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Acetylcholine Prevents Toxic Effects of Paraoxon on Mouse Sperm

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Environmental chemicals such as tricresyl phosphate (Chapin *et al.* 1988) and di(2-ethylhexyl)phthalate (Siddiqui and Srivastana 1992) may affect reproduction in mammals. We have previously shown that paraoxon inhibits *in vitro* fertilization of mouse gametes by affecting sperm not oocytes (Chou and Cook 1994). In this study we report evidence that paraoxon acts by inhibiting sperm capacitation and acetylcholine prevents the inhibition.

MATERIALS AND METHODS

Mouse sperm were collected from B6D2-F1 males 4-7 months of age (Chou and Cook). Sperm from each pair of excised cauda epididymides were collected in 0.3 ml of Brinster's medium for ovum culture-3 (BMOC-3) (Brinster 1971) or BMOC-3+ BSA (20 g/l) at 37°C and diluted with an equal volume of the same medium. Sperm were incubated 115 min. with or without test chemicals at 37°C, 100% humidity, and in an atmosphere maintained by adding 5% CO_2 to air. Aliquots of incubation media were examined at 0, 3, 10, 15, 35, 55, 75, 95, and 115 min. The chlorotetracycline (CTC) fluorescence assay was used to measure capacitation and the acrosome reaction (Ward and Storey 1984). Twenty μl of 500 μM CTC in 20 mM TRIS (pH 7.8), 130 mM NaCl and 5 mM cysteine and 20 μl of incubation media were mixed on a glass slide at 37°C, incubated 10 sec. and fixed with 40 μl of 12.5% glutaraldehyde in 1 mM TRIS (pH 7.8). After adding a cover slip, sperm were examined using a Nikon Biological Labophot Microscope equipped with epifluorescence optics, a Nikon B Excitation Unit (410-485 nm), and a 515W banier filter (515-545 nm). Sperm with uniform fluorescence over the head with or without a line of brighter fluorescence across the equatorial segment were recorded as fresh (Pattern F) sperm. Those with a dark band separating the fluorescent anterior portion and midpiece (Pattern C) were considered capacitated. Sperm with a florescent midpiece as the other two patterns but with

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faint fluorescence over the entire head region had undergone the acrosome reaction (Pattern A).

Paraoxon was purified by dissolving in glass distilled trichloroethylene followed by two washes with Na_2CO_3 (pH 9.8) and two washes with water. The solution was dried over amhydrous Na_2SO_4 and trichloroethylene was removed using a rotating vacuum evaporator.

RESULTS AND DISCUSSION

The progress of sperm capacitation and acrosome reaction is shown in Figure 1. Three fluorescent patterns similar to those described by Ward and Storey (1984) were observed. The fresh sperm (F) showed uniform fluorescence over the entire head and midpiece. Capacitated sperm (C) had a fluorescent midpiece and anterior portion of the head. There was minimal fluorescence in the post acrosomal region. Sperm that had undergone the acrosome reaction (A) had barely detectable fluorescence over the entire surface of the head but the midpiece was highly fluorescent as in F and C.

At zero time, pattern F constituted approximately 50% of the sperm population while patterns C and A were 30 and 20%, respectively. As F declined, pattern C increased, and as C declined, pattern A increased. The assay showed the transformation of fresh sperm to capacitated to acrosome reacted sperm (Fig. 1).

Sperm capacitation was prevented by adding paraoxon to the incubation media at a concentration of 50 μM (Fig. 2). The % pattern F and A were constant from 10 to 95 min. There was 10% conversion of F to A from zero to 3 min. At 115 min. there appeared to be a small conversion of pattern F to pattern A. This resembles the "false" acrosome reaction of degenerating sperm.

When the media contained 2% BSA (BMOC-3+) in the absence of paraoxon, the rate of disappearance of pattern F and appearance of pattern C dramatically increased (Fig. 3). After 55 min of incubation, an accelerated conversion of C to A (Fig. 3) occurred. Again, paraoxon at a concentration of 50 mM completely inhibited capacitation and acrosome reaction (Fig. 4).

