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Changes in responsiveness of preimplantation mouse embryos to serum

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SUMMARY

Changes in uptake of radioactive uridine and its incorporation into RNA were determined in preimplantation mouse embryos, from the 2-cell to the blastocyst stage, as a measure of their responsiveness to extracellular conditions. Two media were tested, one contained serum and the other contained bovine serum albumen as a control. An increase in the acid-soluble pool occurred at the 8-cell stage and a marked increase in RNA synthesis occurred at the early blastocyst stage when the embryos were incubated with serum.

INTRODUCTION

Changes in the metabolism of the preimplantation mouse embryo occur during development from the 2-cell to the blastocyst stage (Epstein, 1975) but little is known about the changes in their metabolism in response to alterations in environmental factors. Increasing responsiveness to environmental factors is suggested to occur during the course of early development (Surani, 1977). Enhanced responsiveness of the embryo to environmental factors may be partly associated with the establishment of membrane transport systems (Borland & Tasca, 1974; Powers & Tupper, 1977) along with other changes in cell surface properties (Pinsker & Mintz, 1973; Nilsson, Lindquist, Ronquist, 1973).

This study is an attempt to examine the embryonic responsiveness to serum macromolecules by measuring RNA synthesis at different stages of development. Two culture media were devised, one contained serum to stimulate embryonic cell metabolism and the other contained only bovine serum albumin (BSA) to serve as a control.

MATERIALS AND METHODS

Animals. Random-bred CFLP female mice (Anglia Laboratory Animals) were used. Mice were kept under standard animal house conditions and on a

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lighting schedule of 05.00 h to 19.00 h. They were mated with CFLP males. Occasionally, for confirmatory experiments, mice were superovulated before mating. An injection of 5 i.u. pregnant mares serum gonadotrophin followed 45 h later by 2·5 i.u. of human chorionic gonadotrophin was used to induce superovulation.

Culture medium. Two types of culture media were used, designated optimal and sub-optimal. Both media contained the constituents of Whittingham's medium (Whittingham, 1971) and were also supplemented with vitamins and amino acids as specified for Eagle's Minimum Essential Medium (Flow Laboratories). The medium used had a pH of 7·2–7·4 after equilibration with 5% CO_2 in air. Optimal media contained 10% heat-inactivated dialysed foetal calf serum. Dialysis was carried out at 2 °C for 3 days against isotonic saline which was stirred continuously and changed every 8 h. Sub-optimal medium contained 4 mg/ml BSA. Embryos were cultured in 30 μl of either optimal or sub-optimal medium containing 10 μCi [5,6-^{3}H]uridine (sp. act. 49 Ci/mmol: Radiochemical Centre, Amersham) in 100 μl medium. For each embryo stage, except the blastocyst stage, the media with and without the radioactive precursor contained a final concentration of 20 μM unlabelled uridine to saturate the endogenous UTP pool (Daentl & Epstein, 1971). Medium for incubating blastocysts contained a final concentration of 10 μM unlabelled uridine.

Collection of embryos and culture in radioactive media. 2-cell embryos were obtained on the morning of day 2 of pregnancy (day 1 = day of vaginal plug) and 4-cell embryos late in the evening on day 2 of pregnancy. 8-cell and early compacting embryos were obtained at 08.00 h and 12.00 h of day 3 respectively. Early and late blastocysts were obtained on the morning and evening of day 4 respectively. Mice were killed by cervical dislocation, the fallopian tubes or uterine horns were dissected out and flushed with prewarmed phosphate buffered saline (PBS) containing 10 mg ml^{-1} polyvinylpyrrolidine at 37 °C. Embryos were washed four times in sub-optimal medium at 37 °C and transferred to 50 μl of optimal or sub-optimal medium. Initial experiments were carried out on blastocysts. Group 1 blastocysts were incubated in optimal medium overnight, groups of 12 embryos were removed at 0, 7, 15 and 23 h and incubated in optimal medium containing radioactive precursor. Group 2 blastocysts were incubated in sub-optimal medium overnight, 12 embryos were removed at 0, 1, 2, 3 and 23 h and transferred to optimal medium containing radioactive precursor. Group 3 embryos were incubated in sub-optimal medium overnight and groups of 12 blastocysts were removed at 0, 1, 2, 3 and 23 h and transferred to sub-optimal medium containing radioactive precursor. Cultures were maintained under light liquid paraffin at 37 °C in an atmosphere of 5% CO_2 in air. Embryos from other experiments were left for 3 h in the unlabelled optimal or sub-optimal media before transfer to 30 μl of medium containing the radioactive precursor. Incubation of embryos with [5,6-^{3}H]-uridine was carried out for 1 h.

