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Influence of Environmental Factors on the Metabolism of Glucose by Preimplantation Mouse Embryos *in vitro*

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Abstract

The metabolism of glucose by late preimplantation mouse embryos was studied in a variety of media whose composition had been changed to reflect the environmental conditions in the uterus more closely than do standard culture media. The effects of combinations of energy substrates, the presence or absence of amino acids and the level of potassium in the medium were investigated.

The use of energy substrates for *in vitro* culture at levels present in the uterine environment resulted in rates of synthesis and degradation of glycogen pools similar to those obtained using standard *in vitro* culture conditions but elevated incorporation into non-glycogen macromolecules. Amino acids influenced the metabolism of glucose by limiting the entry of glucose carbon into the non-glycogen macromolecular pool and directing more glucose into the synthesis of acid-soluble glycogen.

Increasing the K^+ concentration to 60 mM in the culture medium caused a small but significant increase in the number of eight-cell embryos degenerating during culture for 24 h but the metabolism of glucose was unaffected over this time. At the time of morula transformation to the blastocyst this level of potassium ions suppressed glycogen synthesis by 50% over 5 h but did not affect its turnover during chase culture.

It is concluded that factors other than those studied here contribute to the maintenance of the low glycogen levels found in uterine embryos.

Introduction

Earlier work (Ozias and Stern 1973; Ozias and Weitlauf 1971; Snyder *et al.* 1971; Edirisinghe *et al.* 1984*a*, 1984*b*) indicates that the uterine environment influences the metabolism of late preimplantation mouse embryos and in particular the utilization of glycogen stores. This environment is more complex than, and differs in the range and concentration of components from, the standard chemically defined media normally used for *in vitro* culture (Hamner 1971; Wales 1973; Beier 1974; Edirisinghe and Wales 1985). Changes in the environment may well be important in modifying the metabolism of glucose by embryos and be responsible for the differences in the turnover of glycogen *in vivo* as compared to its utilization *in vitro*.

The possible control of glycogen metabolism by the level of exogenous energy substrate has been studied using different concentrations of the major energy substrates, glucose, lactate and pyruvate (Pike and Wales 1982). It is clear from this study that embryos, especially at the late preimplantation stages, have the ability to synthesize large amounts of glycogen and that the presence of the alternative energy substrates, lactate and pyruvate during culture influences the synthesis of glycogen, particularly when the medium contains a low concentration of glucose. Recently, the levels of lactate, pyruvate and glucose in the uterine fluid of mice have been measured and found

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to be in the region of 1·0, 0·1 and 1·0 mM respectively (W. R. Edirisinghe and R. G. Wales, unpublished data). Thus a further investigation using these new values seems to be warranted in order to clarify the significance of the availability of energy substrates in the uterus on the control of glucose metabolism *in vivo*.

Free amino acids constitute part of the organic substrate pool in uterine fluid. They appear in relatively high concentrations in uterine secretions (Gwatkin 1969; Iritani *et al.* 1971; Jaszczak and Hafez 1972; Wales 1973) and could act as an important alternative energy source for the developing embryo. If this is so, their presence in the environment during development could influence the metabolism of simple energy substrates such as glucose and affect the resultant accumulation of glycogen during development *in vivo*.

The ionic content of the environment is another important factor which may influence the development of embryos (Wales 1970) and play a role in the differentiation of cells (McMahon 1974). Whilst the role of K^+ during embryonic development has not been studied extensively, this ion is known to affect the metabolism of energy substrates by cells such as spermatozoa (Wallace and Wales 1964). Thus the high concentration of K^+ found in the uterine fluid of mice (Edirisinghe and Wales 1980) and other animals (Olds and VanDemark 1957; Howard and De Feo 1959; Ringler 1961; Heap 1962; Wales 1973) may have some significance in the control of the metabolism of the differentiating embryo.

The present experiments were carried out to study the influence of the environmental factors discussed above on the metabolism of glucose by the preimplantation mouse embryo and to determine the possible significance of these factors in maintaining a low level of glycogen in embryos developing *in utero*.

Materials and Methods

General

Mouse embryos at the eight-cell, morula–early blastocyst and late blastocyst stages were collected from random-bred Quackenbush [Q(S)] mice 72, 96 and 120 h post-hCG injection according to the methods described previously (Pike and Wales 1982). For collection, washing and culture of embryos, culture medium based on Kreb's Ringer bicarbonate was used (Brinster 1965). All incubations were carried out in droplets of medium under oil at 37°C in a humidified atmosphere of 5% CO_2 : 95% air.

