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Isolation of Inner Cell Masses from Mouse Blastocysts by Immunosurgery or Exposure to the Calcium Ionophore A23187

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Abstract

Two techniques have been evaluated for their use in routinely isolating inner cell masses from mouse blastocysts by destroying the trophectoderm. The most efficient method of immunosurgery was a 15-min incubation in a 1:50 dilution of rabbit anti-mouse spleen antiserum followed by a 30–60-min incubation in guinea pig complement (1:10). Alternatively, inner cell masses were isolated by incubating blastocysts in 10^{-5}M calcium ionophore A23187 in medium devoid of calcium and magnesium ions.

Inner cell masses re-exposed to immunosurgery or the ionophore were less susceptible to lysis than the trophectoderm had been. The presence of the zona pellucida reduced trophectoderm lysis by immunosurgery in antiserum dilutions greater than 1:100, but had no effect when in the presence of ionophore.

Inner cell masses were consistently isolated from expanded blastocysts which had been collected 78 h after ovulation and cultured *in vitro* for 24 h before exposure to ionophore or immunosurgery, whereas blastocysts which had developed for the full 102 h *in vivo* were frequently unaffected.

Introduction

On the fourth day of development, the cells of the developing mouse embryo segregate into two morphologically distinct groups. One is a population of small, round inner cell mass (ICM) cells and the other one of larger, flat trophectoderm cells. The properties of these two cell groups have been investigated after they had been separated by microsurgery (Gardner and Johnson 1972; Rossant 1975*a*, 1975*b*, 1976; Van Blerkom *et al.* 1976; Surani and Barton 1977).

Recently, methods other than microsurgery have been described which permit the simultaneous isolation of ICMs from many blastocysts. The technique of immunosurgery (Solter and Knowles 1975; Handyside and Barton 1977) involves the selective lysis of the trophectoderm layer of the blastocyst by complement-dependent antibody cytotoxicity. The presence of tight junctions between the outer trophectoderm cells (Calarco and Brown 1969; Ducibella *et al.* 1975; McLaren and Smith 1977) is thought to prevent the antiserum from gaining access to the ICM which is thereby protected from lysis. It has also been recently shown that when blastocysts are incubated in the calcium ionophore A23187, the trophectoderm lyses leaving the ICM intact (Surani *et al.* 1978). It is possible that these alternative methods to microsurgery, which is usually performed on fully expanded blastocysts, could be used to isolate the interior blastomeres from early blastocysts or morulae.

The present work was undertaken to compare the methods of immunosurgery and exposure to calcium ionophore, and to assess their efficiency in routinely isolating ICMs from mouse blastocysts. A study was also made on the stage of development at which inside cells or ICMs could first be isolated.

Materials and Methods

Embryos

Mice of the F_1 hybrid cross (C57BL × CBA, or reciprocal) were exposed to light from 0730 to 2130 h. Virgin females, 4–8 weeks old, were superovulated by intraperitoneal injection of 5 or 10 i.u. of pregnant mare serum gonadoptrophin (Folligon, Organon Laboratories) given at 1500–1700 h, followed 46–50 h later with the same dose of human chorionic gonadoptrophin (Chorulon, Organon Laboratories). After the second injection, the females were placed with fertile males of a similar F_1 hybrid type and checked the following morning for copulation plugs (= day 1 of pregnancy).

The medium used for collection and culture of embryos was that of Whitten (WM_1) prepared as described by Hoppe and Pitts (1973).

To determine when inside cells or ICMs could first be isolated, embryos were flushed from the reproductive tract at specific times after ovulation (taken to be 0300 h on day 1 of pregnancy). Embryos from each mouse were kept separated, those collected at 78 and 84 h after ovulation being further subdivided into groups of morulae and blastocysts.

Embryos used in the evaluation of the techniques of immunosurgery and exposure to calcium ionophore were flushed from the uterus between 1000 and 1200 h on the fourth day of pregnancy. They were pooled, washed twice, then cultured in 10 μl of medium in microtest plates (Falcon, No. 3034) under 10 ml of paraffin oil (Fisher Scientific Company) for 24 h at 37°C under a humidified atmosphere of 5% CO_2:5% O_2:90% N_2. The paraffin oil was equilibrated with protein-free culture medium by bubbling with the gas mixture for 15 min and then clarifying by centrifugation (Hoppe and Pitts 1973).