Paraoxon added at 15 min of incubation instead of at zero time tended to delay capacitation, but once sperm were capacitated, the acrosome reaction was not affected (Fig. 5). Similar results were obtained when paraoxon was added after 10 min of incubation.

Since paraoxon at 10-13 nM blocks acetylcholinesterase in mammals

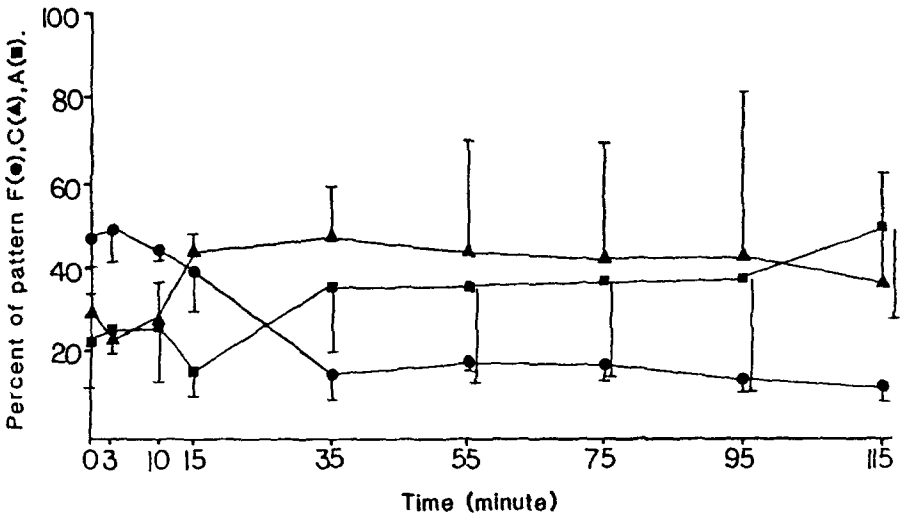

Figure 1. Time course of the changes in the percentage of CTC fluorescence patterns in mouse sperm incubated in BMOC-3. Each time point represents the mean ± SD of three separate experiments with an average of 100 sperm scored in each sample. Symbols represent the percentage of fresh sperm (●), capacitated sperm (■), or acrosome reacted sperm (▲).

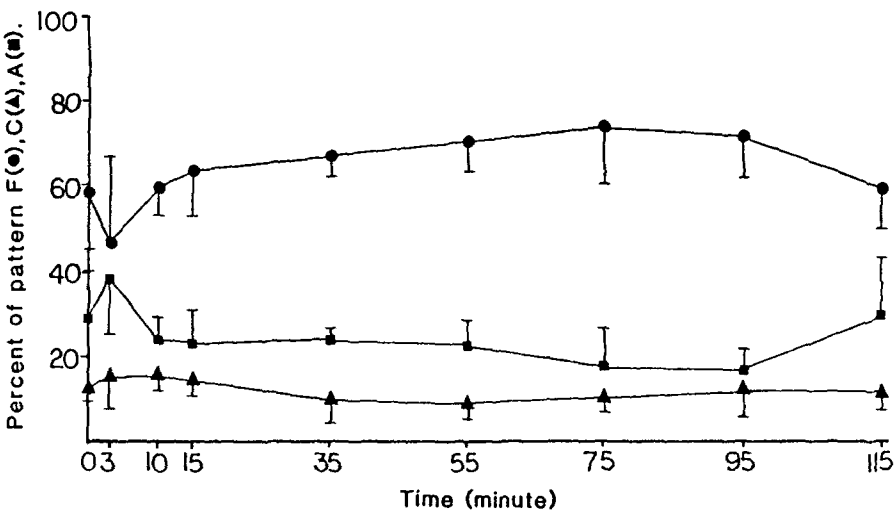

Figure 2. Time course of the changes in the percentage of CTC fluorescence patterns in mouse sperm incubated in BMOC-3 containing 50 mM paraoxon. Each time point represents the mean ± SD of three separate experiments with an average of 100 sperm scored in each sample. Symbols represent the percentage of fresh sperm (●), capacitated sperm (■), or acrosome reacted sperm (▲).

