Although utilization of acid-insoluble glycogen

appeared to occur during chase culture in the presence of simple energy substrates, this change did not reach the level of statistical significance. Increased accumulation of glucose carbon into non-glycogen, acid-insoluble compounds was observed during chase especially in media lacking glucose.

Table 6. Glucose carbon accumulated within mouse morulae–early blastocysts following 1 h pulse culture and after 5 h chase culture in medium with and without sample energy substrates

Medium for pulse culture contained 0·28 mM [U-^{14}C]glucose, 25·0 mM lactate and 0·25 mM pyruvate. Values are the means ± s.e.m. for three replicates

Incubation[A]	No. of embryos per replicate	Glucose carbon (pg-atoms) accumulated per embryo in: Acid-soluble fraction		Acid-insoluble fraction	
		Glycogen	Non-glycogen	Glycogen	Non-glycogen
Pulse	37	4·66±0·81	1·72±0·15	1·03±0·29	1·54±0·13
Chase + L+P+G	41	5·06±0·88	0·59±0·04	0·37±0·04	1·72±0·10
Chase + L+P	37	4·95±0·57	0·62±0·07	0·40±0·13	2·27±0·36
Chase − substrate	34	3·53±0·29	0·92±0·13	0·70±0·10	2·49±0·33

[A] L, 25·0 mM lactate; P, 0·25 mM pyruvate; G, 0·28 mM glucose.

To study the possible turnover of acid-soluble and acid-insoluble glycogen at early cleavage stages, a pulse–chase culture experiment was carried out using two-cell embryos. The changes in the glucose carbon accumulated in the biochemical fractions during 2 h pulse and 5 h chase of these embryos are given in Table 7. No significant changes were observed in any of the biochemical fractions studied during chase culture.

Table 7. Glucose carbon accumulated in various fractions of two-celled mouse embryos following 2 h pulse culture and after 5 h chase culture

Medium for pulse culture contained 0·28 mM [U-^{14}C]glucose. Values are the means ± s.e.m. for three replicates

Incubation[A]	Mean No. of embryos per replicate	Accumulation of glucose carbon (pg-atoms) per embryo in: Acid-soluble fraction		Acid-insoluble fraction	
		Glycogen	Non-glycogen	Glycogen	Non-glycogen
Pulse − 2 h	143	0·04±0·01	1·10±0·39	1·17±0·74	1·05±0·35
Chase − 5 h	178	0·04±0·02	0·89±0·19	1·14±0·64	1·29±0·49

[A] Chase culture medium contained 25·0 mM lactate, 0·25 mM pyruvate and 0·28 mM glucose.

Discussion

The present data and previous observations (Pike and Wales 1982*a*) show that the synthesis of acid-soluble glycogen is minimal at the early cleavage stages and that the acid-insoluble form predominates at this time. The later stages synthesize increasing amounts of acid-soluble glycogen and from the time of compaction this is the major form of glycogen stored within the embryo. Acid-insoluble glycogen represents a form of glycogen which is bound to protein and it has been suggested (Pike and Wales 1982*a*) that this form acts as a primer or core on which acid-soluble glycogen is later synthesized. When the synthesis of acid-insoluble glycogen per cell is calculated, two-cell embryos gave the highest and blastocysts the lowest synthesis per blastomere. This may indicate that at the blastocyst stage only some cells of the embryo are active in the synthesis of acid-insoluble glycogen. Recent autoradiographic studies (Edirisinghe *et al.* 1984) indicate that trophectoderm rather than inner cell mass is active in glycogen synthesis at this stage.

The incorporation of glucose carbon into non-glycogen, acid-soluble compounds increased with the development of the embryo. This fraction represents, in addition to the labelled parent substrate, a common pool of labelled metabolic intermediates which are available for anabolic or catabolic processes in the embryo. At the early cleavage stage, glycolysis is greatly inhibited (Barbehenn *et al.* 1974) and the glucose carbon that accumulates in the non-glycogen, acid-soluble fraction of two-cell embryos probably represents labelled parent substrate rather than metabolic intermediates. As development progresses, the activity of the glycolytic pathway increases due to the increased activity of some of the key enzymes regulating glycolysis (Barbehenn *et al.* 1974). Under these circumstances, the considerable accumulation of carbon from glucose in the non-glycogen, acid-soluble fraction is probably not only due to an increased accumulation of the parent substrate but also to metabolic products such as lactate, pyruvate and acetate (Wales 1969) and amino acids (Wales and Whittingham 1973).

