CAPACITATION OF MOUSE SPERMATOZOA IN VITRO: INVOLVEMENT OF EPIDIDYMAL SECRETIONS AND CUMULUS OOPHORUS

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Summary. Capacitation of mouse spermatozoa *in vitro* is brought about by epididymal secretions released into the medium at the time of sperm collection. Inhibition by glucaro(1→4)lactone indicates that an essential component of these secretions is β -glucuronidase. In the absence of the secretions, capacitation can be induced by components of the cumulus oophorus.

Current concepts of fertilization in mammals indicate that capacitation occurs in the oviduct rather than in the uterus (Edwards, 1972; Zamboni, 1972). Experiments with hamster gametes *in vitro* have shown that the active component is not the secretion of the oviduct, but the cumulus oophorus (Gwatkin, Andersen & Hutchison, 1972; Gwatkin & Carter, 1974). Glycosidases supplied by these cells alter the sperm surface (Gwatkin & Andersen, 1973) so that it can bind to a trypsin-sensitive receptor in the zona pellucida (Hartmann & Gwatkin, 1971; Gwatkin, Williams, Hartmann & Kniazuk, 1973).

We now report experiments indicating that cumulus cells can also capacitate *in vitro* the spermatozoa of the laboratory mouse. To demonstrate this phenomenon, however, it is necessary to remove the epididymal secretions which are released into the medium at the time of sperm collection.

Spermatozoa were obtained by placing four pairs of epididymides in 0.5 ml medium 199M2 (Gwatkin & Andersen, 1973), contained within the cavity of a glass spot-test plate, and making several cuts in their caudae. The epididymides were then removed and the extruded secretions and spermatozoa were mixed and transferred to 2 ml medium 199M2. The final concentration was approximately 2×10^7 motile spermatozoa/ml. Aliquots (20 μ l) were added to a series of 20- μ l drops of medium 199M2 or medium 199M2 containing approximately twenty eggs surrounded by cumulus, under a layer of mineral oil. Eggs in cumulus were obtained from 8- to 9-week-old mice approximately 17 hr after ovulation had been induced by HCG. The cultures were incubated under an atmosphere of 5% CO₂ in air. At hourly intervals, the eggs in cumulus originally added were removed and twenty new cumulus-free eggs were introduced. Incubation was continued for another 90 min. The eggs were then mounted on slides and examined for sperm penetration, as described previously (Gwatkin *et al.*, 1972).

TEXT-FIG. 1. Proportion of mouse eggs penetrated by spermatozoa which were preincubated for various periods of time with medium 199M2 (●) and with medium 199M2 supplemented with cumulus oophorus (○). Each point represents the mean of sixty eggs.

Text-figure 1 shows the effect of preincubating the sperm suspension in medium 199M2 alone (lower curve) or with cumulus cells (upper curve) on the ability of the spermatozoa to enter eggs. Without preincubation, no eggs were penetrated. After 1 hr in medium 199M2, spermatozoa entered approximately 15% of the eggs. After 2 hr, this proportion increased to 45%. Incubation for a longer period reduced the proportion of eggs which were penetrated. Response to preincubation with cumulus cells followed a similar time-response curve, but a greater proportion of the eggs were penetrated, the maximum being 65%.

To determine whether capacitation of the spermatozoa was being produced by the epididymal secretions released into the medium during sperm collection, the secretions were removed by centrifugation at 150 *g* for 4 min and the spermatozoa were resuspended in medium 199M2. When 20- μ l aliquots were incubated for 2 hr with an equal volume of medium 199M2, the spermatozoa failed to penetrate eggs (Table 1) although they remained actively motile. When spermatozoa were incubated with cumulus cells, or resuspended in the original supernatant containing the epididymal secretions, penetration occurred. Capacitation was blocked when the specific β -glucuronidase inhibitor, glucaro-(1 $\rightarrow$ 4)lactone (Levy, 1952), was added at the beginning of preincubation

Table 1. Capacitation of mouse spermatozoa induced by cumulus oophorus and by epididymal secretions

Supplements	% Eggs penetrated by spermatozoa	
	Exp. 1	Exp. 2
None	0	0
Cumulus oophorus	40	50
Epididymal secretions	40	50
Epididymal secretions + cumulus	50	70
Epididymal secretions + GL,† added before capacitation	0	0
Epididymal secretions + GL, added after capacitation	60	60

* Ten eggs per treatment.

† GL, Glucaro(1→4)lactone, 25 µg/ml.

with the secretions. When the inhibitor was added at the end of preincubation, it had no effect.

These experiments demonstrate that capacitation of mouse spermatozoa requires approximately 2 hr to reach a maximum. They also show that capacitation is induced by the cumulus oophorus, but that this may be masked by the capacitation caused by the epididymal secretions, one essential component of which appears to be β -glucuronidase. The 2-hr period required for capacitation of mouse spermatozoa agrees with the results of Toyoda, Yokoyama & Hosi (1971), who capacitated mouse spermatozoa by incubating them in a modified Krebs–Ringer solution. These authors did not remove the epididymal secretions, however, and this led them to conclude that they had induced capacitation in a chemically defined medium when, in fact, their conditions were far from defined by the medium used. Epididymal secretions are known to contain high concentrations of glycosidases (Conchie & Mann, 1957) which have been implicated in the capacitation of hamster spermatozoa (Gwatkin & Andersen, 1973). Even in the presence of these secretions, preincubation with the cumulus oophorus increased the proportion of eggs which became penetrated. These results support the findings of Pavlok & McLaren (1972), who observed a beneficial effect of cumulus cells on the fertilization of mouse ova *in vitro* by a suspension of epididymal spermatozoa from which the epididymal secretions were not removed.

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