Prior to the cytotoxicity tests, the zona pellucida was removed from most embryos by incubating them for 5–10 min in 0·5% pronase (B grade, Calbiochem) prepared in protein-free medium containing 1 mg/ml dialysed polyvinylpyrrolidine. The pronase medium was allowed to self-digest for 1 h at 37°C before use. Zona-free embryos were washed twice and allowed to recover for 10 min in fresh medium.

Antisera and Complement

Rabbit anti-mouse spleen serum was raised by three intravenous injections each of 2×10^8 mouse spleen cells in 0·5 ml saline given 3–6 days apart. The rabbit was bled 7 days after the third injection. Control serum was collected prior to the first injection. Mouse immunoglobulin was precipitated from serum with 40% ammonium sulfate. Rabbit antiserum to the mouse immunoglobulin was raised by intracutaneous injection at eight sites on the back given three times at fortnightly intervals, 100 μg of protein in 0·5 ml Freund's complete adjuvant being used on each occasion (Lynch and Shirley 1975). The rabbit was bled 8–10 days after the third injection. Rabbit anti-mouse spermatozoa serum was prepared in a similar manner as described for the rabbit anti-mouse immunoglobulin serum, using 50 million freshly collected washed epididymal spermatozoa in 0·5 ml adjuvant for each injection.

All sera were heated at 56°C for 30 min to inactivate rabbit complement. Dilutions of antisera were prepared in WM_1 medium buffered with 4 mM phosphate (Quinn and Wales 1973) and stored in 1-ml aliquots at −70°C.

The immunoglobulin fraction of each antiserum was prepared by ammonium sulphate precipitation (Heide and Schwick 1973) and its protein content estimated (Lowry *et al.* 1951).

Stock solutions of guinea pig complement (Commonwealth Serum Laboratories, Melbourne) were stored at −70°C in 50-μl aliquots. A 1:10 dilution in saline was prepared on each day of use.

Calcium Ionophore

Stock solutions of 10^{-2}, 10^{-3} and 10^{-4} M calcium ionophore A23187 (Lilly Industries) were prepared in dimethyl sulfoxide and stored in the dark at −20°C. Immediately before use, 10 μl of

the stock solutions were added to 1 ml of medium, giving final concentrations of 10^{-4}, 10^{-5} and 10^{-6} M. Control medium contained 1% dimethyl sulfoxide. Two types of media were used. One (WM_1) contained calcium and magnesium ions while the other was a modification of WM_1 omitting the divalent cations. The NaCl and KCl levels in the modified medium were adjusted to maintain the osmolarity and Na:K ratio at similar levels to those in WM_1 medium and the lactate concentration was maintained by increasing the sodium lactate level.

Cytotoxicity Tests

In all tests, 10 μl of solution were covered by 10 ml of paraffin oil in microtest plates which were incubated at 37°C under an atmosphere of 5% CO_2:5% O_2:90% N_2. Three different tests were used:

(1) Zona-free blastocysts were incubated in antiserum for 15–30 min, washed in phosphate-buffered WM_1, then transferred to complement for 30–60 min.
(2) Blastocysts with and without the zona pellucida were incubated simultaneously in antiserum and complement for 3 h.
(3) Blastocysts with and without the zona pellucida were exposed to ionophore for 30 min in the dark.

Embryos were examined and photographed using a Leitz Diavert microscope with phase optics. The extent of lysis was determined visually. To confirm these results, some embryos were treated with 0·4% Trypan blue in saline and the extent of dye exclusion examined. Inner cell masses were isolated by pipetting up and down in a narrow-bore siliconized pipette.