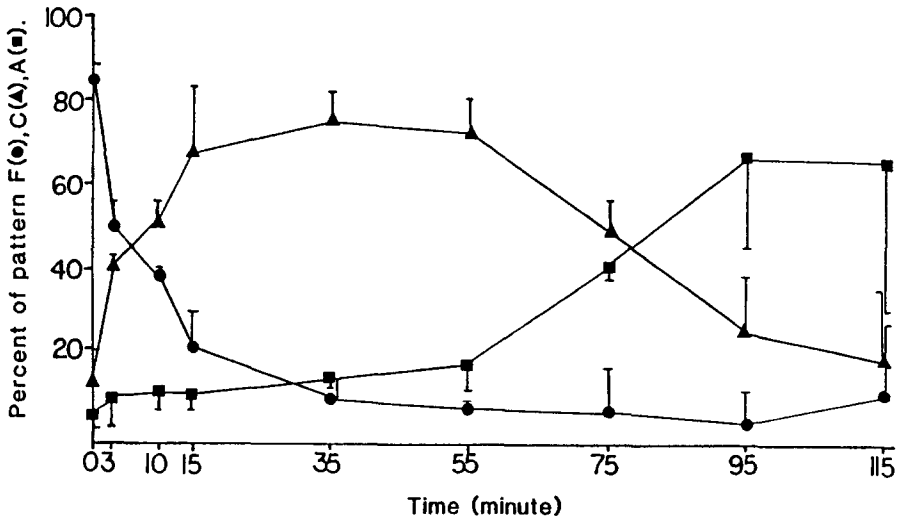

Figure 3. Time course of the changes in the percentage of CTC fluorescence patterns in mouse sperm incubated in BMOC-3+. Each time point represents the mean ± SD of three separate experiments with an average of 100 sperm scored in each sample. Symbols represent the percentage of fresh sperm (●), capacitated sperm (■), or acrosome reacted sperm (▲).

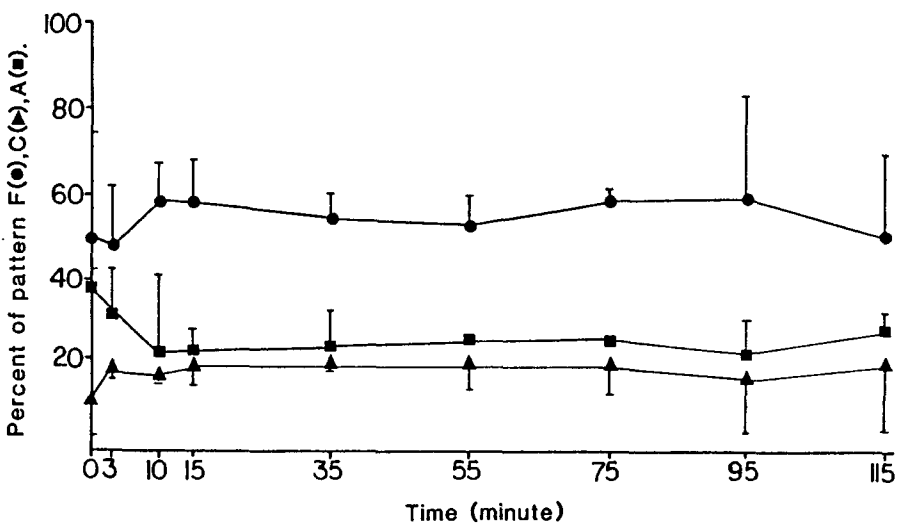

Figure 4. Time course of the changes in the percentage of CTC fluorescence patterns in mouse sperm incubated in BMOC-3+ containing 50 mM paraoxon. Each time point represents the mean ± SD of three separate experiments with an average of 100 sperm scored in each sample. Symbols represent the percentage of fresh sperm (●), capacitated sperm (■), or acrosome reacted sperm (▲).

