EFFECT OF α -CHLOROHYDRIN ON THE FERTILIZING ABILITY OF HAMSTER EPIDIDYMAL SPERMATOZOA

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Summary. Male hamsters of known fertility were injected subcutaneously with α -chlorohydrin to determine the minimal antifertility dose and to elucidate its site of action. Fertility tests at 4-day intervals showed that α -chlorohydrin (66 mg/kg) induced infertility within 4 days and fertilization did not occur after artificial insemination with spermatozoa from the cauda epididymidis of such animals. Comparable daily treatment produced the same antifertility effect subsequent to bilateral ligation of the corpus epididymidis and also in castrated animals given daily treatment (100 μ g/day) with either testosterone, 5 α -dihydrotestosterone or 3 α -androstenediol. The infertility was not related to deficiency of androgen, as judged by the concentration of fructose in the seminal vesicles, or to impaired ejaculation.

INTRODUCTION

Treatment with α -chlorohydrin (3-chloro-1,2 propanediol) will induce reversible sterility in the male rat (Coppola, 1969; Samojlik & Chang, 1970), guinea-pig (Ericsson & Baker, 1970), monkey (Kirton, Ericsson, Ray & Forbes, 1970), sheep (Kreider & Dutt, 1970) and pig (Johnson & Pursel, 1972). The precise mode of action of α -chlorohydrin is still uncertain but it is generally agreed that this compound induces sterility in males without interfering with spermatogenesis or disturbing mating behaviour. The prompt antifertility effect of the drug has been attributed to its influence on mature spermatozoa stored in the cauda epididymidis and ductus deferens (Turner, 1971). In a brief report, Ericsson & Norland (1970) stated that α -chlorohydrin induces infertility in male hamsters but the lack of precise information led to the present study to determine the minimal antifertility dose and probable site of action of the compound.

MATERIALS AND METHODS

Adult male and female hamsters (125 to 140 g) were maintained under controlled conditions of temperature and lighting. Before treatment of male hamsters of known fertility, α -chlorohydrin was diluted with 0.9% NaCl solution to the desired concentration and the appropriate daily dose (mg/kg

body wt) was injected subcutaneously in 0.1 ml/100 g body wt. Control animals received the same amount of 0.9% NaCl solution.

In the first experiment to determine the minimal antifertility dose of α -chlorohydrin, males were treated daily with 33, 49 or 66 mg α -chlorohydrin/kg and were paired with females in oestrus on the 4th day of treatment. Thereafter, only animals receiving the two lower doses of α -chlorohydrin were permitted to mate again at 4-day intervals because treatment with 66 mg/kg induced infertility within 4 days. Mating was confirmed by the presence of spermatozoa in the vagina and females were killed approximately 40 hr after mating. Eggs were flushed from the uterine tubes with 0.2 ml Hanks' solution and examined with a phase-contrast microscope for evidence of fertilization (the presence of a spermatozoon in the vitellus of a cleaved egg). Throughout this study, fertility was assessed by the total number of fertilized eggs recovered from mated or artificially inseminated females.

In a second experiment to test if infertility following treatment with 66 mg α -chlorohydrin/kg results from impaired ejaculation, a ligature (size 5/0 chromic, Ethicon) was placed bilaterally around the distal corpus epididymidis in ten animals (Lubicz-Nawrocki & Glover, 1973). Five animals were injected daily with α -chlorohydrin whilst control animals received 0.9% NaCl solution. Treated and control males were permitted to mate on the 4th day after ligation and were killed the morning after mating. The cauda epididymidis from both sides was macerated with fine scissors in 3 ml Hanks' solution and 0.01 ml of sperm suspension was diluted to 10 ml with 10% formalin; the total sperm content was estimated from haemocytometer counts. Sperm numbers were compared with those for five unmated males that received the same daily treatment with α -chlorohydrin and were killed 4 days after epididymal ligation.

In a third experiment to test if α -chlorohydrin inhibits fertilizing ability in castrated hamsters treated with testosterone or its more active metabolites, 5 α -dihydrotestosterone (5 α -androstan-17 β -ol-3-one) and 3 α -androstanediol (5 α -androstan-3 α ,17 β -diol) (Lubicz-Nawrocki, 1973), thirty-five male hamsters (five/group) were bilaterally castrated under sodium pentobarbitone anaesthesia (Diabutal; Diamond) and epididymides were ligated as before. One group of five castrated control animals received four consecutive daily subcutaneous injections of 0.1 ml sesame oil; animals in the other groups were injected daily with 100 μ g of testosterone, 5 α -dihydrotestosterone or 3 α -androstanediol (Sigma Chemical Co.) alone or in combination with 66 mg α -chlorohydrin/kg (30 min after injection of androgen), as shown in Table 4. Animals in each group were tested for mating ability and fertility 4 days after castration and were killed the morning after mating to determine sperm numbers in the cauda epididymidis.