The increase in the glucose carbon incorporated into the non-glycogen, acid-insoluble compounds from one-cell to the blastocyst stage is in accordance with the findings of Pike and Wales (1982*a*). It has been shown that glucose is incorporated into various lipid components (Flynn and Hillman 1978), into protein (Schneider *et al.* 1976) and nucleic acids (Pike *et al.* 1977). These reports indicate that small amounts of glucose carbon are incorporated into these macromolecules and Pike and Wales (1982*a*) have suggested that a substantial amount of the label recovered in the non-glycogen, acid-insoluble fraction may represent the incorporation of glucose into various glycoproteins following conversion into other hexoses.

The accumulation of glucose carbon in acid-soluble glycogen showed a small and variable reduction during chase culture in the absence of glucose (Table 4), a result which was not confirmed in a subsequent experiment (Table 6). Pike and Wales (1982*b*) were unable to demonstrate any breakdown of acid-soluble glycogen during 24 h chase in the presence of simple energy substrates and thus it would appear that the embryonic glycogen does not act as a major energy source when substrates are present in the environment. In the absence of simple energy substrates the glucose carbon accumulated in the acid-soluble glycogen pool degraded significantly even during a short period of chase *in vitro* (Table 6). This effect is similar to that observed by Pike and Wales (1982*b*) using a longer period of chase (24 h). Thus, under conditions of energy starvation, stored embryonic glycogen can act as a source of energy for the embryo.

The pulse–chase experiments described in the present study show that the late preimplantation embryo accumulates large amounts of acid-soluble glycogen during culture due to the increased rate of synthesis and low rate of degradation of this form of glycogen. This increase occurs despite the presence of adequate phosphorylase enzyme (E.C. 2.4.1.1) at this stage of embryonic development (Hsieh *et al.* 1979). No data are available for the concentration of energy substrates in the reproductive fluids of mice. However, on the basis of information available on other animals (Holmdahl and Mastroianni 1965; Iritani *et al.* 1969, 1971; Wales 1973) it is likely that adequate amounts of glucose and energy substrates are present in mouse reproductive fluids to support glycogen synthesis and to minimize its breakdown *in vivo*. Thus the net effect should be elevated glycogen levels in uterine embryos. Contrary to this expectation, direct measurements of glycogen in uterine embryos have indicated low and falling levels at the later stages of preimplantation development (Ozias and Stern 1973). Thus the control of glycogen metabolism in the uterine embryo must occur as a result of environmental factors other than the availability of simple energy substrates. Such environmental factors could trigger glycogen degradation in the embryo perhaps by converting the inactive form of the enzyme phosphorylase to its active form.

Breakdown of acid-insoluble glycogen occurs at later stages of embryonic development when the chase medium contains energy substrates especially glucose. If the acid-insoluble glycogen acts as a core from which primer oligosaccharide fragments are released and used

for the synthesis of acid-soluble glycogen, as suggested by Pike and Wales (1982*a*), breakdown of acid-insoluble glycogen could be expected to occur to meet the high demand for the primer molecules when conditions such as the abundance of the precursor favour increased glycogen synthesis. During energy starvation acid-soluble glycogen is utilized as a source of energy and the acid-insoluble glycogen molecules remain unaffected.

By contrast, embryos at the early cleavage stages do not utilize any of the glycogen pools accumulated during *in vitro* culture. This could be explained by the low phosphorylase (E.C. 2.4.1.1) activity (Hsieh *et al.* 1979) and the inhibition of glycolysis (Barbehenn *et al.* 1974) at this stage of embryonic development. Accumulation of more glucose carbon in non-glycogen, acid-insoluble compounds occurred during chase particularly in the absence of glucose. This increase probably arises from conservation and recycling of carbon through the non-glycogen, acid-soluble pool for the synthesis of proteins, lipids and nucleic acids.

The findings of the present experiments indicate that the metabolism, especially the glycogen metabolism, of the cultured mouse embryo differs from that of the uterine embryo. As embryos are cultured *in vitro* in a chemically defined medium without exposure to the influence of the maternal environment one might expect some discrepancy between fresh and cultured embryos. Hormones such as ovarian steroids or those involved in glycogen metabolism or other factors in the uterine environment could influence the regulatory mechanisms of the developing embryo *in vivo* in order to synchronize the embryonic and uterine events for successful development and implantation to occur.

Acknowledgments

The technical assistance of Rosalie Taylor is gratefully acknowledged. The work was aided by a grant from the Australian Research Grants Scheme. One of us (W.R.E.) was supported by a Murdoch University Postgraduate Studentship.

