EFFECT OF COPPER ON ISOLATED RABBIT BLASTOCYSTS

M. H. CROSS

*Laboratory of Reproductive Physiology, School of Veterinary Medicine,
University of Pennsylvania, Philadelphia, Pennsylvania 19104, U.S.A.*

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The addition of metallic copper to an IUD has been shown to enhance its contraceptive effectiveness (Zipper, Tatum, Pastene, Medel & Rivera, 1969). A change occurs in the intrauterine environment (Chang, Tatum & Kincl, 1970), which may interfere with the development of the blastocyst. Since copper may act directly on the free-lying blastocyst, its effect was studied on the electrical membrane properties of the isolated-perfused 6-day rabbit blastocyst.

Blastocysts (2 to 3 mm average diameter) were collected and perfused as previously described (Cross, 1971) in an environmental chamber (Cross & Brinster, 1970) designed to secure the embryo during manipulations and maintain constant temperature (37° C), humidity and gas phase (5% CO₂ in air). Electrical measurements, made by techniques described previously (Cross, 1971), included short-circuit current (SCC), transmural potential difference (p.d.) and conductance. Values of SCC were recorded continuously except when the external current was briefly opened to obtain values of p.d. Conductance was computed from the ratio of open circuit p.d. to SCC since the blastocoele wall behaves as a simple linear resistor (Cross, 1971). Blastocysts were perfused and bathed with the same solution (Krebs-Ringer bicarbonate containing 1 mg bovine serum albumin/ml) to eliminate concentration gradients or chemical forces. The p.d. across the blastocoele wall was reduced to zero by passing current from an external source. This removes electrical forces acting on ions. Under these short-circuit conditions, no electrochemical gradient exists, no net passive transfer of ions takes place, and the SCC is the resultant of the currents produced by all the net active ionic transport processes. After complete replacement of the blastocoele cavity with the bathing solution, the p.d. and SCC progressively increase, reaching steady values by 40 to 60 min. Once attained, the electrical parameters were stable ($\pm 5\%$ /hr) for 3 to 4 hr. Steady electrical parameters (mean $\pm$ S.E.) of twenty blastocysts maintained up to 4 hr were as follows: p.d. was -13.2 ± 0.6 mV, blastocoele cavity negative; SCC was 5.3 ± 0.2 μ A/cm²; conductance was 0.4 mmho/cm².

Text-figure 1 and Table 1 show the results of nine experiments in which serial additions of CuCl₂ were added to the bathing solution. Each serial addition was a 10- μ l aliquot of a stock solution of CuCl₂ prepared in Krebs-Ringer solution. Similar serial additions of Krebs-Ringer solution to twenty

TEXT-FIG. 1. Effects of CuCl_2 on short-circuit current (SCC), potential difference (p.d., blastocoele cavity negative with respect to outside solution) and conductance of the rabbit blastocoele wall. Serial additions of CuCl_2 were made to the outside solution. Arrows indicate time of addition of CuCl_2 to reach the final concentrations noted above the arrows. Each point represents mean $\pm$ S.E. of nine blastocysts obtained from nine rabbits.

blastocysts, used to determine steady electrical parameters, produced no significant changes in the electrical values. After a control period of 30 min (each embryo was its own control), when stable SCC and p.d. readings were obtained, the addition of CuCl_2 , 10^{-5} M final concentration, caused no significant change in any of the electrical parameters. When the concentration of copper in the outside solution was increased to 2.5×10^{-5} M, a reduction in the average p.d. (25%) and average SCC (12%) was seen after 30 min. During the sub-

Table 1. Changes in potential difference and short-circuit current across the blastocyst wall with serial additions of CuCl_2 to the outside bathing solution

Treatment	Time† (min)	p.d. (mV)	SCC ($\mu\text{A}/\text{cm}^2$)
Control‡		-12.5 ± 1.0	5.25 ± 0.45
10^{-5} M- CuCl_2	30	-12.2 ± 1.1	5.62 ± 0.59
2.5×10^{-5} M- CuCl_2	30	-9.4 ± 1.4	4.74 ± 0.67
5.0×10^{-5} M- CuCl_2	30	$-6.5 \pm 1.2^{**}$	$3.27 \pm 0.65^*$
7.5×10^{-5} M- CuCl_2	10	$-1.9 \pm 1.6^{***}$	$1.57 \pm 0.41^{***}$
7.5×10^{-5} M- CuCl_2	30	$-1.4 \pm 1.5^{***}$	4.07 ± 1.97

For abbreviations, see text. Mean values $\pm$ S.E. are given for nine blastocysts.

† Time after final concentration was reached.

‡ Average of three 10-min control periods before addition of CuCl_2 .

* $P < 0.02$ when compared with control.