To study the metabolism of glucose, [U-^{14}C]glucose (Radiochemical Centre, Amersham, England) at the required concentration and specific activity was incorporated into the culture medium. At the completion of treatments the embryos were fractionated into the major biochemical components, acid-soluble glycogen, non-glycogen acid-soluble compounds, acid-insoluble glycogen and non-glycogen acid-insoluble compounds using the standard extraction procedure described previously (Edirisinghe *et al.* 1984*a*). The radioactivity in each fraction was determined by the use of scintillation spectrometry and the pg-atoms of glucose carbon incorporated into each biochemical fraction was estimated using the radioactivity in the samples and the specific activity of the parent substrate.

All experiments were of a balanced factorial design. For each replicate of an experiment, a pool of embryos was collected and approximately equal number of embryos (35–70 morulae–early blastocysts or 10–20 late blastocysts per treatment depending on the requirements of each experiment) were assigned at random to each treatment group. Results as appropriate, were subjected to *t*-tests or analysed by standard analyses of variance after logarithmic transformation to equalize variances. The levels of significance of the treatment effects are quoted in the text.

Effect of Energy Substrates

In these experiments the incorporation of [^{14}C]glucose into the various biochemical fractions of embryos, especially into the glycogen pools, during culture in media containing glucose, lactate and pyruvate at concentrations found to be present in the uterine fluid of mice (W. R. Edirisinghe and R. G. Wales, unpublished data) was studied. In the first experiment, glucose incorporation over 5 h using these substrate concentrations was compared with incorporation at the substrate concentrations used in previous studies (Edirisinghe

et al. 1984*a*, 1984*b*). In the second experiment, the pulse–chase technique, using a 2 h pulse and a 24 h chase, was used to compare turnover of label through the biochemical pools under these conditions.

Morula–early blastocyst stage embryos were collected, washed and the pooled embryos were divided into two groups. One group of embryos was cultured in a medium containing 0·28 mM [U-^{14}C]glucose (specific activity 1·11 MBq/μmol), 25 mM lactate and 0·25 mM pyruvate. The other half of the embryos were transferred into medium containing 1 mM [U-^{14}C]glucose (specific activity 0·56 MBq/μmol), 1 mM lactate and 0·1 mM pyruvate and cultured as above after being washed twice (2 ml per wash) through similar, but non-radioactive medium. At the completion of culture in labelled glucose, embryos were washed twice (2 ml per wash) through non-radioactive media identical in composition to those used during culture.

In the first experiment, embryos cultured for 5 h as described above were stored for fractionation. In the second experiment, embryos were pulse-labelled under the conditions described above. After the 2 h pulse, some of the embryos were stored for fractionation after washing whilst the remainder were placed in droplets of the non-radioactive glucose medium identical in composition to that used for pulse culture. Following 24 h chase culture in these droplets, the embryos were washed twice (2 ml per wash) and stored at −70°C prior to fractionation.

Effect of Amino Acids

To study the effect of amino acids on the incorporation of [^{14}C]glucose, morula–early blastocyst and late blastocyst stage embryos were used. These embryos were collected, washed, divided into two pools and cultured for 5 h in media containing 0·28 mM [U-^{14}C]glucose (specific activity 1·11 MBq/μmol) with or without the addition of 5 mM alanine, 2·5 mM glycine, 2·5 mM lysine, 2·5 mM leucine plus 2·5 mM valine to the medium. These amino acids and their concentrations were selected because they are the major amino acids shown to be present in the uterine fluid of the ewe (Wales 1973). After culture the embryos were collected, washed through two changes of medium containing non-radioactive glucose (2 ml per wash) and stored at −70°C for fractionation.

Effect of Potassium

The effects of increasing the K^+ concentration was investigated by culturing eight-cell, morula–early blastocyst and late blastocyst stage mouse embryos for 5 or 24 h in medium containing 0·28 mM [U-^{14}C]glucose (specific activity 1·11 MBq/μmol) and increasing concentrations of KCl. Following culture each group of embryos was washed twice (2 ml per wash) in medium containing 0·28 mM non-radioactive glucose and the same concentration of K^+ used during culture. Following the wash, the embryos were stored at −70°C for subsequent fractionation. The development of the eight-cell embryos after culture for 24 h in medium containing 6, 30 or 60 mM K^+ was scored in order to gauge the effect of K^+ on development.