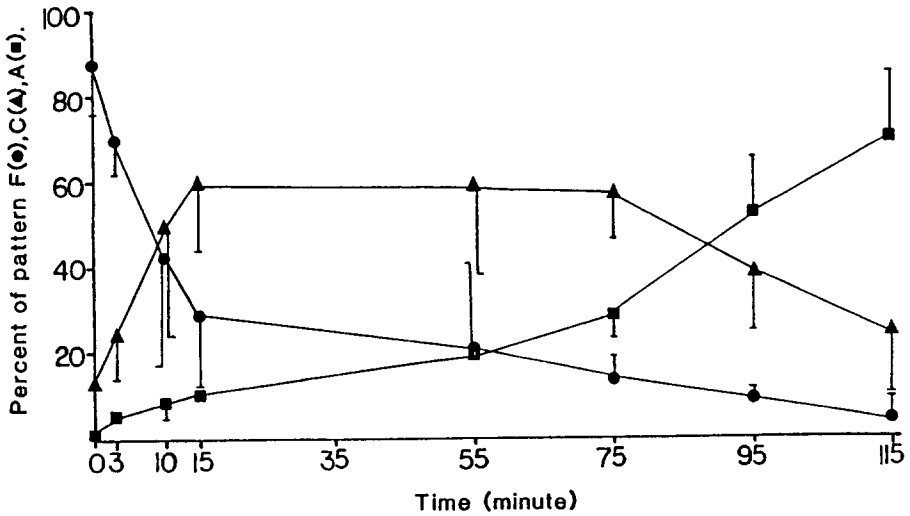

Figure 5. Time course of the changes in the percentage of CTC fluorescence patterns in mouse sperm when paraoxon was added 15 minutes after the beginning of incubation. Sperm were incubated in BMOC-3+. Each time point represents the mean ± SD of three separate experiments with an average of 100 sperm scored in each sample. Symbols represent the percentage of fresh sperm (●), capacitated sperm (■), or acrosome reacted sperm (▲).

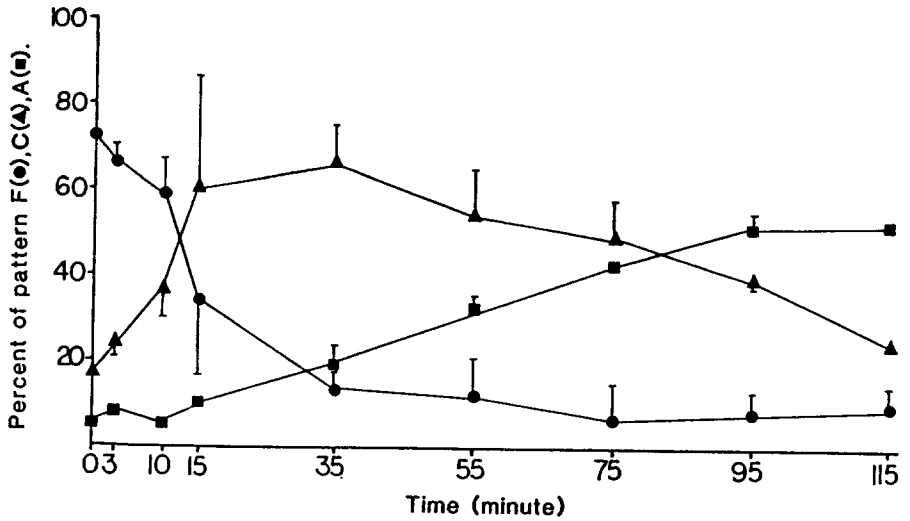

Figure 6. Time course of the changes in the percentage of CTC fluorescence patterns in mouse sperm incubated in BMOC-3+ containing Ach. Each time point represents the mean ± SD of three separate experiments with an average of 100 sperm scored in each sample. Symbols represent the percentage of fresh sperm (●), capacitated sperm (■), or acrosome reacted sperm (▲).