Results

Cytotoxicity Test 1

When zona-free blastocysts were incubated in anti-spleen serum for 15–30 min, washed, then transferred to complement for 30–60 min, more than half had lysed trophectoderm in dilutions of antiserum up to 1:10,000 (Fig. 1). Lysis of the ICM

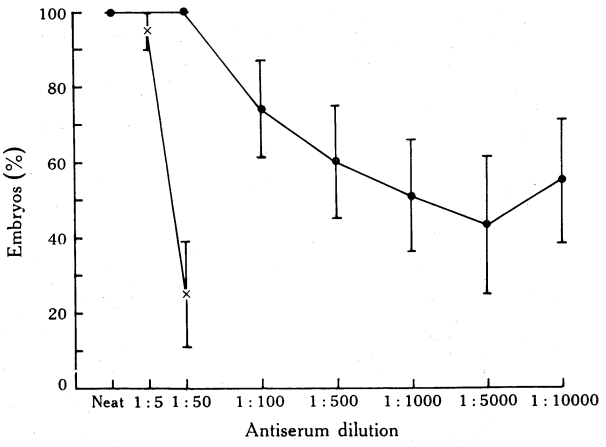

Fig. 1. Percentage of zona-free blastocysts with lysed trophectoderm and of isolated ICMs which lysed after incubation in anti-spleen serum followed by complement. Points represent means with standard error bars based on 4–7 replicates with 5 blastocysts or ICMs per replicate. ● Whole blastocysts with lysed trophectoderm. × ICMs which lysed after re-exposure to antiserum and complement.

as well as trophectoderm was never observed in this test. If, however, isolated ICMs were re-exposed to antiserum then complement, most lysed if a 1:5 antiserum dilution was used. One-quarter of the ICMs lysed when the antiserum dilution was 1:50, the

dilution at which all blastocysts had lysed trophectoderm. No cell lysis occurred when blastocysts were exposed to complement, antiserum nor control serum alone, nor in dilutions of control serum followed by complement.

Antisera (1:5 dilutions) raised against spleen cells or spermatozoa also lysed trophectoderm of all blastocysts (Table 1). Antiserum raised against mouse immunoglobulin was only 70% as effective as the other two antisera in causing lysis of trophectoderm. When isolated ICMs were re-exposed to the different antisera followed by complement, most lysed in anti-spleen, about half lysed in anti-spermatozoa and approximately one-tenth in anti-immunoglobulin antiserum.

Table 1. Percentage of naked blastocysts with lysed trophectoderm and of isolated ICMs which lysed after incubation in three types of antiserum (1:5) followed by complement (1:10)

Values given are means ± standard errors based on 3–6 replicates with 5–20 blastocysts or ICMs per replicate. The total number of embryos used is given in parentheses

	Antiserum		
	Anti-spleen	Anti-spermatozoa	Anti-Ig
Blastocysts with lysed trophectoderm (%)	100±0 (48)	100±0 (43)	70±19 (46)
Lysed ICMs after re-exposure (%)	95±5 (42)	58±14 (25)	11±11 (27)
Ig concentration (mg/ml of neat antiserum)	18·4	25·9	5·4

An estimate of the immunoglobulin concentration in each of the antiserum preparations was made and the results are also shown in Table 1. The anti-spermatozoa antiserum had one-third more protein than the anti-spleen antiserum, although it was only half as active in lysing ICMs. Furthermore, the anti-immunoglobulin antiserum had one-third less protein than the anti-spleen antiserum, although it was only 70% as effective as anti-spleen antiserum in lysing trophectoderm, and only 10% as effective in lysing ICMs.

Cytotoxicity Test 2

Lysis of blastocysts exposed simultaneously to antiserum and complement is shown in Fig. 2. The 50% end-point dilution for lysis of trophectoderm from naked blastocysts was 1:2000. In dilutions greater than 1:20, less than 10% of the blastocysts had lysed ICM. Even in a 1:10 dilution, less than 30% of the blastocysts had ICM lysed. No cell lysis occurred when blastocysts were exposed to control serum and complement simultaneously for 3 h.

The presence of the zona pellucida reduced the immune lysis of trophectoderm cells, especially in antiserum dilutions greater than 1:80. It completely prevented the lysis of ICMs in dilutions greater than 1:10.