In a second experiment, pulse–chase culture was used to study the effect of K^+ concentration in the medium on the turnover of labelled biochemical pools in morulae–early blastocysts. These embryos were pulse-labelled by culturing for 2 h in the radioactive glucose medium (0·28 mM) containing either 6 or 60 mM K^+. The pulse-labelled embryos were washed and after storing some embryos for fractionation, the remainder were chase cultured for 24 h in 0·28 mM non-radioactive glucose medium containing 6 or 60 mM K^+. Following chase the embryos were washed through two changes of medium identical to that used in chase culture (2 ml per wash) and stored for fractionation.

Results

Effect of Energy Substrates

The data obtained for the incorporation of glucose carbon into morula–early blastocyst stage embryos cultured for 5 h in the presence of the energy substrates, glucose, lactate and pyruvate, either at the concentrations used in previous studies (medium 1) or at the concentrations found to be present in uterine fluid (medium 2) are included in Table 1. Total incorporation of label was approximately one-third greater in medium 2 than medium 1 (49·21 *v.* 37·50 pg-atom glucose carbon incorporated per embryo). Incorporation into the non-glycogen acid-soluble and acid-insoluble fractions increased approximately 50% when culture was carried out in medium 2 rather than medium 1 ($P < 0·05$). However, in the case of the glycogen fractions, which contribute more

than half to the total label incorporated, incorporation was not significantly affected by change in substrate concentrations.

The turnover of glucose carbon incorporated in the biochemical pools of morula–early blastocyst stage embryos during culture in the above two media was studied by pulse–chase techniques and the results are also shown in Table 1. The pattern of incorporation during the 2 h pulse was similar to that observed during the 5 h culture period in the previous experiment. Total incorporation was 40% higher in medium 2 than medium 1 and there was no significant effect of medium on the incorporation of glucose into the acid-soluble and acid-insoluble glycogen fractions. During the period of chase, there was a small, but significant ($P < 0{\cdot}01$), turnover of acid-soluble glycogen but this effect was not influenced by the medium used. Substantial degradation of the

Table 1. Effect of different combinations of substrate on the incorporation of glucose carbon during 5 h culture (Experiment A) and its distribution in mouse morulae–early blastocysts following 2 h pulse culture and a subsequent 24 h chase (Experiment B)

Medium 1 contained 0·28 mM glucose + 25 mM lactate + 0·25 mM pyruvate. Medium 2 contained 1 mM glucose + 1 mM lactate + 0·1 mM pyruvate. Values are means ± standard errors of the means for three replicates of each experiment

Culture	Glucose carbon accumulated (pg atoms per embryo) in:			
	Acid-soluble fraction		Acid-insoluble fraction	
	Glycogen	Non-glycogen	Glycogen	Non-glycogen
	Experiment A			
Culture 5 h				
Medium 1	20·54 ± 2·45	7·14 ± 0·70	2·81 ± 0·35	7·01 ± 0·65
Medium 2	23·95 ± 3·47	11·93 ± 1·57	2·89 ± 0·52	10·44 ± 1·03
	Experiment B			
Pulse 2 h				
Medium 1	6·26 ± 0·69	3·61 ± 0·33	1·95 ± 0·25	2·85 ± 0·48
Medium 2	8·35 ± 1·61	7·19 ± 1·50	1·71 ± 0·32	3·75 ± 0·39
Chase 24 h				
Medium 1	5·01 ± 0·93	0·56 ± 0·14	0·10 ± 0·01	1·73 ± 0·26
Medium 2	5·01 ± 0·72	0·58 ± 0·09	0·17 ± 0·02	2·43 ± 0·30

acid-insoluble glycogen pool occurred during chase culture ($P < 0{\cdot}01$). As with acid-soluble glycogen, degradation of this pool was not affected significantly by the medium used. Chase culture resulted in the utilization of the major portion of the glucose carbon incorporated into non-glycogen acid-soluble components of the embryo and the amount remaining at the completion of 24 h chase was the same in the two media even though the amount of label present in this fraction at the commencement of chase was greater when using medium 2 rather than medium 1. Approximating 60% of the carbon incorporated into the non-glycogen acid-insoluble fraction during pulse culture remained at the completion of 24 h chase culture. Whilst embryos cultured in medium 2 incorporated significantly more carbon into this fraction ($P < 0{\cdot}01$), there was no difference between the two media in the proportion of this component degraded during chase culture.

