J. Reprod. Fert. (1977) **50**, 137–139

Lack of effect of α-chlorohydrin on the ATP content of rat, mouse and human spermatozoa

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α-Chlorohydrin (3-chloro-1,2-propanediol) has been shown to cause inhibition of glycolysis of spermatozoa *in vivo* and *in vitro* (Mohri, Suter, Brown-Woodman, White & Ridley, 1975; Suter, Brown-Woodman, Mohri & White, 1975; Homonnai, Paz, Sofer, Yedwab & Kraicer, 1975). The drug also leads to reduced sperm motility (Coppola, 1969; Samojlik & Chang, 1970; Kreider & Dutt, 1970; Johnson & Pursel, 1972; Brown & White, 1973; Vickery, Erickson & Bennett, 1974; Homonnai *et al.*, 1975). It has been suggested that the inhibition of glycolysis prevents normal ATP production, leading to insufficient energy supply for motility (Mohri *et al.*, 1975; Suter *et al.*, 1975; Homonnai *et al.*, 1975). We have therefore investigated, *in vivo* and *in vitro*, the effect of α-chlorohydrin on the ATP content of rat, mouse and human spermatozoa.

In-vivo experiments

Two groups of male albino rats (200–300 g) were kept in the same room and thereby exposed to the same environmental conditions. The experimental animals were given orally α-chlorohydrin (99% pure: Aldrich Chemical Co.) in propylene glycol (1,2-propanediol) at a dose of 5 mg/kg body weight/day for 7 days. The control group was given only the vehicle. The drug treatment was sufficient to reduce the fertility of the animals (Vickery *et al.*, 1974). After such treatment, the animals were anaesthetized with ether, the epididymides were removed, separated into caput and cauda portions and placed in Hank's balanced salt solution containing 4% bovine serum albumin (HBSS–4% BSA) at room temperature. The spermatozoa from the caput and the cauda epididymidis were extruded after puncturing the tubules with a fine hypodermic needle. The freshly extruded spermatozoa from the cauda epididymidis of the experimental rats showed vigorous motility only for 1–2 h while those of the control animals were motile for several hours. Glycolysis was measured by the use of [U-^{14}C]glucose and the [^{14}C]lactate formed was estimated after purification by the method of Brown & Garratt (1974). After washing with HBSS–4% BSA, the spermatozoa from the experimental animals (4) exhibited a lower mean (± S.E.M.) glycolytic activity (7·2 ± 0·8 nmol lactate/h/10^8 cells) than did those of the control rats (4) (10·2 ± 1·5 nmol lactate/h/10^8 cells). The reduced motility and glycolysis were taken to indicate that α-chlorohydrin had affected the spermatozoa.

Without any washing, freshly extruded spermatozoa were immediately acidified with 5% ice-cold

Table 1. The ATP content (pmol/10^6 cells) of epididymal spermatozoa of rats (one in each exp.) treated with propylene glycol (controls) or α-chlorohydrin (experimental animals)

	Control rats		Experimental rats	
Exp.	Caput	Cauda	Caput	Cauda
1	186	600	186	778
2	193	825	288	1280
3	193	756	185	425
4	299	1002	453	762
5	343	530	340	506
6	346	523	414	610
Mean ± S.D.	260 ± 78*	706 ± 190*	311 ± 113**	727 ± 304**

Significantly different (paired Student's *t* test): *$P < 0.01$, **$P < 0.02$.

trichloroacetic acid. Before assay for ATP, the acidic sperm extract was neutralized with NaOH and the ATP content was determined in triplicate by the luciferase technique of Stanley & Williams (1969). During epididymal maturation, the ATP content of rat spermatozoa was found to increase significantly (Table 1) as it does in maturing bovine spermatozoa (Hoskins, Munsterman & Hall, 1975). However, treatment with α-chlorohydrin did not significantly alter the ATP content of the rat spermatozoa (Table 1) thus contradicting the suggestion that α-chlorohydrin causes lack of the ATP essential for motility.

Because the epididymal spermatozoa were not washed before the acid extraction, contamination by the epididymal fluid and cytoplasmic droplets was expected in the control and experimental groups. It was not known if the fluid or the droplets contained any ATP. The fluid remaining after the removal of spermatozoa by centrifugation contained negligible amounts of ATP.

In-vitro experiments

When freshly extruded spermatozoa were incubated *in vitro* for 1 h, the ATP content of spermatozoa incubated with 0·6 M-, but not with 0·06 M-, α-chlorohydrin was consistently lower than that of the cells incubated with propylene glycol or the buffer alone (Table 2). Incubation with propylene

Table 2. The ATP content (pmol/10^6 cells) of spermatozoa incubated *in vitro* at 37°C for 1 h with buffer (HBSS–4% BSA), propylene glycol or α-chlorohydrin

Exp.	Source of sperm.	HBSS–4% BSA	Propylene glycol (0·6 M)	α-Chlorohydrin (0·6 M)
Rat†				
1	Caput	23	32	9
	Cauda	16	23	8
2	Caput	16	36	6
	Cauda	108	56	9
3	Caput	22	34	6
	Cauda	102	48	10
4	Caput	24	59	11
	Cauda	14	21	9
5	Caput	21	32	12
	Cauda	16	19	11
6	Caput	41	43	13
	Cauda	52	82	5
Mean ± S.D.	Caput	25 ± 9	39 ± 10	10 ± 3*
	Cauda	51 ± 44	42 ± 25	8 ± 2*
Mouse‡				
7	Caput	22	26	7
	Cauda	14	19	5
8	Caput	39	44	6
	Cauda	16	16	3
9	Caput	49	53	11
	Cauda	45	44	7
10	Caput	34	42	6
	Cauda	39	51	6
Mean ± S.D.	Caput	36 ± 11	41 ± 11	8 ± 2*
	Cauda	29 ± 16	33 ± 18	5 ± 2*
Man§				
11	Ejaculated	26	27	18
12	Ejaculated	26	25	15

* $P < 0·05$ when compared to the corresponding value obtained by incubation in propylene glycol.

† One rat was used in each experiment.

‡ Spermatozoa from 8–10 mice were pooled and used in each experiment.

§ One ejaculate sample was used in each experiment. Each sample was washed by centrifuging through 10% ficoll in 0·9% NaCl at room temperature.

glycol sometimes resulted in an increase of ATP content. The motility of the spermatozoa incubated for 1 h with 0·6 M-α-chlorohydrin was much less than that of the cells incubated in propylene glycol or buffer alone, irrespective of maturity or species.

The effective concentration of the drug was greater than the estimated physiological level of 0·01 mg/ml or 0·1 mM in the epididymis of rats treated with 10 mg α-chlorohydrin/kg (Jones & Jackson, 1974). Thus, the effect obtained *in vitro* was non-physiological and probably due to inhibition of some sperm enzymes essential for ATP regeneration, as shown for spermatozoa (Mohri *et al.*, 1975).

Steps beyond that of ATP synthesis should now be studied to understand the molecular action of α-chlorohydrin in inhibiting fertility of the male.

This work was supported by the World Health Organization. I. H. was a Rockefeller Foundation Postdoctoral Fellow from the Department of Biochemistry, Faculty of Medicine, University of Northern Sumatra, Medan, Indonesia.

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Received 22 October 1976