THE REACTION OF THE MOUSE BLASTOCYST AND ITS ZONA PELLUCIDA TO pH CHANGES *IN VITRO*

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Once the implantation reaction has been initiated, the zona pellucida of the mouse undergoes rapid lysis. This occurs irrespective of whether the zona has been shed several days earlier, as in lactational delay of implantation (McLaren, 1967, 1968a), or whether it is in the process of being shed, as in the case of normal implantation, or whether it surrounds a passive vitellus, such as that of the unfertilized egg (McLaren, 1968b). The zona lysis is probably produced by the uterus rather than by the blastocyst.

Lysis of the zona pellucida of the mouse and rat has been reported to occur in solutions of pH 4.0 to 5.0 (Hall, 1935; Gwatkin, 1964). In contrast, a study of the pH tolerance of mouse eggs *in vitro* showed that the development of 2-cell stages was inhibited by a pH of 6.08, while optimal development occurred in the pH 6.40 to 7.21 (Brinster, 1965a).

These data make it unlikely that zona lysis could be brought about *in vivo* by any shift in uterine pH, which would allow the blastocyst to continue normal development. The possibility remains, however, that at the time of loss of the zona the blastocyst might be less sensitive to low pH, or the zona more sensitive, than earlier in development. To investigate this possibility, mouse blastocysts were maintained in culture during the period when hatching from the zona is normally observed, and the pH of the culture medium was varied.

The random bred Q strain of mice was used. Blastocysts were recovered (3½ days *post coitum*) from pregnant females by flushing the uterine horns with phosphate-buffered saline. Implantation *in vivo* would normally occur on the following day. A standard egg culture technique was used (Brinster, 1963); the control culture medium was Krebs-Ringer bicarbonate incorporating lactate and pyruvate (Brinster, 1965b). Media of different pH were prepared by interchanging NaHCO₃ and NaCl while the other components of the medium were kept constant. After equilibration in 5% CO₂ in air, the actual pH of the drops of medium was measured using a Beckman Zeromatic pH meter fitted with a dual-glass micro-electrode. The blastocysts were then transferred to the media and cultured for 48 hr at 36° C. The results are shown in Table 1.

At a pH below 6, development was inhibited and the blastocysts died within 24 hr. At a pH of 6.5 or higher, almost 100% of the blastocysts survived for 48 hr; 68%, 55% and 35% of the blastocysts hatched at a pH of 6.5, 7.2 and

7.6, respectively. The negative regression of percentage hatching on pH of the medium was significant at the 5% level. Lysis of the zona occurred in 45% of the eggs at pH 5.1. At pH 5.9 an intermediate stage was found, where the zonae had become swollen and slightly thinned, rather like the appearance in the first stage of pronase digestion (Mintz, 1962). At pH 6.5 to 7.6, where hatching had occurred, the shed zonae, in all cases, were still present in the culture medium 2 days after hatching.

As a test for elasticity, the zonae were gently squeezed with a pair of fine forceps and watched to see whether they would return to their original shape. At pH 5.9 most of the zonae remained squashed, but at higher pH levels they very quickly regained their original form.

TABLE 1

pH of the culture medium	No. of blastocysts			% of surviving blastocysts hatched	No. of zonae lysed
	Cultured	Surviving	Hatched		
5.1	20	0	0	—	9
5.9	22	0	0	—	0
6.5	19	19	13	68	0
7.2	22	22	12	55	0
7.6	24	23	8	35	0

It thus appears that the pH which lyses the zonae is lower than that at which development can occur, even at the later stages of egg development *in vitro* which might be expected to be less sensitive to pH change. It is, therefore, unlikely that pH is the chief factor responsible for zona lysis *in vivo*. There is a suggestion, however, that hatching may be more easily accomplished at pH 6.5 than at pH 7.6, although no effect on the zonae can be observed under the dissecting microscope.

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