Effect of Amino Acids

The incorporation of glucose carbon into the biochemical fractions of morulae–early blastocysts and late blastocysts during culture in medium containing [^{14}C]glucose with or without supplementation with amino acids is given in Table 2. Incorporation into the acid-soluble glycogen fraction at both stages of development was higher during culture in medium supplemented with amino acids than when amino acids were absent

Table 2. Effects of amino acids on the incorporation of glucose carbon into mouse morulae–early blastocysts and late blastocysts during 5 h culture

Medium contained 0·28 mM [U-^{14}C]glucose, 25 mM lactate and 0·25 mM pyruvate. Values are means ± standard errors of the means for four replicates

Stage of development and treatment	Incorporation of glucose carbon (pg-atoms per embryo per 5 h) into: Acid-soluble fraction		Acid-insoluble fraction	
	Glycogen	Non-glycogen	Glycogen	Non-glycogen
Morula–early blastocyst				
Amino acids absent	31·36 ± 4·44	12·69 ± 2·21	2·16 ± 0·38	11·60 ± 0·79
Amino acids present	45·32 ± 2·42	14·22 ± 1·68	1·74 ± 0·34	9·53 ± 0·82
Late blastocyst				
Amino acids absent	50·76 ± 12·48	14·27 ± 2·41	1·46 ± 0·57	9·53 ± 1·51
Amino acids present	65·27 ± 9·96	13·39 ± 0·74	0·98 ± 0·16	8·36 ± 1·13

($P < 0·05$). No significant differences in the incorporation of glucose carbon into acid-insoluble glycogen and non-glycogen acid-soluble components occurred as a result of the inclusion of amino acids in the medium. The incorporation of glucose carbon into non-glycogen acid-insoluble compounds decreased significantly ($P < 0·05$) as a result of the addition of amino acids to the culture medium.

Table 3. Effects of different concentrations of K^+ on the incorporation of glucose carbon into eight-cell, morula–early blastocyst and late blastocyst stage mouse embryos during 5 h culture

Medium contained 0·28 mM [U-^{14}C]glucose, 25 mM lactate and 0·25 mM pyruvate. Values are means ± standard errors of the means for three replicates

Stage of development and treatment	Incorporation of glucose carbon (pg-atoms per embryo per 5 h) into: Acid-soluble fraction		Acid-insoluble fraction	
	Glycogen	Non-glycogen	Glycogen	Non-glycogen
Eight-cell stage				
6 mM K^+	0·60 ± 0·18	3·35 ± 0·41	2·67 ± 0·47	2·31 ± 0·16
60 mM K^+	0·75 ± 0·25	3·99 ± 0·18	2·82 ± 0·76	1·98 ± 0·05
Morula–early blastocyst				
6 mM K^+	24·09 ± 4·68	8·14 ± 0·32	4·51 ± 0·52	7·77 ± 0·92
60 mM K^+	13·16 ± 2·30	6·71 ± 0·14	3·51 ± 0·14	5·71 ± 0·61
Late blastocyst				
6 mM K^+	41·39 ± 3·50	10·59 ± 2·03	1·13 ± 0·11	7·49 ± 1·99
60 mM K^+	44·48 ± 9·34	9·79 ± 0·22	1·15 ± 0·04	6·54 ± 1·66

Effect of Potassium

The effect of increasing the concentration of potassium ions in the medium from 6 to 60 mM on the incorporation of glucose carbon over 5 h into embryos at different stages of development from the eight-cell stage onwards is shown in Table 3. Statistical analyses of the data showed that, overall, there was no significant

effect of increasing the concentration of K^+ on incorporation of glucose. In the analysis of the acid-insoluble glycogen, and the non-glycogen acid-soluble and acid-insoluble fractions, there was no significant interaction of stage of development with K^+ concentration, indicating a lack of effect of the change in K^+ concentration at all stages studied. In the case of the acid-soluble glycogen fraction, however, a significant interaction was found. High K^+ had no significant effect at the eight-cell and late blastocyst stages but decreased incorporation into this pool at the morula–early blastocyst stage of development.