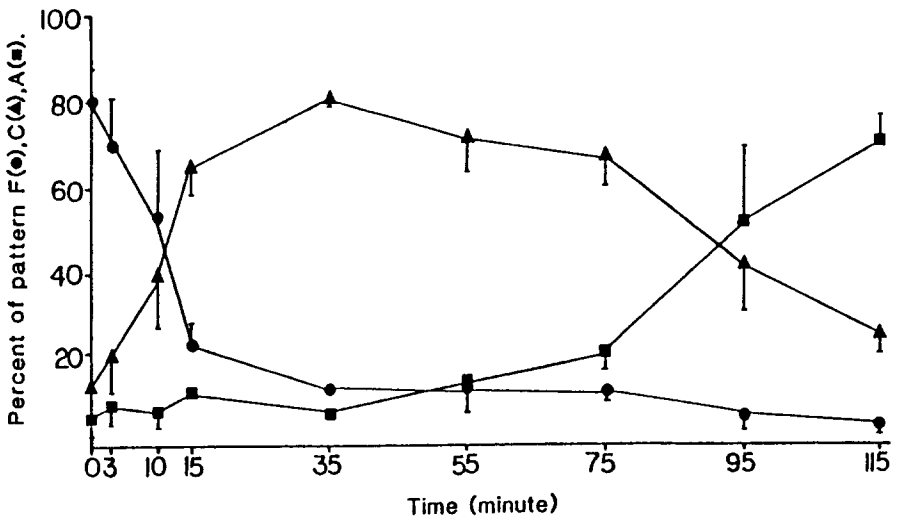

Figure 7. Time course of the changes in the percentage of CTC fluorescence patterns in mouse sperm incubated in BMOC-3+ containing Ach and 50 mM paraoxon. Each time point represents the mean ± SD of three separate experiments with an average of 100 sperm scored in each sample. Symbols represent the percentage of fresh sperm (●), capacitated sperm (■), or acrosome reacted sperm (▲).

(Johnson and Wallace 1987) and since sperm are known to possess elements of the cholinergic system (Robbins and Molenaar 1981; Bishop *et al.* 1976), the protective effect of acetylcholine was studied. Acetylcholine alone tended to delay the onset of capacitation and alter the timing of the acrosome reaction which tended to prolong the development of pattern A sperm (Fig. 6).

However, 50 μM acetylcholine completely prevented the inhibitory effects of paraoxon (Fig. 7 vs Fig. 4). The overall profile was not different from controls (Fig. 3). This work suggests that paraoxon may inhibit an esterase that exists on sperm in a stimulator - and inhibitor - sensitive form until removal of membrane surface components. If the esterase is activated by acetylcholine, capacitation is not inhibited by paraoxon.

The results indicate that acetylcholine prevents the inhibition of sperm capacitation by paraoxon. Understanding the mechanism of these reactions may lead to a better understanding of the effects of environmental chemicals on fertility in mammals.

REFERENCES

Bishop MR, Rawa-Sasty BV, Schmidt DE, Harbison RD (1976) Occurrence of choline acetyltransferase and acetylcholine and other

quaternary ammonium compounds in mammalian spermatozoa. Biochem Pharmacol 25:1617-1622

Brinster RL (1971) *In vitro* culture of the embryo. In: Mach HC (ed). *Pathways to Conception*. Charles B. Thomas Publishing Co, Springfield, IL

Chapin RE, George JD, Lamdio JC (1988) Reproductive toxicity of tricresyl phosphate in a continuous breeding protocol in Swiss (CD-1) mice. Fund Appl Toxicol 10:344-354

Chou K, Cook RM (1994) Paraoxon inhibits fertilization of mouse gametes *in vitro*. Bull Environ Contam Toxicol 53, 6 (In press)

Johnson JA, Wallace KB (1987) Species-related differences in the inhibition of brain acetylcholinesterase by paraoxon and malaoxon. Toxicol Appl Pharmacol 88:234-241

Robbins N, Molenaar PC (1981) Investigation of possible cholinergic mechanisms in fertilization of xenopus eggs. Proc R Soc Lond B 213:59-72

Siddiqui A, Srivastana SP (1992) Effect of di(2-ethylhexyl) phthalate administration on rat sperm count and on sperm metabolic enzymes. Bull Environ Contam Toxicol 48:115-119

Ward CR, Storey BT (1984) Determination of the time course of capacitation in mouse spermatozoa using a chlortetracycline fluorescence assay. Develop Biol 104:287-296