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Antifertility Actions of α-Chlorohydrin in the Male

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Abstract

Non-steroidal chemicals that affect male fertility have been known for over 25 years but only one compound, α-chlorohydrin, possesses most of the attributes of an ideal male contraceptive. In the male rat, for example, continuous daily oral administration of low doses produces an almost immediate and continuous antifertility response that ceases when treatment is withdrawn. Such a dose regime does not interfere with libido, is apparently not toxic and the action is specific towards mature sperm. Furthermore, the action of the compound is species-specific: it is effective in the rat, ram, boar, guinea pig, hamster, rhesus monkey and upon ejaculated human sperm but it is ineffective in the mouse and the rabbit. High doses of α-chlorohydrin can be neurotoxic, nephrotoxic and, in rats, lead to prolonged or permanent infertility. However, the antifertility response and the toxicity of racemic α-chlorohydrin may be due, respectively, to the separate enantiomers. No other antifertility chemical has been investigated to such an extent as α-chlorohydrin; this article reviews the progress that has been achieved with α-chlorohydrin during the past six years.

Introduction

The first report that α-chlorohydrin [3-chloropropan-1,2-diol (*1*)] possessed antifertility activity in the male was published by Coppola (1969) and the first major review of its activity was written in 1977 (Jones 1978*a*). While this article outlines the progress that has been achieved over the period 1977–82, several aspects of the antifertility action of α-chlorohydrin have been included in books (Brooks 1979*a*; Mann and Lutwak-Mann 1981) and in more general reviews of male contraception and fertility (Gomes 1977; Jackson and Morris 1979; Lobl 1980; Waites 1980; Feghali and Feghali 1982).

Before the work of the past six years can be discussed, it is important to outline the events that are involved in the production of mature sperm as well as the state of knowledge that existed in 1977 regarding the mechanism of action of α-chlorohydrin. A diagrammatic representation of the testis–epididymis complex of the rat is depicted in Fig. 1. The testicular phase of spermatogenesis, which takes approximately 8 weeks, occurs within the epithelium of the seminiferous tubules (*A*) of the testis culminating in the shedding of immature sperm into the lumen of the tubules. These immature sperm are transported in a specific fluid to a region called the rete testis which is located towards one pole of the testis (*B*). From here the dilute suspension of sperm is directed via several efferent ducts (*C*) to the head of the epididymis, or caput epididymis (*D*). Much resorption of fluid takes place in the caput region and as

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the more concentrated suspension of sperm traverses the epididymis, through the corpus (*E*) to the cauda (*F*), the environment is continually being modified by the addition and the absorption of many specific physiological substances. During their passage along the epididymis, which takes approximately 10 days, the sperm undergo a process of maturation. The mature sperm are contained within the cauda (*F*) from where they are ejaculated through the muscular vas deferens (*G*).

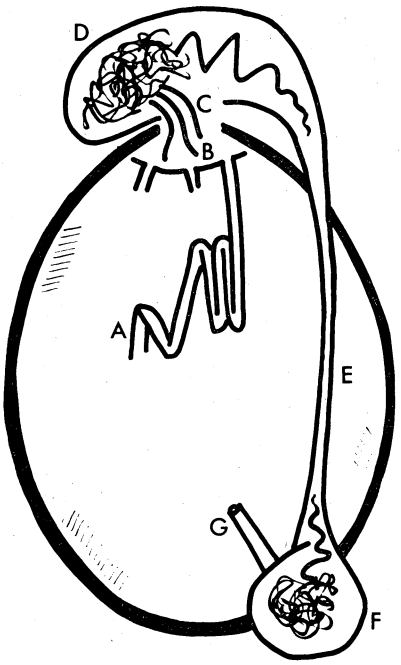


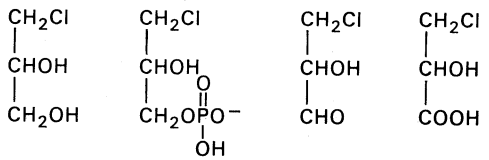
Fig. 1. The testis–epididymis complex of the rat. *A*, seminiferous tubule within the testis; *B*, the rete testis; *C*, efferent ducts; *D*, caput epididymis; *E*, corpus epididymis; *F*, cauda epididymis; *G*, vas deferens.