References

Allen, J., and McLaren, A. (1971). Cleavage rate of mouse eggs from induced and spontaneous ovulation. *J. Reprod. Fertil.* **27**, 137–40.

Barbehenn, E. K., Wales, R. G., and Lowry, O. H. (1974). The explanation for the blockade of glycolysis in early mouse embryos. *Proc. Natl Acad. Sci. U.S.A.* **71**, 1056–60.

Barlow, P., Owen, D. A. J., and Graham, C. (1972). DNA synthesis in the preimplantation mouse embryo. *J. Embryol. Exp. Morph.* **27**, 431–45.

Bowman, P., and McLaren, A. (1970). Cleavage rate of mouse embryos *in vivo* and *in vitro*. *J. Embryol. Exp. Morph.* **24**, 203–7.

Brinster, R. L. (1969). Incorporation of carbon from glucose and pyruvate into the preimplantation mouse embryo. *Expl Cell Res.* **58**, 153–8.

Edirisinghe, W. R., Wales, R. G., and Pike, I. L. (1984). Studies of the distribution of glycogen between the inner cell mass and trophoblast cells of mouse embryos. *J. Reprod. Fertil.* (In press.)

Flynn, T. J., and Hillman, N. (1978). Lipid synthesis from [U-^{14}C]glucose in preimplantation mouse embryos in culture. *Biol. Reprod.* **19**, 922–6.

Holmdahl, T. H., and Mastroianni, L. (1965). Continuous collection of rabbit oviduct secretions at low temperature. *Fertil. Steril.* **16**, 587–95.

Hsieh, B., Chi, M. M. Y., Knor, J., and Lowry, O. H. (1979). Enzymes of glycogen metabolism and related metabolites in preimplantation mouse embryos. *Dev. Biol.* **72**, 342–9.

Iritani, A., Gomes, W. R., and Vandemark, N. L. (1969). Secretion rates and chemical composition of oviduct and uterine fluids in ewes. *Biol. Reprod.* **1**, 72–6.

Iritani, A., Nishikawa, Y., Gomes, W. R., and Vandemark, N. L. (1971). Secretion rates and chemical composition of oviduct and uterine fluids in rabbits. *J. Anim. Sci.* **33**, 829–35.

Ozias, C. B., and Stern, S. (1973). Glycogen levels of preimplantation mouse embryos developing *in vitro*. *Biol. Reprod.* **8**, 467–72.

Pike, I. L., and Wales, R. G. (1982*a*). Uptake and incorporation of glucose especially into the glycogen pools of preimplantation mouse embryos during culture *in vitro*. *Aust. J. Biol. Sci.* **35**, 195–206.

Pike, I. L., and Wales, R. G. (1982*b*). Turnover of carbon pools labelled with [^{14}C]glucose during *in vitro* culture of preimplantation mouse embryos. *Aust. J. Biol. Sci.* **35**, 637–44.

Pike, I. L., Kaye, P. L., and Wales, R. G. (1977). Glucose incorporation into nucleic acids of mouse morulae. *Proc. Aust. Biochem. Soc.* **10**, 75 (Abstr.).

Pike, I. L., Murdoch, R. N., and Wales, R. G. (1973). The use of *in vitro* pulse/chase culture techniques in studies of the incorporation of radioactive energy substrates by the preimplantation mouse embryo. *J. Reprod. Fertil.* **32**, 314–15 (Abstr.).

Schneider, H. J. (Jr), Olds, D., Tucker, R. E., and Mitchell, G. E. (Jr) (1976). Mouse embryos use glucose in protein synthesis. *J. Anim. Sci.* **42**, 271 (Abstr.).

Stern, S., and Biggers, J. D. (1968). Enzymatic estimation of glycogen in the cleaving mouse embryo. *J. Exp. Zool.* **168**, 61–6.

Wales, R. G. (1969). Accumulation of carboxylic acids from glucose by the preimplantation mouse embryos. *Aust. J. Biol. Sci.* **22**, 701–7.

Wales, R. G. (1973). The uterus of the ewe. II. Chemical analysis of uterine fluid collected by cannulation. *Aust. J. Biol. Sci.* **26**, 947–59.

Wales, R. G., and Whittingham, D. G. (1973). The metabolism of specifically labelled lactate and pyruvate by two-cell mouse embryos. *J. Reprod. Fertil.* **33**, 207–22.

Manuscript received 14 November 1983, accepted 21 March 1984