In a fourth experiment, the corpus epididymidis was bilaterally ligated in six hamsters. Three of these animals received four daily injections of α -chlorohydrin and three were injected with 0.9% NaCl solution. Animals in both groups were killed on the 4th day after ligation and six sperm suspensions were prepared, one from each animal, by macerating the bilateral caudae in 3 ml sterilized Hanks' solution followed by filtration through sterilized gauze. Each suspension (0.2 ml) was then injected into both uterine horns of a female under

pentobarbitone-sodium anaesthesia (60 mg/kg, intraperitoneally) to test for fertilizing ability. The percentage of partly stained (live) spermatozoa (Lubicz-Nawrocki & Glover, 1973) in each sperm suspension was estimated in smears stained with aqueous nigrosin-eosin and the concentration of spermatozoa was estimated from haemocytometer counts; thus, it was possible to estimate the number of live spermatozoa injected into the uterine horns.

For the estimation of fructose, seminal vesicles were thoroughly blotted on filter paper to remove the secretion and stored at -20°C . Both lobes were weighed and fructose was assayed in one lobe of each gland using a slight modification (Lubicz-Nawrocki & Glover, 1973) of the method originally described by Lindner & Mann (1960). The other lobe was dried to constant weight at 150°C to determine the percentage of dry matter and fructose concentration was expressed as mg/100 g dry wt of seminal vesicle.

Student's *t* test was used for statistical analysis.

RESULTS

Effects on fertilizing ability of spermatozoa

Table 1 shows that daily subcutaneous injections of α -chlorohydrin (49 mg/kg) for 16 days had no effect on the fertilizing ability of intact male hamsters whereas at 66 mg/kg, fertilizing ability was inhibited within 4 days. Following bilateral ligation of the corpus epididymidis, the higher dosage produced the same antifertility effect (Table 2) without impairing ejaculation since the total number of spermatozoa in the cauda epididymidis after mating (Table 3) was not significantly different from that found in the fertile ligated control animals ($P > 0.05$).

Table 4 shows that animals failed to mate 4 days after castration whereas daily treatment with 100 μg of testosterone, 5 α -dihydrotestosterone or 3 α -androstenediol maintained their fertilizing ability. Four daily injections of α -chlorohydrin (66 mg/kg) still inhibited fertilizing ability in castrated animals

Table 1. Fertilizing ability of male hamsters treated with various doses of α -chlorohydrin for 4 to 16 days

<i>Days of treatment</i>	<i>Dose (mg/kg/day)</i>	<i>No. of females mated*</i>	<i>Total no. of eggs recovered</i>	<i>Total no. of eggs fertilized</i>
4	33	5	48	48
	49	5	50	50
	66	5	57	0
8	33	5	56	56
	49	5	54	54
12	33	5	51	51
	49	5	56	56
16	33	5	59	59
	49	5	54	54

* Each female was paired with a treated male.

Table 2. Fertilizing ability of male hamsters 4 days after ligation of the corpus epididymidis and daily treatment with α -chlorohydrin

<i>Treatment</i> (five males/group)	<i>Seminal vesicle fructose</i> (mg/100 g dry wt) (mean $\pm$ S.E.)	<i>No. of females mated</i>	<i>Total no. of eggs recovered</i>	<i>Total no. of eggs fertilized</i>
Saline (control)	1.72 $\pm$ 0.13	5	59	59
α -Chlorohydrin (66 mg/100 g)	1.66 $\pm$ 0.32	5	57	0

Table 3. Effect of four daily injections of α -chlorohydrin on ejaculation in hamsters following bilateral ligation of the corpus epididymidis

<i>Males</i>	<i>Treatment</i> (five males/group)	<i>No. of spermatozoa $\times 10^6$ in the cauda epididymidis from both sides</i> (mean $\pm$ S.E.)
Unmated	α -Chlorohydrin	265.0 $\pm$ 9.0
Mated	Saline (control)	156.0 $\pm$ 11.9
Mated	α -Chlorohydrin	135.2 $\pm$ 9.3

The dose of α -chlorohydrin was 66 mg/kg body wt/day injected subcutaneously.