Cytotoxicity Test 3

Table 2 shows the effects of incubating blastocysts or ICMs in the calcium ionophore. No lysis occurred in control medium with or without calcium and magnesium ions or in 10^{-6}M ionophore. In a 10^{-5}M ionophore solution, more than

90% of blastocysts had lysed trophectoderm in the absence of calcium and magnesium but only half of the blastocysts had lysed trophectoderm in the presence of the divalent cations. In neither case were ICMs lysed. Even when isolated ICMs were

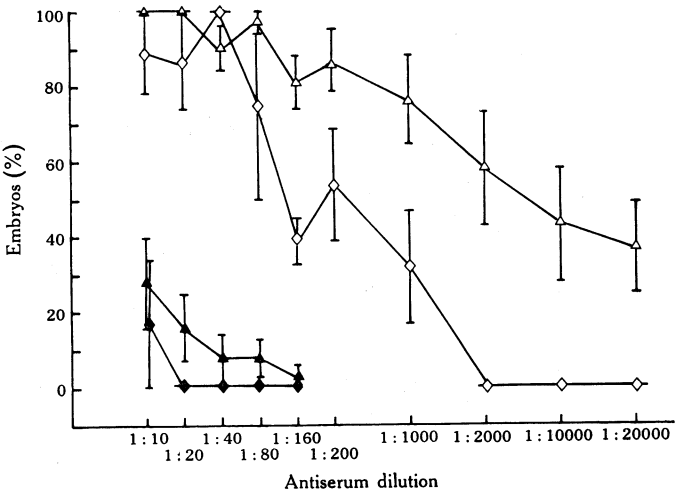

Fig. 2. Percentage of blastocysts with trophectoderm lysed and ICM intact after incubation in antiserum and complement simultaneously for 3 h. The insert shows those with both trophectoderm and ICM lysed. Points represent means with standard error bars based on 6–8 replicates with 5–10 blastocysts per replicate. △ Zona pellucida absent, trophectoderm lysed. ▲ Zona pellucida absent, trophectoderm and ICM lysed. ◊ Zona pellucida present, trophectoderm lysed. ◆ Zona pellucida present, trophectoderm and ICM lysed.

Table 2. Number (out of 10) of blastocysts and ICMs which lysed after a 30-min incubation in the calcium ionophore A23187

Values are means ± standard errors based on three replicates with 10 embryos per replicate. T, trophectoderm; n.e., not examined

Medium	Zona pellucida	Embryos	Ionophore concentration (M) 0	10^{-6}	10^{-5}	10^{-4}
Ca^{2+}, Mg^{2+} present	Absent	Blastocysts with T lysed	0·0	0·0	5·7	10·0
	Absent	Blastocysts with T + ICM lysed	0·0	0·0	0·0	1·0
Ca^{2+}, Mg^{2+} absent	Absent	Blastocysts with T lysed	0·0	0·0	9·3	10·0
	Absent	Blastocysts with T + ICM lysed	0·0	0·0	0·0	6·0
	Absent	ICMs lysed on re-exposure	n.e.	n.e.	0·0	10·0
	Present	Blastocysts with T lysed	n.e.	n.e.	10·0	n.e.
	Present	Blastocysts with T + ICM lysed	n.e.	n.e.	0·0	n.e.

re-exposed to 10^{-5}M ionophore, they were not lysed (Fig. 3). The zona pellucida had no effect on the extent of trophectoderm lysis.

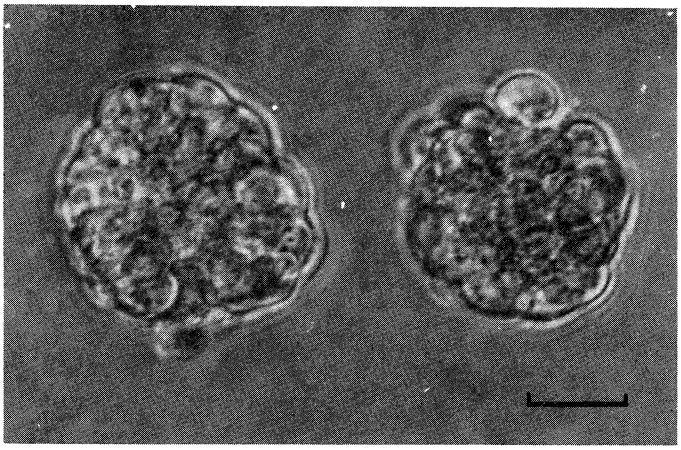

Fig. 3. Inner cell masses isolated after incubation of blastocysts in calcium ionophore (10^{-5}M) in the absence of Ca^{2+} and Mg^{2+} for 30 min. They were then returned to 10^{-5}M ionophore. Bar = 20 μm.

