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# The Effect of Inhibitors of Trypsin and Phospholipase A<sub>2</sub> on the Penetration of Zona Pellucida-free Hamster Eggs by Acrosome-reacted Hamster Sperm

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The involvement of a trypsin-like enzyme and a phospholipase A<sub>2</sub> in hamster sperm-egg fusion was investigated. Previously acrosome-reacted sperm and zona pellucida-free eggs were incubated in a low-K<sup>+</sup> medium in the presence of nontoxic levels of low molecular-weight synthetic inhibitors of these enzymes. The level of K<sup>+</sup> used in the fertilization droplet (0.6 mM) did not support further acrosome reactions and thus results were not due to inhibitor effects on the acrosome reaction. The operational definition of "sperm-egg fusion" used in the present work includes both attachment and fusion, since we could not distinguish between these events in these experiments. Sperm-egg fusion, as assayed by counting the number of decondensed sperm heads per egg, and the percentage of eggs penetrated were significantly reduced in the presence of the trypsin inhibitors benzamidine (1.2 mM) and p-nitrophenyl p'-guanidinobenzoate (55 μM) but not in the presence of the phospholipase A<sub>2</sub> inhibitors p-bromophenacylbromide (12 μM) or Upjohn Compound #1002 (100 μM). Preincubation of the sperm but not eggs in p-nitrophenyl-p'-guanidinobenzoate reduced subsequent fusion in the absence of inhibitor. Examination of semi-thin sections revealed that all sperm which penetrated eggs were decondensed. These results support a role for a sperm trypsin-like enzyme but not a sperm or egg phospholipase A<sub>2</sub> in hamster sperm-egg fusion.

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