Table 4. Effect of four daily injections of α -chlorohydrin on the fertilizing ability of castrated hamsters treated with various androgens

<i>Treatment*</i> (five males/group)	<i>Seminal vesicle fructose</i> (mg/100 g dry wt) (mean $\pm$ S.E.)	<i>No. of females mated</i>	<i>Total no. of eggs recovered</i>	<i>Total no. of eggs fertilized</i>
Castrated controls + saline	1.10 $\pm$ 0.10†	0	54	0
Testosterone + saline	1.72 $\pm$ 0.32	5	64	64
Testosterone + α -chlorohydrin	1.90 $\pm$ 0.23	5	70	0
5 α -Dihydrotestosterone + saline	1.92 $\pm$ 0.21	5	59	59
5 α -Dihydrotestosterone + α -chlorohydrin	2.3 $\pm$ 0.60	5	54	0
3 α -Androstanediol + saline	1.87 $\pm$ 0.32	5	61	61
3 α -Androstanediol + α -chlorohydrin	1.68 $\pm$ 0.14	5	65	0

* The dose of α -chlorohydrin was 66 mg/kg body wt/day injected subcutaneously. The dose of the various androgens was 100 μ g/day injected subcutaneously.

† Significantly different from castrated animals treated with the various androgens ($P < 0.05$).

given the androgen treatment. Sperm counts after mating showed that infertility was not due to impaired ejaculation since the total number of spermatozoa in the cauda epididymidis from both sides was not significantly different ($P > 0.05$) from that of androgen-treated castrated animals exhibiting normal fertilizing ability (Table 5).

The results presented in Table 6 show that α -chlorohydrin suppressed the fertilizing ability of spermatozoa in the cauda epididymidis. None of the eggs was fertilized by spermatozoa from animals receiving α -chlorohydrin.

Table 5. Effect of four daily injections of α -chlorohydrin on ejaculation in castrated hamsters treated with various androgens

Androgen	Treatment* (five males/group)	No. of spermatozoa in the cauda epididymidis from both sides (mean $\pm$ S.E.)
Testosterone	Unmated controls + α -chlorohydrin	264.0 $\pm$ 26.5
	Mated { controls + saline + α -chlorohydrin	141.0 $\pm$ 11.5 127.4 $\pm$ 12.4
5 α -Dihydrotestosterone	Unmated controls + α -chlorohydrin	240.2 $\pm$ 7.0
	Mated { controls + saline + α -chlorohydrin	128.6 $\pm$ 6.5 147.6 $\pm$ 6.7
3 α -Androstanediol	Unmated controls + α -chlorohydrin	222.0 $\pm$ 39.0
	Mated { controls + saline + α -chlorohydrin	150.0 $\pm$ 14.2 115.0 $\pm$ 10.2

* The dose of α -chlorohydrin was 66 mg/kg body wt/day injected subcutaneously. The dose of the various androgens was 100 μ g/day injected subcutaneously.

Table 6. Fertilizing ability of spermatozoa in the cauda epididymidis of males treated daily with α -chlorohydrin for 4 days

Treatment (three males/group)	No. of spermatozoa $\times 10^6$ / uterine horn (mean $\pm$ S.E.)		No. of females inseminated*	Total no. of eggs recovered	Total no. of eggs fertilized
	Total	Live			
Saline (control)	10.6 $\pm$ 1.1	2.5 $\pm$ 0.5	3	62	62
α -Chlorohydrin (66 mg/kg body wt)	16.0 $\pm$ 4.0	2.6 $\pm$ 0.1	3	58	0

* One female/male was artificially inseminated to test the fertilizing ability of the spermatozoa.

Effects on seminal vesicle fructose

In contrast to its antifertility effect, α -chlorohydrin had no effect on seminal vesicular fructose either in intact hamsters (Table 2) or castrated animals treated daily with 100 μ g of testosterone, 5 α -dihydrotestosterone or 3 α -androstanediol (Table 4). Estimations showed that the mean seminal vesicular fructose concentration remained high in each group or that it was slightly greater than in controls at the end of the experimental period.

DISCUSSION

The results of the present study amplify a previous report (Ericsson & Norland, 1970) that α -chlorohydrin has an antifertility effect in hamsters. As in rats (Ericsson & Baker, 1970; Turner, 1971), the effect is due to loss of the fertilizing ability of the spermatozoa since recovered eggs were not fertilized. Hamsters are, however, less sensitive to α -chlorohydrin than rats since approximately 4.3 to 5.5 mg/kg given subcutaneously will induce sterility in rats within 8 days of the beginning of treatment (Ericsson & Baker, 1970).