Table 4. Effects of different concentrations of K^+ on the incorporation of glucose carbon into eight-cell mouse embryos during culture for 24 h

Medium contained 0·28 mM [U-^{14}C]glucose, 25 mM lactate and 0·25 mM pyruvate. Values are means ± standard errors of the means for three replicates

Treatment	Incorporation of glucose carbon (pg-atoms per embryo) into:			
	Acid-soluble fraction		Acid-insoluble fraction	
	Glycogen	Non-glycogen	Glycogen	Non-glycogen
6 mM K^+	11·46 ± 3·40	10·07 ± 1·69	10·08 ± 1·29	13·71 ± 1·53
30 mM K^+	14·55 ± 4·05	11·58 ± 1·37	10·59 ± 1·41	14·97 ± 2·21
60 mM K^+	14·24 ± 0·66	11·98 ± 0·28	14·46 ± 2·21	14·40 ± 0·91

Data on the development of eight-cell mouse embryos cultured for 24 h in the presence of 6, 30 and 60 mM K^+ (100 embryos per treatment) showed that, overall, 90% of all embryos developed normally through to the morula–blastocyst stage. However, the results revealed a significant ($P < 0·05$) effect of K^+ concentration on the number of embryos degenerating, with a larger proportion of degenerated embryos (10%) when the medium contained 60 mM K^+ than when 6 or 30 mM K^+ was used (3%). In view of this, the incorporation of [^{14}C]glucose into eight-cell mouse embryos cultured in medium containing 6, 30 or 60 mM K^+ was studied (Table 4). Changes in the concentration of potassium in the medium had no effect upon the incorporation of glucose carbon into any of the biochemical pools isolated.

Table 5. Effects of 6 or 60 mM K^+ on the distribution of glucose carbon in mouse morulae–early blastocysts after 2 h pulse culture and following 24 h chase in non-radioactive medium

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

Culture	Accumulation of glucose carbon (pg-atoms per embryo) in:			
	Acid-soluble fraction		Acid-insoluble fraction	
	Glycogen	Non-glycogen	Glycogen	Non-glycogen
Pulse 2 h				
6 mM K^+	9·23 ± 0·99	6·00 ± 1·46	2·20 ± 0·14	3·34 ± 0·36
60 mM K^+	6·92 ± 0·56	5·51 ± 0·86	1·68 ± 0·12	2·74 ± 0·24
Chase 24 h				
6 mM K^+	7·53 ± 0·94	1·01 ± 0·30	0·52 ± 0·05	1·89 ± 0·26
60 mM K^+	4·49 ± 0·42	0·71 ± 0·20	0·45 ± 0·03	1·55 ± 0·03

The effect of high K^+ on turnover of glucose carbon in the various biochemical pools of morulae–early blastocysts was studied by the pulse–chase technique. Embryos were pulse-labelled for 2 h, then chase cultured for 24 h in the presence of either 6 or 60 mM K^+. In this experiment (Table 5), high K^+ significantly depressed

incorporation of glucose carbon into acid-insoluble ($P < 0{\cdot}05$) as well as acid-soluble ($P < 0{\cdot}01$) glycogen. As in the previous experiment (Table 3), non-glycogen acid-soluble and acid-insoluble fractions were not significantly affected by the change in K^+ concentration. During chase there was significant utilization of label in all fractions and this turnover of label was unaffected by changing the concentration of K^+.

Discussion

The rate of incorporation of [^{14}C]glucose into the major biochemical fractions of eight-cell, morula–early blastocyst and late blastocyst stage embryos during *in vitro* culture for 2 or 5 h in the presence of 0·28 mM glucose, 25 mM lactate and 0·25 mM pyruvate is similar to that observed in the previous experiments (Edirisinghe *et al.* 1984*a*, 1984*b*). Thus these results confirm the earlier findings that morulae and blastocysts synthesize large amounts of acid-soluble glycogen during *in vitro* culture and that the synthesis of the acid-insoluble glycogen pool decreases during the later development of the blastocyst. The results obtained for eight-cell embryos during 24 h culture are similar to those obtained by Pike and Wales (1982) using a similar medium. Comparison of incorporation over 5 and 24 h for these embryos indicates that there is a relatively linear increase in incorporation into all fractions except the acid-soluble glycogen fraction whose incorporation reflects the considerable acceleration in the rate of synthesis of this compound as the embryo develops from the 8-cell stage towards morula formation (Edirisinghe *et al.* 1984*a*).

