THE EFFECTS OF pH ON CULTURE OF ONE-CELL RABBIT OVA TO BLASTOCYSTS IN BICARBONATE-BUFFERED MEDIUM

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Studies on the effects of pH on growth of preimplantation mammalian embryos have so far been limited to the mouse. Brinster (1965a) found that blastocysts developed in culture from two-cell mouse ova in media with pH values ranging from 5.87 to 7.78 with an apparent optimum of 6.82. He later found, however, that the optimum pH appeared to depend on the carbohydrate energy source present in the medium (Brinster, 1965b). These studies were carried out using bicarbonate-buffered media.

The work reported here extends the study of these problems to the rabbit, using a synthetic medium similar to that developed by Kane & Foote (1970) for culture of rabbit ova. One-cell rabbit ova were cultured in media of various bicarbonate concentrations. Two experiments were carried out.

Experiment 1 was a preliminary study in which bicarbonate concentrations were varied between 1.875 and 120 mM, corresponding to a pH range of 6.43 to 8.2. Experiment 2 was a detailed study of the range between 4.6 and 80 mM, corresponding to a pH range of 6.64 to 7.88.

All media had in common the following composition: 1.5% bovine serum albumin, 4.78 mM-KCl, 1.71 mM-CaCl₂.2H₂O, 1.19 mM-KH₂PO₄, 1.19 mM-MgSO₄.7H₂O, with penicillin G 100,000 i.u. and streptomycin sulphate, 50 mg/litre. This was supplemented with the amino acids, trace elements, vitamins and cofactors of Ham's F10 medium (Ham, 1963). In Exp. 1, the treatment with the lowest level of bicarbonate contained 131.2 mM-NaCl and 1.875 mM-NaHCO₃ and in all the other treatments of Exps 1 and 2, increased bicarbonate levels were obtained by the substitution of NaHCO₃ for NaCl on an equimolar basis. The embryos were cultured in 0.5-ml drops of medium under Merck liquid paraffin in Falcon plastic tissue culture dishes. Embryos were cultured at 37°C under a gas phase of approximately 5% CO₂ in air. The pH of the droplets in which ova were cultured was determined at the end of the culture period using a Radiometer blood micro-system pH meter. Spot checking of some droplets indicated that the pH did not change significantly during the culture period.

The method of collection and handling of ova was that described by Kane & Foote (1970) with minor modifications. Superovulated one-cell ova were recovered from New Zealand does 20 to 21 hr after the ovulatory injection of HCG. The ova were washed with three separate washes of 3 ml medium each

Table 1. Effect of bicarbonate concentration and pH on growth of blastocysts from one-cell rabbit ova *in vitro*

	Experiment 1							
	Bicarbonate (mM)	pH	1.875 6.43 ± 0.02*	3.75 6.69 ± 0.02	7.5 6.91 ± 0.02	15 7.31 ± 0.06	30 7.6 ± 0.04	60 7.91 ± 0.04
No. of ova tested	34		34	34	34	33	34	34
% Hatched blastocysts	0		0	0	44	52	59	41
								120 8.2 ± 0.00
	Experiment 2							
	Bicarbonate (mM)	pH	4.6 6.64 ± 0.01*	7.0 6.81 ± 0.02	10.5 6.95 ± 0.03	15.8 7.20 ± 0.04	23.8 7.33 ± 0.01	35.6 7.50 ± 0.03
No. of ova tested	64		64	61	61	61	60	67
% Hatched blastocysts	17		17	36	46	67	67	61
Mean blastocyst diameter (μm)	275 ± 37*		312 ± 16	280 ± 16	339 ± 15	332 ± 20	383 ± 22	375 ± 29
								53.4 7.73 ± 0.04
								80.0 7.88 ± 0.04
								63 43
								298 ± 22

* Mean value ± S.E.

time and were then randomly allocated as equally as possible to the treatments. Usually one drop was used per treatment for ova collected on one day. The numbers of ova per drop varied from six to twelve. The ova were examined over a period of 5 days at magnifications of $\times 40$ and $\times 100$. Blastocyst formation began after 3 days of culture. In this culture system, blastocysts expand and escape or 'hatch' from the zona pellucida (Kane, 1972). Recent work in this laboratory suggests that this hatching *in vitro* of rabbit blastocysts cultured from the one-cell stage is due to the absence of the mucin coat which is normally acquired by rabbit embryos during passage through the oviduct and uterus (Kane, 1974). The number of hatched blastocysts per drop was recorded on Day 5 of culture and, in Exp. 2, the diameter of the hatched blastocysts was also recorded. Hatching was used as the criterion because it was a definite end-point indicating a degree of blastocyst growth capable of pushing the blastocyst out through the zona. Failure of an expanded blastocyst to escape from the zona was very rare in these experiments.

TEXT-FIG. 1. Percentages of hatched blastocysts (○) and mean blastocyst diameters (●, $\pm$ S.E.M.) for rabbit embryos cultured from the one-cell stage in bicarbonate-buffered media of differing pH. (See details of Exp. 2 in text.)

Table 1 and Text-fig. 1 show the results of the bicarbonate and pH treatments. It may be noted that the pH values for Exp. 1 are, on average, almost 0.1 unit higher than the predicted values for a nominal 5% CO₂ in air gas phase. This may have been due to a lower content of CO₂ in the gas mixture used in this experiment.

Blastocyst formation from one-cell ova occurred between a pH of about 6.6 to 8.0 corresponding to a bicarbonate concentration range of about 4 to 100 mM. The numbers of hatched blastocysts formed in Exp. 2 were analysed by orthogonally partitioned χ^2 (Li, 1964) using regression polynomials (Fisher & Yates, 1938). There were highly significant ($P < 0.005$) linear and quadratic components indicating that the relationship of % blastocysts formed to log

($\overline{\text{HCO}_3}$) is best fitted by a parabola. The optimum pH, as judged by proportion of blastocysts formed, appeared to be about 7.3, corresponding to a bicarbonate concentration of about 22 mM, but there was little variation between pH 7.2 and 7.7. Blastocyst expansion, however, appeared to be greatest at pH 7.55 corresponding to a bicarbonate concentration of about 40 mM. It is possible that the processes of blastocyst formation and blastocyst expansion may have different optimum pH requirements.

These results indicate a lower optimum pH and bicarbonate concentration than data on oviducal and uterine fluid might indicate. Vishwakarma (1962) reported mean values for pH and bicarbonate concentration of 7.91 and 52.19 mM in rabbit oviducal fluid, and 7.86 and 50.10 mM in rabbit uterine fluid. Bishop (1957) reported a pH value of 7.75 in oestrous rabbit oviducal fluid. Hamner & Williams (1964), however, found a bicarbonate concentration of only 28.3 mM in oviducal fluid. It is difficult to compare the results of the experiments reported here with the work of Brinster (1965a, b) in the mouse since the pH effects in the mouse are confounded by interaction with the type and concentration of carbohydrate energy source in the medium. The medium used in these experiments did not contain any such energy sources.

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