When a 10^{-4}M ionophore solution was used, all blastocysts had lysed trophectoderm in medium with or without calcium and magnesium ions. However, ICMs were also lysed, more so in the absence of the divalent cations. Any ICMs surviving exposure to 10^{-4}M ionophore were re-exposed to this concentration after being freed from lysed trophectoderm and were subsequently lysed.

Stage of Development

Embryos collected at various times post-ovulation were exposed to immunosurgery (cytotoxicity test 1 using a 1:50 dilution of anti-spleen antiserum) or to the calcium ionophore (10^{-5}M in medium without Ca^{2+} and Mg^{2+} for 30 min) after the zona pellucida was removed. The percentage of ICMs obtained is shown in Fig. 4. At 54 and 60 h post-ovulation (5 cells to compacted 8 cells), embryos exposed to immunosurgery or the ionophore showed superficial swelling of the blastomeres but were not totally lysed. After immunosurgery, inside cells were isolated from a few 78-h morulae, while ICMs were obtained from 60% of 78-h blastocysts. One-third of morulae and 5% of blastocysts were totally lysed. No inside cells or ICMs could be isolated after exposing 78-h post-ovulatory morulae or blastocysts to the ionophore. One-third of morulae and half of the blastocysts showed some swelling and disaggregation of their outer blastomeres, while the remainder appeared unaffected.

Inside cells were isolated from one-fifth of 84-h morulae after immunosurgery or exposure to ionophore. Morulae were not totally lysed using either technique. A few blastocysts, however, were totally lysed after immunosurgery. Inner cell masses were isolated from 70% of blastocysts after immunosurgery but only from 10% of blastocysts after incubation in ionophore.

By 102 h post-ovulation, few embryos were flushed from the uterus, as attachment had begun. Those that were collected had hatched and were elongate with developing ectoplacental cones. Inner cell masses were isolated in 10% of blastocysts after immunosurgery and 30% of blastocysts after exposure to calcium ionophore. The other blastocysts showed little evidence of lysis. When blastocysts were collected 78 h post-ovulation and cultured for 24 h, all yielded ICMs using either immunosurgery or exposure to ionophore.

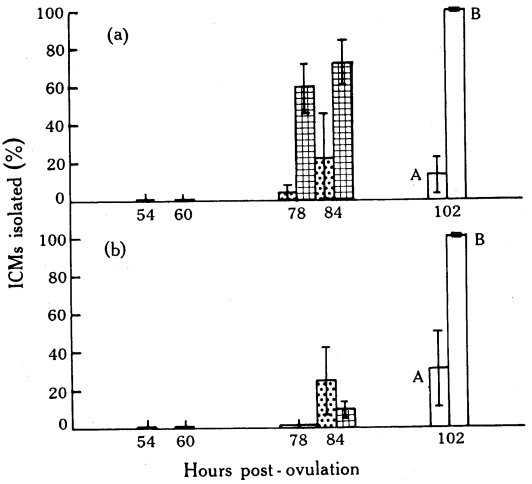

Fig. 4. Isolation of ICMs from preimplantation mouse embryos using (*a*) immunosurgery and (*b*) exposure to the calcium ionophore. Points represent means with standard error bars based on 3–8 mice with 5–40 embryos per mouse. *A*, embryos developed for 102 h *in vivo*. *B*, embryos developed for 78 h *in vivo* then 24 h *in vitro*. Stippled hatching, morulae; square hatching, blastocysts.

Discussion

Inner cell masses may be isolated from mouse blastocysts by destroying the trophectoderm using immunosurgery or exposure to the calcium ionophore A23187. The most efficient method of immunosurgery found in the present study was a 15-min incubation in a 1:50 dilution of rabbit anti-mouse spleen antiserum, followed by a 30–60-min incubation in guinea-pig complement (1:10). There was no relationship between the extent of cell lysis caused by the three antisera tested and the concentration of immunoglobulin in each of the antisera.

