PERMEABILITY OF THE MOUSE ZONA PELLUCIDA TO IMMUNOGLOBULIN

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During the preimplantation stages of pregnancy, the mammalian embryo is surrounded by an acellular envelope, the zona pellucida. Although little is known of the functions of this membrane it has been suggested that it may be impermeable to antibody molecules (immunoglobulins), thereby protecting the embryo from the potentially adverse effects of maternal immunity (Simmons & Russell, 1966, 1967). In the present study, we have investigated the permeability of the mouse zona pellucida to immunoglobulins by comparing the complement-mediated cytotoxic effect of rabbit anti-mouse serum and two of its immunoglobulin components, IgG and IgM, on the development of zona-free and zona-enclosed embryos *in vitro*.

Mouse embryos at the 8-cell stage were recovered in phosphate-buffered saline (PBS) from the oviducts of randomly bred hybrid mice 48 hr after the finding of a vaginal plug. Half of the embryos were left with the zona pellucida intact, and the other half were transferred to 0.5% pronase in PBS and incubated at 37°C until partial digestion of the zona occurred, when removal was completed by mechanical disruption with a glass micropipette in pronase-free PBS. After a short recovery period in culture medium (RPMI+10% fetal calf serum), groups of embryos, zona-free and zona-enclosed, were preincubated for 1 hr at 37°C in various dilutions of either whole rabbit anti-mouse serum or its IgG- and IgM-containing fractions obtained by ammonium sulphate precipitation followed by ion-exchange chromatography on DEAE cellulose DE 23. Following exposure to serum, embryos were washed twice in PBS and transferred either to culture medium containing 5% detoxified guinea-pig serum as a source of complement (Cohen & Schlesinger, 1970) or to culture medium alone. Control cultures in medium + complement but without preincubation in antiserum were also carried out. To determine the time taken for antibody to cross the zona pellucida, a second series of experiments was performed in a similar manner except that preincubation in antiserum was varied from 1 to 30 min.

All cultures were carried out in 0.1-ml volumes in Cooke Microtitre plates under 5% CO₂ in air, and were observed for 48 to 72 hr for evidence of cytotoxic effect or development to morula and blastocyst stages.

The results are summarized in Tables 1 and 2. Over the effective range of antiserum dilutions (1/10 to 1/1500), 8-cell mouse embryos treated with antiserum and complement underwent destruction, without further development,

Table 1. The effect of the zona pellucida on the survival of 8-cell mouse embryos exposed to rabbit anti-mouse serum and serum fractions

<i>Treatment</i>	<i>Presence of zona</i>	<i>No. of embryos treated</i>	<i>No. developing to morula</i>	<i>No. developing to blastocyst</i>
Antiserum 1/10 to 1/500	+	19	19	14
	—	14	14	12
Complement	+	15	15	11
	—	36	35	33
Serum 1/10 to 1/500	+	59	0	0
+ complement	—	50	0	0
Serum 1/1000 to 1/1500	+	18	2	1
+ complement	—	15	1	1
Serum 1/2000	+	20	20	15
+ complement	—	19	17	13
IgG fraction 1/10*	+	8	8	6
	—	5	5	3
IgG fraction 1/10	+	19	7	0
+ complement	—	11	0	0
IgG fraction 1/50	+	14	11	4
+ complement	—	4	1	0
IgM fraction 1/10	+	12	12	10
	—	13	13	11
IgM fraction 1/10	+	14	0	0
+ complement	—	10	0	0
IgM fraction 1/50	+	5	2	1
+ complement	—	5	0	0
IgM fraction 1/100 to 1/1000	+	12	11	11
+ complement	—	9	8	6

* Fractions were made up in PBS at serum concentration and then diluted in culture medium in the same way as whole serum.

irrespective of the presence of the zona pellucida. Similarly, zona-free and zona-enclosed embryos exposed either to the IgG- or IgM-containing fraction and complement were also destroyed. Control cultures of embryos incubated in serum or serum fractions alone, or in complement alone, survived and developed normally to the blastocyst stage, indicating that the effect observed in experi-

Table 2. The effect of duration of exposure to rabbit anti-mouse serum on the survival of zona-free and zona-enclosed 8-cell mouse embryos

<i>Preincubation time (min)</i>	<i>Presence of zona</i>	<i>No. of embryos treated</i>	<i>No. developing to morula or beyond</i>
1 to 5	+	26	26
	—	24	5
10	+	10	0
	—	5	0
15 to 30	+	19	3
	—	14	0

mental cultures was not due to non-specific toxicity of the antisera or complement used.

