HEAT SENSITIVITY OF THE CORTICAL GRANULE PROTEASE FROM HAMSTER EGGS

R. B. L. GWATKIN AND D. T. WILLIAMS

*Merck Institute for Therapeutic Research,
Rahway, New Jersey 07065, U.S.A.*

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Summary. Before fertilization, the protease present within the cortical granules of the hamster egg is relatively stable to heat. Once discharged into the medium, however, it is extremely heat sensitive, undergoing inactivation even at 37°C. Thus, body temperature would be expected to prevent the zona reaction of one egg from rendering neighbouring eggs infertile.

Previous studies (Gwatkin, Williams, Hartmann & Kniazuk, 1973) showed that the zona reaction of the hamster ovum is produced by a cortical granule protease, which inactivates the species-specific receptor-for-spermatozoa located in the zona pellucida. This receptor was earlier shown to be sensitive to low concentrations of pancreatic trypsin (Hartmann & Gwatkin, 1971).

Several years ago Austin & Braden (1954) showed that exposure of the oviducts of oestrous rats to 45°C for 8 to 12 min, just before the expected time of fertilization, increased the proportion of polyspermic eggs. The cause of this loss in the efficiency of the zona reaction was not known. We now report that exposure of hamster ova to heat before fertilization does not increase polyspermy significantly until the temperature is raised to 40°C. At this temperature, cortical granule discharge is inhibited, resulting in massive polyspermy. In contrast to the relative stability of the enzyme confined within the cortical granules, the free protease is sensitive to very slight elevations of temperature, undergoing partial inactivation even at 37°C. This fact has important physiological implications.

Ova were collected from the oviducts of golden hamsters (*Mesocricetus auratus*) either 21 or 24 hr after the induction of ovulation with HCG, and their cumuli oophori were removed with hyaluronidase, as previously described (Gwatkin *et al.*, 1973). The eggs were placed in 20- μ l drops of medium 199M2 (Gwatkin & Andersen, 1973) on the bottom of plastic Petri dishes (60 mm in diam.) containing 10 ml mineral oil, previously equilibrated with Krebs–Ringer solution. The dishes were then submerged, up to the level of the oil, in water-baths adjusted to various temperatures between 37°C and 42°C. After 30 min, the eggs were removed, washed twice in 0.5-ml vols of medium 199M2 and exposed at 37°C to capacitated spermatozoa (Gwatkin, Andersen & Hutchison, 1972). Penetration of the eggs by the spermatozoa was recorded 90 min later. The presence of cortical granules in the penetrated eggs was

determined by oil-immersion phase-contrast microscopy. Table 1 shows that there was greater polyspermy in the older group of eggs, but that polyspermy did not increase further in either group until the temperature was raised to 40°C. It then became massive and was associated with an inhibition of cortical granule discharge.

Table 1. Induction of polyspermy in hamster eggs by heat exposure before fertilization

Temperature (°C)*	Eggs collected 21 hr after HCG		Eggs collected 24 hr after HCG	
	Mean no. of sperm./egg	% Eggs with cortical granules after sperm penetration	Mean no. of sperm./egg	% Eggs with cortical granules after sperm penetration
37	1.3	0	8.6	0
38	1.7	0	5.6	0
39	3.1	0	8.5	0
40	9.2	35	12.8	100
42	7.6	100	17.3	100

* Twenty eggs were exposed for 30 min at each temperature.

Heat sensitivity of the cortical granule protease was determined as follows. Zonae pellucidae were removed and 150 of the resulting naked vitelli were placed in each of a series of 10- μ l drops of medium 199M2 under mineral oil. An electrical square-wave pulse (150 V for 1.0 msec) was delivered across the vitelli with microelectrodes to cause discharge of the cortical granule contents into the medium (Gwatkin *et al.*, 1973). After incubation for 60 min at 37°C, the supernatants were pooled and 20- μ l aliquots were placed in 0.5-ml centrifuge

Table 2. Heat-instability of the material derived from the cortical granules of hamster eggs

Material	Conditions of incubation	% Eggs penetrated by sperm. after pretreatment with material*	
		Exp. 1	Exp. 2
Cortical granule contents	5 min at 37°C	0	0
	30 min at 37°C	15	20
	30 min at 40°C	65	50
	30 min at 43°C	100	—
Medium only	30 min at 43°C	100	100

* Twenty eggs per treatment.

tubes, which were closed with aluminium foil and incubated in water-baths adjusted to various temperatures between 37°C and 43°C. Drops (10 μ l) were then placed under equilibrated mineral oil and groups of twenty cumulus-free eggs were added to each of them. After incubation on a rocker (five to six oscillations per min) for 2 hr at 37°C, the eggs were removed, washed and exposed to capacitated spermatozoa. Table 2 shows that even after exposure

to 37°C for 30 min, there was partial inactivation of the free protease. Loss in activity was greater at 40°C and was complete at 43°C. Such sensitivity has also been observed with pancreatic trypsin (Anson & Mirsky, 1934).

Since Chen, Powell & Maze (1945) have reported that the body temperature of the golden hamster, determined rectally, is $38.7 \pm 0.3^\circ\text{C}$, the free cortical granule protease would be inactivated relatively rapidly by body heat. This would be advantageous for the reproductive process, since otherwise release of this protease from an egg, either by accident or by fertilization, might render neighbouring eggs infertile. On the other hand, the protease within the granules would be unaffected by normal body temperatures, possibly because it may exist as a zymogen. The polyspermy induced by heating hamster eggs to 40°C or above before exposure to spermatozoa can now be attributed to an interference with the mechanism of cortical granule discharge and is unrelated to the instability of the cortical granule protease.

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