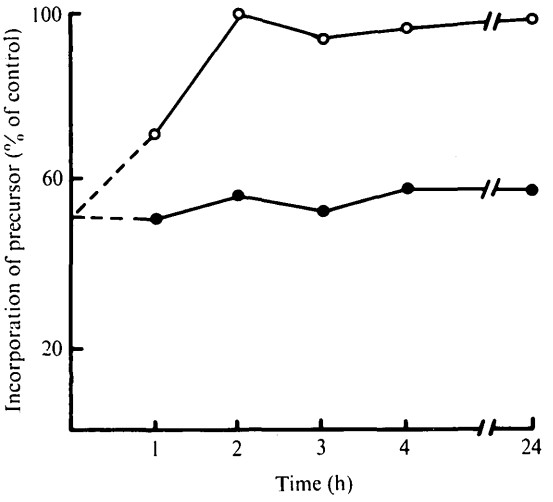

Fig. 1. Incorporation of [5,6-^{3}H]uridine into RNA of blastocysts from Group 2 (○—○) and Group 3 (●—●). Results are expressed as a percentage of the control values (Group 1).

Determination of radioactivity. After 1 h incubation, embryos were removed from the radioactive medium and washed six times in PBS which contained 20 μM unlabelled uridine. After washing, the embryos were transferred to 100 μl solubilizing buffer consisting of 0·14 M 2-mercaptoethanol and 0·1 % sodium dodecyl sulphate in 0·01 M sodium phosphate buffer. Samples were heated at 65 °C for 1 h in a water bath, 10 μl aliquots of the samples were removed, added to glass fibre discs (GF/C 2·5 cm, Whatman) and dried in air. For precipitation of RNA, 30 ml ice-cold 10 % trichloracetic acid (TCA) was run slowly through the discs, under reduced pressure, followed by an equal volume of ethanol. The discs were ether dried and added to scintillation vials. As a control the precipitate on the discs were incubated at 37 °C with 0·5 N-NaOH for 1 h. To determine total uptake of precursor the air-dried discs were added to scintillation vials without treatment with TCA and ethanol. Acid-soluble values were obtained by subtracting the acid-insoluble from the total uptake. 10 ml Scintillation fluid containing 5·5 g Packard-Permablend 111/1 litre of toluene was added to the vials. All samples were prepared in duplicates. The vials were left overnight in the dark and counted in a Tracerlab Scintillation Spectrophotometer. The efficiency of counting was calculated at 38 %.

RESULTS

Radioactivity incorporated into acid-precipitable material was completely solubilized by dilute alkali hydrolysis, indicating that labelled uridine was incorporated into RNA. The results do not indicate true rates of RNA synthesis since the value of the intracellular pool of uridine and its metabolites is un-

Table 1. *Uptake of radioactive uridine into preimplantation mouse embryos incubated in optimal and sub-optimal media*

Embryo stages	No. of experiments (no. of embryos)	[^{3}H]uridine (p-mole/embryo/h × 10^4 ± S.E.M.) Optimal (So)	[^{3}H]uridine (p-mole/embryo/h × 10^4 ± S.E.M.) Sub-optimal (Ssb)	[^{3}H]uridine (p-mole/embryo/h × 10^4 ± S.E.M.) Optimal (Io)	[^{3}H]uridine (p-mole/embryo/h × 10^4 ± S.E.M.) Sub-optimal (Isb)	Difference in the incorporation of [^{3}H]uridine between optimal and sub-optimal media (%)
2-cell	4 (72)	36·5 ± 11	27·8 ± 8	10·3 ± 4	8·8 ± 1	15
4-cell	4 (102)	103·8 ± 4	100·3 ± 19	18·3 ± 4	17·0 ± 4	8
8-cell	6 (170)	503·8 ± 13	357·7 ± 9	44·2 ± 5	33·2 ± 5	25*
Early compaction	7 (154)	619·6 ± 17	444·9 ± 23	49·9 ± 4	36·0 ± 4	25**
Early blastocyst	5 (118)	1358·4 ± 63	926·4 ± 34	661·4 ± 17	341·0 ± 10	49***
Late blastocyst	9 (192)	1712·7 ± 181	951·8 ± 146	671·8 ± 10	336·0 ± 9	50***

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}0005$ (Student's t-test).