In rats, α -chlorohydrin apparently affects spermatozoa in the cauda epididymidis and ductus deferens because ligation of the corpus epididymidis did not influence the antifertility effect of the drug (Turner, 1971). Other workers have concluded that α -chlorohydrin is not anti-androgenic since they found no effect on libido, coitus and ejaculation or on the weights of accessory sexual organs in rats (Coppola, 1969) and monkeys (Setty, Kar, Roy & Chowdhury, 1970). The present study also shows that α -chlorohydrin has no effect on coitus or on ejaculatory ability in hamsters, whilst the concentration of fructose in the seminal vesicles remained high in intact animals and castrated animals treated with various androgens in combination with α -chlorohydrin. The maintenance of fertilizing ability in hamsters is androgen-dependent since epididymal spermatozoa lose their fertilizing ability 12 days after castration unless the animals are given daily subcutaneous injections of 100 μ g testosterone, 75 μ g 5 α -dihydrotestosterone or 12.5 μ g 3 α -androstanediol (Lubicz-Nawrocki, 1973). The present study shows that α -chlorohydrin inhibited fertilizing ability in castrated hamsters treated with the same androgens and that even 3 α -androstanediol given at eight times the minimal effective dose (Lubicz-Nawrocki, 1973) did not overcome the effect of the drug. Although there is no evidence that androgens directly control biochemical factors associated with the maintenance of fertilizing ability in mature spermatozoa, there is some indirect evidence (Lubicz-Nawrocki, 1973) that exogenous 3 α -androstanediol acts directly on epididymal spermatozoa to maintain fertilizing ability in castrated hamsters. Since 3 α -androstanediol did not prevent the antifertility effect of α -chlorohydrin, it is feasible that infertility results from a direct effect of the drug on spermatozoa in the cauda epididymidis. This conclusion supports the observation of Crabo & Appelgren (1972) who reported accumulation of radioactive material in the rat cauda epididymidis following intravenous injection of ^{14}C -labelled α -chlorohydrin and is consistent with the present finding that fertilization did not occur after artificial insemination with spermatozoa from the cauda epididymidis of males treated with 66 mg α -chlorohydrin/kg. An indirect antifertility effect of α -chlorohydrin must induce temporary changes in the epididymis or elsewhere since normal fertilizing ability has been shown to return within 5 days in hamsters after comparable treatment with the drug (C. M. Lubicz-Nawrocki and M. C. Chang, in preparation).

Conclusive proof concerning the nature and site of the primary action of α -chlorohydrin must await further study. It still remains a possibility that

metabolites of α -chlorohydrin are involved in mediating its effect (Jackson, Campbell & Jones, 1970).

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REFERENCES

- COPPOLA, J. A. (1969) An extragonadal male antifertility agent. *Life Sci.* **8**, 43.
- CRABO, B. & APFELGREN, L.-E. (1972) Distribution of [^{14} C] α -chlorohydrin in mice and rats. *J. Reprod. Fert.* **30**, 161.
- ERICSSON, R. J. & BAKER, V. F. (1970) Male antifertility compounds: biological properties of U-5897 and U-15,646. *J. Reprod. Fert.* **21**, 267.
- ERICSSON, R. J. & NORLAND, J. F. (1970) Androgen involvement with male antifertility compound α -chlorohydrin. *Excerpta Med. Int. Congr. Ser.* **210**, 174.
- JACKSON, H., CAMPBELL, I. S. C. & JONES, R. A. (1970) Is glycidol an active intermediate in the antifertility action of α -chlorohydrin in male rats? *Nature, Lond.* **224**, 83.
- JOHNSON, L. A. & PURSEL, V. G. (1972) Reversible infertility in male swine fed α -chlorohydrin. *J. Anim. Sci.* **34**, 241.
- KIRTON, K. T., ERICSSON, R. J., RAY, J. A. & FORBES, A. D. (1970) Male antifertility compounds: efficacy of U-5897 in primates (*Macaca mulatta*). *J. Reprod. Fert.* **21**, 275.
- KREIDER, J. L. & DUTT, R. H. (1970) Induction of temporary infertility in rams with an orally administered chlorohydrin. *J. Anim. Sci.* **31**, 95.
- LINDNER, H. R. & MANN, T. (1960) Relationship between the content of androgenic steroids in the testes and the secretory activity of the seminal vesicles in the bull. *J. Endocr.* **21**, 341.
- LUBICZ-NAWROCKI, C. M. (1973) The effect of metabolites of testosterone on the viability of hamster epididymal spermatozoa. *J. Endocr.* **58**, 193.
- LUBICZ-NAWROCKI, C. M. & GLOVER, T. D. (1973) The influence of the testis on the survival of spermatozoa in the epididymis of the golden hamster, *Mesocricetus auratus*. *J. Reprod. Fert.* **34**, 315.
- SAMOJLIK, E. & CHANG, M. C. (1970) Antifertility activity of 3-chloro-1,2-propanediol (U-5897) on male rats. *Biol. Reprod.* **2**, 299.
- SETTY, B. S., KAR, A. B., ROY, S. K. & CHOWDHURY, S. R. (1970) Studies with sub-toxic doses of alpha-chlorohydrin in the male monkey (*Macaca mulatta*). *Contraception*, **1**, 279.
- TURNER, M. A. (1971) Effects of α -chlorohydrin upon the fertility of spermatozoa of the cauda epididymidis of the rat. *J. Reprod. Fert.* **24**, 267.