α-Chlorohydrin has two specific actions on the reproductive tract of the male rat. These actions are dose-dependent and have been classified as the low-dose effect and the high-dose effect. The low-dose effect is directed at mature sperm contained in the cauda epididymis. This action, which is evident 2–3 days after continuous daily oral doses of 5–10 mg/kg, renders the sperm incapable of fertilization without causing any visible changes to their morphology. In 1977, evidence suggested that the mechanism of action involved the entry of α-chlorohydrin into the sperm and its conversion by glycerol kinase to α-chlorohydrin-1-phosphate (*2*). This metabolite was thought to inhibit glyceraldehyde-3-phosphate dehydrogenase thereby blocking the glycolytic pathway so that ATP levels could not be maintained for adequate sperm motility and, consequently, for successful fertilization (Jones 1978*a*).

The effect of high doses of α-chlorohydrin leads to prolonged or even permanent infertility in male rats. A single dose of 90–120 mg/kg leads to an occlusion of the efferent ducts or the tubule, or both, of the caput epididymis. This pathological lesion, which is caused by the development of bilateral sperm retention cysts or spermatocoeles, prevents the passage of immature testicular sperm to the epididymal tract (Cooper and Jackson 1973). The back-pressure of testicular fluid causes oedema, the inhibition of spermatogenesis and, ultimately, atrophy of the testes.

Effects on the Epididymis, Testis and Sperm of the Rat

The direct effect of α-chlorohydrin on the motility and fertility of rat epididymal spermatozoa *in vivo* has been confirmed (Chulavatnatol *et al.* 1981; Tsang *et al.* 1981; Paz and Homonnai 1982). Epithelial cells isolated from the caput epididymides of rats treated with α-chlorohydrin (20–73 daily doses of 6–8 mg/kg) showed a 50% decrease in the activity of β-galactosidase, when compared to those from untreated animals, but there was no change in the activities of β-D-glucuronidase or *N*-acetyl-β-D-glucosaminidase (Kemp and Killian 1978). The activities of other enzymes isolated from the testis and epididymis of rats treated with a daily dose of α-chlorohydrin (6·5 mg/kg) for 9 days have also been assessed (Gill and Guraya 1980). On the tenth day acid phosphatase activity was normal in the testis, caput and corpus epididymis but was decreased by 40% in the cauda; throughout the epididymis alkaline phosphatase and glucose-6-phosphatase activities were increased, and that of hyaluronoglucuronidase decreased. In contrast to the findings of Kemp and Killian (1978), these authors showed that there was an increase in the activity of β-D-glucuronidase in all sections of the epididymis. The rates of secretion of potassium ions and proteins were not affected when isolated cauda epididymides obtained from rats treated daily with α-chlorohydrin (2 mg/kg for 7 days) were perfused *in vitro* (Wong *et al.* 1977). There was, however, 50% inhibition of the absorption of sodium ions and water which paralleled the findings of a previous study (Wong and Yeung 1977).



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Rats treated with a daily subcutaneous injection of α-chlorohydrin (6·5 mg/kg) for 9 days were killed on day 10 and the activities of various enzymes determined (Kaur and Guraya 1981*a*). A conspicuous decrease in the activities of isocitrate-, succinate-, malate- and glutamate- dehydrogenases, DPN diaphorase, TPN diaphorase and monoamine oxidase (but not of 3β-hydroxy-Δ^5-steroid dehydrogenase) in the epithelial cells of all regions of the epididymis was interpreted as illustrative of a defect in the TCA cycle and amino acid metabolism. In a parallel study the same authors showed that testicular and epididymal DNA content was unaffected by treatment with α-chlorohydrin; RNA and protein concentrations decreased, the activities of proteinase and ribonuclease increased and that of γ-glutamyltransferase decreased. These results indicated defective metabolic pathways (Kaur and Guraya 1981*b*; Guraya and Kaur 1982). The phospholipids of the testis–epididymis complex of rats that were killed 1 day after receiving the last of daily doses of α-chlorohydrin (6·5 mg/kg) for 14 days have been analysed (Kalla and Singh 1978). There was no alteration in the amounts of total phospholipids, phosphatidyl-choline, -ethanolamine, -inositol, -serine or sphingomyelin in the testis, caput or corpus epididymis, whereas all but the last two substances showed a marked decrease in both the cauda epididymis and the vas deferens. The authors suggested that the decrease in these two moieties is related to a decrease in sperm motility. In a similar study, Guraya and Gill (1978)

demonstrated a decrease in the amounts of phospholipids in the testis and epididymis of rats 11 days after a single subcutaneous dose of α-chlorohydrin (14 mg/kg). Accompanying this was a decrease in glycogen, the activities of ATP pyrophosphatase and 5′-nucleotidase and an increase in the amount of triglycerides. When rats were killed on the day after they had received the last of a daily subcutaneous dose of α-chlorohydrin (0·5 mg/kg) for 9 days, the activities of all glycolytic enzymes in epididymal and testicular tissue were reduced (Kaur and Guraya 1981*c*). Two of the enzymes of the pentose phosphate pathway, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, showed lower activities in the epididymis but those from the testis were unaffected.