A more rapid method for obtaining ICMs is to expose the blastocysts to 10^{-5}M calcium ionophore in medium without calcium and magnesium ions for 30 min. Surani *et al.* (1978) claimed that the response of embryos to the ionophore was similar in the presence or absence of the divalent cations. However, in the present study, in which lower ionophore concentration was used, cell lysis occurred more rapidly in the absence of calcium and magnesium ions. The ionophore acts by transporting divalent cations across membranes (Reed and Lardy 1972; Babcock *et al.* 1976) resulting in an influx of calcium ions into the cytosol which normally maintains low calcium levels (approximately 10^{-3}mM; Rasmussen 1970). Extra-cytosolic calcium comes from the medium (1·7 mM), the blastocoele (6–9 mM; Borland *et al.* 1977) and

mitochondria (approximately 1 mM; Rasmussen 1970). The alteration in ionic balance would lead to the diffusion of water into the cell, swelling and subsequent lysis, as occurs in complement-dependent antibody cytotoxicity. When the divalent cations are absent from the medium, calcium still flows into the cytosol from the blastocoel and mitochondria. Furthermore, as the tight junctions between blastomeres are dependent on calcium in the medium (Wales 1970; Ducibella and Anderson 1975), they would be disrupted in its absence leading to decompaction. The ICMs would then be exposed to the ionophore more rapidly than in the presence of calcium and undergo more extensive lysis as was observed.

Surani *et al.* (1978), using 2×10^{-5}M ionophore in the presence of calcium and magnesium, observed that ICMs re-exposed to ionophore were partly destroyed. However, in the present study, ICMs remained intact if 10^{-5}M ionophore without divalent cations in the medium was used. Inner cell masses re-exposed to immunosurgery are also less susceptible to lysis than the trophectoderm. A similar finding was reported by Moskalewski and Koprowski (1972). Solter and Knowles (1975), using blastocysts approximately 24 h younger than those in the present study, claimed that most ICMs were totally lysed when transferred back to antiserum and complement. Such a difference in results may occur if the synthesis of antigens cross-reacting with anti-spleen antiserum is turned off when the ICM differentiates as suggested by Searle and Jenkinson (1978) for the synthesis of antigens to ectoplacental–cone-defined products.

It was observed that the presence of the zona pellucida reduced trophectoderm lysis by immunosurgery in antiserum dilutions greater than 1:100, but had no effect when using the ionophore. This is most likely due to the difference in molecular weights of the antiserum (approx. 10^5) and the ionophore (mol. wt. 523).

Inner cell masses were consistently isolated from expanded blastocysts which had been collected 78 h after ovulation and cultured for 24 h. In contrast to the work of McLaren and Smith (1977) and Magnuson *et al.* (1978), embryos developing entirely *in vivo* often remained unaffected after immunosurgery. The ionophore also lysed few embryos which had developed *in vivo*. Culture of blastocysts *in vitro* for 24 h may, therefore, render the embryos more susceptible to osmotic stresses than the *in vivo* environment. Embryos from hybrid mice used in the present study may also be more resistant to osmotic stresses than those from random-bred mice used by other workers. A more thorough knowledge of the mechanism of action of the two techniques under study and the reasons for the reduced lysis of the ICM and embryos developing *in vivo* would lead to a further understanding of when and how the ICM of the blastocyst differentiates giving it different properties to the trophectoderm.

Experiments are in progress to examine the further development *in vitro* and *in vivo* of ICMs isolated by immunosurgery or exposure to the calcium ionophore.

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References

Babcock, D. F., First, N. L., and Lardy, M. A. (1976). Action of ionophore A23187 at the cellular level. *J. Biol. Chem.* **251**, 3881–6.

Borland, R. M., Biggers, J. D., and Lechene, C. P. (1977). Studies on the composition and formation of mouse blastocoele fluid using electron probe microanalysis. *Dev. Biol.* **55**, 1–8.

Calarco, P. G., and Brown, E. H. (1969). An ultrastructural and cytological study of preimplantation development of the mouse. *J. Exp. Zool.* **171**, 253–84.

Ducibella, T., and Anderson, E. (1975). Cell shape and membrane changes in the 8-cell mouse embryo: prerequisites for morphogenesis of the blastocyst. *Dev. Biol.* **47**, 45–58.