Exposure of zona-free embryos to antiserum for as little as 1 min allowed binding of sufficient antibody to the surface of the embryonic cells to cause lysis on subsequent transfer to complement (Table 2). Zona-enclosed embryos needed exposure to antiserum for 10 min for a cytotoxic effect to be observed.

The results clearly indicate that heterologous immunoglobulin and the effective components of complement can pass across the zona pellucida of the 8-cell mouse embryo and that the passage of effective concentrations takes place in as little as 10 min. The finding that zona-enclosed embryos were destroyed, not only by the smallest of the immunoglobulins (IgG, mol. wt 150,000), but also by the largest (IgM, mol. wt 900,000), further suggests that molecules of considerable size can penetrate the zona. In a recent study of the effect of oestrogen on the susceptibility of mouse blastocysts to xenoantisera, it has been shown that the zona at this later stage is similarly unable to exert a protective effect (Hakansson, 1973).

Several reports exist on the permeability of the zona pellucida to substances of different molecular weight. Austin & Lovelock (1958) reported that dyes of mol. wt 1200 could pass through the zona of the rabbit, rat and hamster embryo, whilst heparin (mol. wt 16,000) could not. On this basis, immunoglobulins (mol. wt range 150,000 to 900,000) might be expected not to pass through the zona, a view consistent with the findings of Heyner, Brinster & Palm (1969) and James (1969) that zona-enclosed embryos were less susceptible to specific allo-antibody *in vitro* than those with the zona removed. Recently, however, electron microscope studies have shown that ferritin (mol. wt 480,000 to 1,000,000) can pass through the zona in the mouse, rat and rabbit (Hastings, Enders & Schlafke, 1972), and our own results further indicate that the mouse zona is permeable to high molecular weight immunoglobulins. A number of explanations exist for the apparent discrepancy between these findings. It is possible that the pronase treatment used to remove the zona in the studies of Heyner *et al.* (1967) may also have affected the cell surface of the embryos, rendering them more susceptible to complement-mediated destruction by antibody than untreated embryos. The use of rabbit anti-mouse serum in the present investigation has enabled us to examine directly the permeability of the zona to immunoglobulins. At the same time, since this serum probably recognizes a diversity of determinants on the embryonic cell surface, the possibility that the differential susceptibility of zona-free and zona-enclosed embryos is due to slight modification of antigen expression by pronase treatment has been avoided.

The ability of molecules to pass through the zona pellucida may depend not only on molecular weight but also on molecular configuration, degree of aggregation or the charge characteristics of the molecule in relation to those of the zona itself. Evidence in the rat and mouse suggests that the zona contains compounds, including sialic acid (Soupart & Noyes, 1964), that could give rise to a negative charge within the substance of the zona, and impede the entry of molecules also bearing a net negative charge.

In view of the similarity between immunoglobulins of different species,

the finding that heterologous IgG and IgM can pass through the zona of the mouse embryo makes it seem unlikely that homologous maternal immunoglobulin can be excluded by this acellular membrane. Other explanations must therefore be sought for the ability of the preimplantation embryo to avoid the potentially adverse effects of maternal humoral immunity, particularly in preimmunized females. It is possible that survival may be due to a lack or low level of paternal antigen expression on the cleavage stages or the failure of effective levels of immunoglobulin, or some of the components of complement, to gain access to the oviduct and uterus during these stages of development. Kirby (1969) has shown that homozygous allogeneic mouse blastocysts held for long periods in a state of delayed implantation in the uterus of ovariectomized, progesterone-maintained females are unharmed if the female is preimmunized to donor antigens. Since it is known that histocompatibility antigens are expressed by the mouse blastocyst (Searle, Johnson, Billington, Elson & Clutterbuck-Jackson, 1974), this survival might suggest that some component(s) of the antibody/complement-mediated lysis system is not present in the reproductive tract during this time.

The apparently high degree of permeability of the zona pellucida and the speed with which even high molecular weight substances can traverse it may have other implications for the relationship between the embryo and its environment. It seems likely that substances present in the fluids of the reproductive tract, including drugs, teratogenic agents and maternal products (e.g. uteroglobin-like substances) which may have a rôle in the control of development (Daniel, 1970; McLaren & Menke, 1971), will have access to the embryo during the entire preimplantation period. Conversely, the outward passage of materials such as metabolites and waste products should also be unimpeded. This may be an important consideration in view of the increasing level of metabolic activity of the embryo, and the possible rôle of the blastocyst in providing a chemical stimulus for the induction of the uterine decidual reaction (Bitton-Casimiri & Psychoyos, 1970), which is initiated before zona loss (McLaren, 1969).

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