The effects of administration to rats of a single dose of α-chlorohydrin (90 mg/kg), which causes bilateral lesions in the caput epididymis, have been compared with those of efferent duct ligation (Brown-Woodman *et al.* 1976). Both treatments reduced the quantity of glycerylphosphorylcholine in all segments of the epididymis which suggested that the secretion or release of this compound is probably a response to efferent duct occlusion rather than a direct effect of α-chlorohydrin. As well, neither treatment caused significant changes in the pattern of blood flow in the epididymis (Brown-Woodman and White 1978).

While *in vivo* fertility studies with α-chlorohydrin are commonly performed with various strains of laboratory rats, the susceptibility of two other strains have been reported. The oral administration of single high doses (100–300 mg/kg) to the Polynesian rat (*Rattus exulans*) caused microscopic lesions within the initial segment of the caput epididymis (Cummins and Wodzicki 1980). Seven days later, testicular and epididymal cytology were normal and there was no evidence of gross epididymal lesions indicating that there was no permanent high-dose effect. By contrast, subcutaneous doses of 25 mg/kg each day for 20–30 days to house rats (*Rattus rattus rufescens*) caused gross disorganization of testicular and epididymal cells and inhibited spermatogenesis (Dixit and Agrawal 1980). The absence of sperm in the epididymis suggested that this strain of rat is susceptible to the high-dose effect.

The High-dose Effect and Toxicity

The high-dose effect of α-chlorohydrin occurs in the rat following a single oral or intraperitoneal dose of 90–120 mg/kg, depending on the strain (Jones 1978*a*). The testis becomes enlarged and increases in weight for approximately 5 days and this is followed by a constant decrease in testicular weight due to a pressure-mediated necrosis caused by the development of spermatocoeles in the caput epididymis. The consequences of this effect on the seminiferous tubules has been the subject of a histological study (Kalla and Chohan 1980). When the dose is increased by some 20–30%, the compound is toxic and death follows 2–5 days later. In a study of the oxidative metabolism of α-chlorohydrin, it was observed that intraperitoneal administration of a single dose (100 mg/kg) caused a prolonged phase of diuresis (Jones *et al.* 1978). It was proposed that the diuretic action was not due to the compound itself but to a metabolite, oxalic acid, since a number of compounds which also initiated the formation of spermatocoeles in the rat also caused diuresis and were metabolized to oxalic acid (Jones 1978*b*). A further study showed that the diuresis induced by a single high dose of α-chlorohydrin was accompanied both by glucosuria and the excretion of oxalic acid (Jones *et al.* 1979). Due, perhaps, to the similar

embryological origins of the kidney and the testis–epididymis complex, these two effects of a high dose of α-chlorohydrin may be related. Both the kidney and the caput epididymis are involved in fluid reabsorption and an impairment of this physiological function could be responsible for inducing diuresis and in causing the development of spermatocoeles.

The nephrotoxic action has been studied by Morris and Williams (1980) who confirmed that the effect on the kidney was dose-dependent. As the dose was increased the effects on the kidney became more severe so that at 120 mg/kg, the mortality rate was nearly 50% which was preceded by a loss in appetite and body weight, proteinuria and anuria. The effect on the caput epididymis, however, has not been studied in detail. Water and sodium ion reabsorption in the cauda epididymis of the rat was impaired by α-chlorohydrin (Wong and Yeung 1977; Ngai *et al.* 1978). Treatment of rats with a single oral dose of the vasodilator sodium nitrite (20 mg/kg) 30 min before a dose of α-chlorohydrin (90 mg/kg) inhibited the high-dose effect (Kalla and Singh, 1979). The weights of the testes and epididymides were normal 6 days after treatment and the incidence of spermatocoeles was reduced from 89% (α-chlorohydrin alone) to 8·5% (pretreatment with sodium nitrite).

The development of bilateral spermatocoeles in male rats by α-chlorohydrin results in aspermia, due to occlusion of the efferent ducts, with no impairment of libido. Attempts to exploit this physiological effect and cause negative population growth by the 'sterile male approach' have been effective in isolated rat colonies (Anderson 1977). Acceptance by wild rats of baits containing α-chlorohydrin has been successful and the compound is now marketed as a rodenticide in many countries under the name Epibloc* (Anon. 1982; Ericsson 1982), a use which, incidentally, was suggested nearly 40 years ago (Leffingwell and Lesser 1945).