Ducibella, T., Albertini, D. F., Anderson, E., and Biggers, J. D. (1975). The preimplantation mammalian embryo: characterization of intercellular junctions and their appearance during development. *Dev. Biol.* **45**, 231–50.

Gardner, R. L., and Johnson, M. H. (1972). An investigation of inner cell mass and trophoblast tissues following their isolation from the mouse blastocyst. *J. Embryol. Exp. Morph.* **28**, 279–312.

Handyside, A. H., and Barton, S. C. (1977). Evaluation of the technique of immunosurgery for the isolation of inner cell masses from mouse blastocysts. *J. Embryol. Exp. Morph.* **37**, 217–26.

Heide, K., and Schwick, M. G. (1973). Salt fractionation of immunoglobulins. In 'Handbook of Experimental Immunology'. (Ed. D. M. Weir.) pp. 6.1–6.11 (Blackwell Scientific Publications: Oxford.)

Hoppe, P. C., and Pitts, S. (1973). Fertilization *in vitro* and development of mouse ova. *Biol. Reprod.* **8**, 420–6.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265–75.

Lynch, S. S., and Shirley, A. (1975). Production of specific antisera to follicle-stimulating hormone and other hormones. *J. Endocrinol.* **65**, 127–32.

McLaren, A., and Smith, R. (1977). Functional test of tight junctions in the mouse blastocyst. *Nature* (*London*) **267**, 351–3.

Magnuson, T., Jacobson, J. B., and Stackpole, C. W. (1978). Relationship between intercellular permeability and junction organization in the preimplantation mouse embryo. *Dev. Biol.* **67**, 214–24.

Moskalewski, S., and Koprowski, H. (1972). Presence of egg antigen in immature oocytes and preimplantation embryos. *Nature* (*London*) **237**, 167–8.

Quinn, P., and Wales, R. G. (1973). Growth and metabolism of preimplantation mouse embryos cultured in phosphate-buffered medium. *J. Reprod. Fertil.* **35**, 289–300.

Rasmussen, H. (1970). Cell communication, calcium ion, and cyclic adenosine monophosphate. *Science* **170**, 404–11.

Reed, P. W., and Lardy, M. A. (1972). A23187: A divalent cation ionophore. *J. Biol. Chem.* **247**, 6970–7.

Rossant, J. (1975*a*). Investigation of the determinative state of the mouse inner cell mass. I. Aggregation of isolated inner cell masses with morulae. *J. Embryol. Exp. Morph.* **33**, 979–90.

Rossant, J. (1975*b*). Investigation of the determinative state of the mouse inner cell mass. II. The fate of isolated inner cell masses transferred to the oviduct. *J. Embryol. Exp. Morph.* **33**, 991–1001.

Rossant, J. (1976). Investigation of inner cell mass determination by aggregation of isolated rat inner cell masses with mouse morulae. *J. Embryol. Exp. Morph.* **36**, 163–74.

Searle, R. F., and Jenkinson, E. J. (1978). Localization of trophoblast-defined surface antigens during early mouse embryogenesis. *J. Embryol. Exp. Morph.* **43**, 147–56.

Solter, D., and Knowles, B. B. (1975). Immunosurgery of mouse blastocyst. *Proc. Nat. Acad. Sci. U.S.A.* **72**, 5099–102.

Surani, M.A.H., and Barton, S. C. (1977). Trophoblastic vesicles of preimplantation blastocysts can enter into quiescence in the absence of inner cell mass. *J. Embryol. Exp. Morph.* **39**, 273–7.

Surani, M. A. H., Torchiana, D., and Barton, S. C. (1978). Isolation and development of the inner cell mass after exposure of mouse embryos to calcium ionophore A23187. *J. Embryol. Exp. Morph.* **45**, 237–47.

Van Blerkom, J., Barton, S. C., and Johnson, M. H. (1976). Molecular differentiation in the preimplantation mouse embryo. *Nature* (*London*) **259**, 319–21.

Wales, R. G. (1970). Effects of ions on the development of the preimplantation mouse embryo *in vitro*. *Aust. J. Biol. Sci.* **23**, 421–9.

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