known. However, saturation of the endogenous UTP pool with cold uridine allows for a direct comparison of labelled uridine incorporated into RNA (Daentl & Epstein, 1971). The values for RNA synthesis, as determined by the incorporation of [5,6-^{3}H]uridine, in blastocysts from the 8, 16 and 24 h incubations of Group 1 were constant at approximately 0·064 p-mole/embryo/h. This constant value was used as a control for Group 2 and 3. RNA synthesis in blastocysts obtained from sub-optimal media of Group 2 was stimulated to optimal levels resembling values found in Group 1 within 2 h. After the initial increase up to 2 h, a constant high rate of RNA synthesis was maintained (Fig. 1). RNA synthesis in blastocysts from Group 3 was maintained at a constant low level at about half the value obtained for the control blastocysts in Group 1 (Fig. 1). This indicated the positive responsiveness of blastocysts to stimulatory macromolecules in the serum. After a period of 3 h incubation in sub-optimal medium, RNA synthesis reached a basal level. This time period gave reproducible results for blastocysts and other stages of development and was therefor used in all subsequent experiments.

Experiments were performed on 2, 4, 8-cell and early compaction embryos, early and late blastocysts. There was no detectable difference in RNA synthesis by 2- and 4-cell embryos whether they were cultured in optimal or sub-optimal media. Even when the incubation period in the optimal medium was increased to 8 h RNA synthesis was similar to that obtained after 3–4 h incubation in the medium.

Table 1 indicates the amount of radioactivity incorporated into the acid-soluble pool (S) and RNA (I) of the embryo at each developmental stage. The data

Table 2. *Ratios of incorporation into RNA and the acid-soluble pool between optimal and sub-optimal media*

Embryo stage	Io/Isb	So/Ssb	$\%R\left(\frac{Io.Ssb}{Isb.So}\times 100\right)$
2-cell	1·07	1·39	85·75
4-cell	1·10	1·12	105·25
8-cell	1·41	1·41‡	98·50
Early compaction	1·42	1·40	101·57
Early blastocyst	1·94***	1·47	132·60***
Late blastocyst	2·01†††	1·90*††	108·78*

* $P < 0{\cdot}005$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.
† Comparison between early compaction and late blastocyst. †† $P < 0{\cdot}05$. ††† $P < 0{\cdot}001$.
‡ $0{\cdot}05 < P < 0{\cdot}1$.
Values obtained from the Student's *t*-test using the 2P integral.

shows an increase in RNA synthesis at the 8-cell stage, with a further and more marked increase at the blastocyst stage. From these values the % rate of RNA synthesis (R) was calculated as shown (Table 2). These values are calculated assuming that the specific activity of the pool of the precursor is constant throughout the cycle and the mechanisms of uptake are not saturated. The values show that the increase in RNA synthesis between 4- and 8-cell stages are most likely due to increasing pool sizes. Therefore there is no significant change in R where the incorporation values are corrected for alterations in pool size. There is no significant increase in the comparative pool sizes between early compacting embryos and early blastocysts when they are incubated in optimal and sub-optimal media, but there is a highly significant increase in the incorporation of radioactivity into RNA. This is reflected in a significant increase in R between these stages. There is a marked increase in the acid-soluble pool between early blastocyst and late blastocyst without a substantial difference in incorporation. Thus when corrected for pool size the value for R indicates a significant decline. There is also a substantial response in the incorporation values between early compaction and late blastocyst, but due to the increased pool size there is no significant increase in R between these stages.

DISCUSSION

The incorporation values of [5,6-^{3}H]uridine into RNA in sub-optimal medium agree with previously published data (Daentl & Epstein, 1971) when medium containing only BSA was used. The increase in the uptake of the radioactive precursor at the 8-cell stage, in the presence of serum, occurs at the time of major changes in the morphology and organization of the embryo (Hastings & Enders, 1975; Ducibella, Ukena, Karnovsky & Anderson, 1977), when all major classes

of RNA have been detected (Woodland & Graham, 1969; Piko, 1970; Warner & Hearn, 1977). The significant increase in RNA synthesis (R) observed in optimal medium at the early blastocyst stage may be associated with the functional changes in membrane transport systems and other cell-surface properties (Borland & Tasca, 1974; Pinsker & Mintz, 1973). The decline in R which is observed between early and late blastocyst stage is due to an increase in the acid-soluble pool. This value may not however reflect the true rate of RNA synthesis in the late blastocyst partly because the increase in values for acid-soluble pool would occur by virtue of an influx of the precursor in the blastocoelic cavity.

These results therefore indicate that preimplantation mouse embryos respond to environmental factors and, in this instance, to serum. Such factors mediate their effects on uptake of precursor and/or macromolecular synthesis, predominantly at the later preimplantation stages of embryonic development and not at earlier stages. Blastocysts have been shown to be stimulated by serum in previous studies (see Review by McLaren, 1973). This increase in responsiveness of embryos to their environment may be an important feature in the control of blastocyst metabolism at implantation.

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