Effects on Species Other than the Rat

The fertility of male hamsters was inhibited by daily oral doses of α-chlorohydrin (30–100 mg/kg) for 7 days (Das and Yanagimachi 1978). This effect was mediated by an impairment of epididymal sperm motility and was completely reversible, the animals resuming normal fertility 1 week after treatment had ceased. These results, which suggest that the response of the hamster to α-chlorohydrin is similar to that of the rat, are substantiated by the findings that α-chlorohydrin inhibited glycolysis (Ford *et al.* 1979) and the acrosome reaction (Dravland and Meizel 1981) in hamster sperm *in vitro*.

When α-chlorohydrin was given orally (20 mg/kg for 50 days) to male gerbils (*Meriones hurrianae*), testicular damage was evident but this was reversible (Dixit and Lohiya 1976). A single intratesticular injection (100 mg) to male dogs (*Canis familiaris*) caused variable degrees of spermatogenic arrest and biopsies taken 30 days after treatment showed the epididymis and vas deferens to be devoid of sperm (Dixit 1979). This result complements a previous study in which the epididymides of dogs that had been treated with 30 subcutaneous doses of α-chlorohydrin (8 mg/kg) were examined (Dixit 1977). The caput epididymides exhibited denudation of the epithelial lining and the conclusions drawn were that the compound acts directly on this organ and alters its biochemical composition. When α-chlorohydrin was

* α-Chlorohydrin is the active ingredient of Epibloc, the registered trade mark of Gametrics Limited, Sausalito, California, and covered by U.S. Patent 3,659,022 and U.K. Patent 1,221,467.

administered to dogs orally for 50 days at 30 mg/kg, followed by 50 days at 60 mg/kg, there were no adverse effects but no mention was made regarding its antifertility action (Jackson 1977).

Daily oral administration of α-chlorohydrin (5, 10 or 30 mg/kg for 30 days) to miniature boars was followed by an analysis of various epididymal parameters (Crabo *et al.* 1979). Water resorption in the caput epididymis was slightly decreased in all treatment groups; the levels of glycerylphosphorylcholine and of sodium, potassium and chloride ions were not altered significantly following the two higher dose levels but those from the animals subjected to the lower dose regime exhibited elevated levels of sodium, potassium and chloride ions. It was concluded that the antifertility action of the compound was probably not mediated by an impairment of epididymal function. Intramuscular injection of daily doses of α-chlorohydrin (25 mg/kg for 4 days) to mature Merino rams reduced the respiratory and glycolytic activity as well as the motility of ejaculated sperm but there were only minor alterations to the fructose and amino acid concentrations in the seminal plasma (Brown-Woodman and White 1976). The primary mode of action of the compound was proposed to involve a disruption of the metabolism of the caudal sperm.

A histological study of the effect of subcutaneous doses of α-chlorohydrin (10 mg/kg for 30 days) to the Indian langur monkey (*Prebytis entellus entellus*) showed that spermatogenesis was suppressed (Braz *et al.* 1976). The animals were killed 1 day after the final dose and although sperm were present in the cauda epididymis, their fertilizing capacity could not be assessed.

α-Chlorohydrin had no effect on the libido or fertility of cockerels in daily doses of either 10 mg/kg for 30 days or 60 mg/kg for 2 days (Aire and Olusanya 1980).

A synergistic effect of α-chlorohydrin and copper ions on decreasing the motility of human sperm *in vitro* (Kalla and Singh 1981) is thought to be partly due to the enhanced alkylation of cysteine residues with the sperm by the compound (Kalla and Bansal 1977).

Endocrine Effects

The low-dose activity of α-chlorohydrin has no apparent effect on the relative proportion of those hormones involved in the processes of male reproduction (Kalla 1981). The high-dose effect does show some variations in these parameters but these can be attributed to the occlusion of the efferent ducts or the caput epididymides by spermatocoeles since efferent duct ligation results in similar responses (Morris 1979). With the exception of the epididymides, there were no changes in the weights of androgen-dependent organs of those rats that had received an oral sterilizing dose of 80 mg/kg (Morris and Jackson 1978*a*). Serum prolactin and LH levels were elevated for several weeks with the level of serum FSH being raised significantly for 90 days. This was attributed to disturbances in the production of inhibin from the seminiferous epithelium rather than its retention within the testicular tubules (Morris and Jackson 1978*a*, 1978*b*). The levels of plasma and testicular testosterone remained normal in rats receiving a single oral dose (100 mg/kg) (Kalla and Chohan 1980), and no consistent changes in testosterone levels were obtained in rats given a single oral dose of 40–60 mg/kg (Lobl 1980). In rats receiving a single oral dose of 60 mg/kg, α-chlorohydrin caused an increased level (600%) of androgen binding protein in the testis and a concomitant decrease (to 1·6% of controls) in the amount present in the

epididymis by day 20 (Lobl *et al.* 1979). It was concluded that it is the functional ligation produced in the initial segment of the caput epididymis that produces these effects as they are similar to those observed after surgical ligation of the efferent ducts.