** $P < 0.01$ when compared with control.

*** $P < 0.001$ when compared with control.

TEXT-FIG. 2. Effects of CuCl_2 on SCC, p.d. and conductance of the rabbit blastocoele wall. Arrow indicates time at which 10^{-4} M- CuCl_2 was added to the outside solution. Each point represents mean $\pm$ S.E. of nine blastocysts obtained from nine rabbits.

sequent CuCl_2 applications, a final concentration of 5×10^{-5} M further decreased p.d. (48%) and SCC (38%) after an additional 30 min; after a final concentration of 7.5×10^{-5} M was reached, the p.d. was greatly depressed (89%) within 10 min, a third of the p.d. values being positive. The absolute magnitude of the average SCC continued to decrease (71%) and then increased during the next 20 min to 77% of control values. The subsequent changes in p.d. and SCC caused a sixfold increase in membrane conductance.

Table 2. Changes in potential difference and short-circuit current across the blastocyst wall when CuCl_2 is added to outside bathing solution

Treatment	Time† (min)	p.d. (mV)	SCC ($\mu\text{A}/\text{cm}^2$)
Control‡		-14.5 ± 1.2	7.00 ± 0.43
10^{-4} M- CuCl_2	10	-12.9 ± 1.3	6.58 ± 0.50
10^{-4} M- CuCl_2	15	$-8.9 \pm 1.5^{**}$	4.94 ± 0.87
10^{-4} M- CuCl_2	20	$-4.7 \pm 1.3^{***}$	$3.78 \pm 1.00^{**}$
10^{-4} M- CuCl_2	25	$-2.0 \pm 1.4^{***}$	8.35 ± 1.50
10^{-4} M- CuCl_2	30	$+2.1 \pm 1.7^{***}$	9.05 ± 2.00
10^{-4} M- CuCl_2	35	$+2.4 \pm 1.2^{***}$	$11.55 \pm 1.40^{**}$

For abbreviations, see text. Mean values $\pm$ S.E. are given for nine blastocysts.

† Time after addition of 10^{-4} M- CuCl_2 .

‡ Average of three 10-min control periods before addition of CuCl_2 .

** $P < 0.01$ when compared with control.

*** $P < 0.001$ when compared with control.

Text-figure 2 and Table 2 show the results of nine experiments involving a second group of blastocysts. The effects of a single addition of CuCl_2 (10^{-4} M) on the electrical properties of the blastocoele wall were studied. The average p.d. continued to decrease during exposure to copper, with a reversal to positive values after 30 min. A biphasic response of SCC was observed, similar to that for 7.5×10^{-5} M- CuCl_2 . The absolute magnitude of SCC decreased (46%) after 20 min and then increased by 35 min to a value 1.7 times that of the control values. The resultant increase in membrane conductance was about tenfold. The effects seen in the two types of experiments performed (Text-figs 1 and 2) were not reversible when copper was removed from the outside solution. In addition, the majority of blastocysts exposed to 10^{-4} M- CuCl_2 began to collapse after their return to copper-free solution, while three of nine embryos were only slightly collapsed after exposure to the serial additions of copper.

We recently demonstrated, using the short-circuit preparation simultaneously with isotope flux, that the 6-day rabbit blastocyst actively transports Na^+ and Cl^- at about the same rate from the bathing solution to the blastocoele cavity (Cross, 1973). The same experiments strongly suggest that HCO_3^- is actively transported in the same direction. The present results do not indicate which of the active ionic transport processes copper affects, but concentrations of 2.5×10^{-5} and 5×10^{-5} M decrease SCC without greatly affecting conductance. This suggests an alteration of at least one of the active ionic transport processes at lower concentrations of copper.

The effects of copper at higher concentrations (7.5×10^{-5} and 10^{-4} M) appear to be more complex, resulting in a biphasic response. In the first phase, the active ionic transport processes across the blastocoele wall are changed (SCC decreases) as in the case for lower copper concentrations. In the second phase, SCC is stimulated and membrane conductance is greatly increased. A decrease in SCC could indicate an increase in the cation component of SCC, a decrease in anion transport, or a differential inhibition or stimulation of cation and anion transport. An increase in SCC would result from the reverse conditions. To identify which specific actively transported ions are affected by copper and to determine the nature of the effects on passive ionic permeability (conductance), simultaneous short-circuit current— ^{36}Cl isotope flux experiments, carried out under the same conditions as the present experiments, are needed. The collapse noted after exposure to higher CuCl_2 concentrations, and the large increase in membrane conductance, suggest that longer term effects under our experimental conditions would be toxic and would probably evoke conformational changes of membranes.

In other *in-vitro* epithelium preparations, addition of copper (10^{-5} to 10^{-4} M) has been shown to increase $^{36}\text{Cl}^-$ permeability but to decrease its net transport (Ferreira, 1970), to decrease $^{36}\text{Cl}^-$ influx without effect on SCC and $^{22}\text{Na}^+$ fluxes (Koefoed-Johnsen & Ussing, 1958) and to stimulate (10^{-5} to 10^{-4} M) active Na^+ transport (Ferreira, 1970; Brunton, 1972).

Since active ionic transport must be directly related to the accumulation of blastocoele fluid, factors altering these ionic transport processes could inhibit blastocyst expansion. The action of copper noted here may have some bearing on the effectiveness of the copper-containing IUD as a contraceptive agent.

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