Changes in the concentration of substrates in the medium from those used in earlier experiments (0·28 mM glucose, 25 mM lactate and 0·25 mM pyruvate) to those found in the mouse uterine fluid (1 mM glucose, 1 mM lactate and 0·1 mM puruvate) have no significant effect on the incorporation or turnover of glucose carbon in the glycogen pools of the embryo. However, the fourfold increase in the concentration of glucose in the medium caused a 40% increase in overall glucose uptake due to increased incorporation into non-glycogen macromolecules and into the non-glycogen acid-soluble components. Pike and Wales (1982) also found a relatively small increase in incorporation on increasing glucose concentration in the medium 20-fold from 0·28 mM to 5·56 mM and concluded that the glucose carrier system remains close to saturation even with low levels of glucose in the medium.

Amino acids are present in uterine secretions in considerable amounts (Gwatkin 1969; Iritani *et al.* 1971; Jaszczak and Hafez 1972; Wales 1973) and have been implicated in the biology of development. Their uptake and incorporation into the mouse embryo has been demonstrated both *in vitro* (Brinster 1971; Smith and Smith 1971; Epstein and Smith 1973; Epstein 1975) and *in vivo* (Greenwald and Everett 1959; Weitlauf and Greenwald 1965) and some have been shown to be important for the outgrowth of the embryo *in vitro* (Gwatkin 1966). The present findings indicate that amino acids influence the metabolism of glucose by the embryo by limiting the entry of glucose carbon into non-glycogen macromolecules and directing more label into the synthesis of acid-soluble glycogen. Thus amino acids tend to increase, rather than decrease glycogen synthesis from glucose and thus cannot be responsible for the reduced glycogen levels that are found in the late preimplantation mouse embryo *in vivo*.

There is evidence to suggest that *in vivo* the mouse embryo develops in the presence of concentrations of potassium ions much higher than those normally present in culture media. At the eight-cell stage of development, mouse embryos lie in the isthmus of the oviduct where the concentration of K^+ is about 30 mM (Roblero *et al.* 1976).

Within the following 24 h, the developing embryo enters the uterus where the concentration of K^+ has been estimated to be double this value (Edirisinghe and Wales 1985). In the present experiments, increasing the concentration of K^+ from 6 mM to 30 mM had no effect on the development of eight-cell embryos over 24 h in culture. When the concentration of K^+ was raised to 60 mM, a higher percentage of eight-cell embryos degenerated during culture, suggesting that the K^+ concentration in the uterus is close to the upper limit for the survival of late preimplantation embryos. It is possible that this could have the beneficial effect of eliminating less viable embryos in the uterus prior to spacing and the initiation of implantation.

The metabolism of the eight-cell embryo is unaffected by an increase in concentration of potassium ions in the medium from 6 to 60 mM even when the culture period is extended to 24 h. In fact the only stage during uterine development at which high levels of K^+ were found to affect the metabolism of the embryo was the time of morula transformation to the early blastocyst. This is a very active stage in glycogen synthesis and the depression observed in the presence of high K^+ was of the order of 50% over 5 h. However, high concentrations of K^+ had little effect on glycogen turnover and elevated levels of this cation, alone, would seem unlikely to explain the large discrepancy in glycogen content between freshly collected and *in vitro* cultured embryos. Thus factors other than those examined in this paper must contribute to the maintenance of low glycogen levels in uterine embryos.

One component of uterine fluid that changes significantly with the stage of early pregnancy is the luminal protein fraction (Beier 1974; Gore-Langton and Surani 1976; Fishel 1979). In the mouse on day 3–5 of pregnancy, an increase in the concentration of the high molecular weight proteins has been observed (Gore-Langton and Surani 1976). A similar increase in the high molecular weight proteins following administration of oestrogen to progesterone maintained ovariectomized rats has been recorded (Surani 1975). These high molecular weight proteins may regulate the metabolism of the embryo by binding to embryonic receptors (Surani 1977; Tzartos and Surani 1979). It is possible that some of these proteins could play a role similar to that of the specific protein isolated in the rabbit uterus called 'uteroglobin' (Beier 1968) or 'blastokinin' (Krishnan and Daniel 1967). However, the significance of these luminal proteins in the regulation of the metabolism of the preimplantation embryo has yet to be investigated.

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