Various suggestions implicating an anti-androgenic action of α-chlorohydrin are not supported by experimental evidence (Hundal and Mangat 1978; Kakaria *et al.* 1979).

The Active Metabolite of α-Chlorohydrin

When the effect of α-chlorohydrin was examined on ram sperm *in vitro* it was observed that the inhibitory action was not immediate but that it developed over a period of time. This led to the suggestion that α-chlorohydrin was not active *per se* but that it was being converted into a metabolite that was the true inhibitory species (Mohri *et al.* 1975). It was speculated that the inhibitory metabolite was the phosphorylated derivative, α-chlorohydrin-1-phosphate (*2*) though no supporting evidence was forthcoming and the identity of this 'metabolic product of α-chlorohydrin' was not mentioned in a subsequent paper by these authors (Brown-Woodman *et al.* 1978). The assumption that α-chlorohydrin-1-phosphate was the active metabolite became accepted as fact especially since it had been reported that α-chlorohydrin was a substrate for glycerol kinase which would convert it into the phosphorylated derivative (Thorner 1972). Studies with synthetic α-chlorohydrin-1-phosphate confirmed that it was an inhibitor of the pure enzyme glyceraldehyde-3-phosphate dehydrogenase, albeit in high concentrations (Dickinson *et al.* 1977; Mashford and Jones 1978; Fitzpatrick *et al.* 1979).

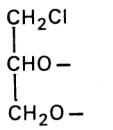
Enzyme studies showed that purified glycerol kinase (from *Candida mycoderma*) and ram spermatozoa subjected to ultrasonic vibration could convert α-chlorohydrin to the phosphorylated derivative (Brooks 1979*a*); however, it was later shown that the α-chlorohydrin used in these experiments was contaminated with glycerol (Brooks 1979*b*). When the glycerol was removed by repeated distillation, the pure α-chlorohydrin was shown not to be a substrate for the purified enzyme (Brooks 1979*b*; Jones *et al.* 1981). Metabolic studies with α-[^{36}Cl]chlorohydrin failed to detect its conversion to the phosphorylated derivative by rat or boar sperm (Hutton *et al.* 1980; Jones *et al.* 1981) but did show that biotransformation was occurring. Several radioactive metabolites were detected and identified as Cl^-, 3-chlorolactaldehyde (*3*) and 3-chlorolactate (*4*), the aldehyde being proposed as the true inhibitory metabolite (Jones *et al.* 1981; Stevenson and Jones 1981).

The inhibitory activity of α-chlorohydrin-1-phosphate towards purified glyceraldehyde-3-phosphate dehydrogenase, therefore, appears to be a coincidence for there is no evidence of its formation within sperm. Its antifertility activity *in vivo* could be due to its hydrolysis to α-chlorohydrin (Brown-Woodman *et al.* 1979).

Structure–Activity Studies with α-Chlorohydrin

Attempts to modify the structure of α-chlorohydrin, thereby improving its therapeutic efficiency and reducing its toxicity, have not been particularly successful. Previous studies had shown that for antifertility activity, the structural requirements were specific and well-defined (see Jones 1978*a*). The compound must possess three saturated carbon atoms; a primary carbon atom (bearing a chlorine atom)

adjacent to a carbon atom (bearing a secondary hydroxyl group) adjacent to a primary carbon atom (bearing a primary hydroxyl group) (see structure *5*). As the only latitude with these strict requirements was that the hydroxyl groups could either be free, esterified or present as ether linkages, structural variations are limited to the choice of the particular ester or ether groups.



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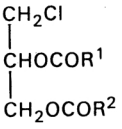
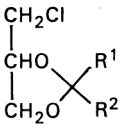
Basic structure necessary for antifertility activity



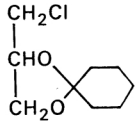
6

6a: $R^1 = R^2$ = acetyl
6b: $R^1 = R^2$ = caproyl
6c: $R^1 = R^2$ = palmitoyl
6d: $R^1 = R^2$ = *m*-nitrobenzoyl
6e: R^1 = H, R^2 = *p*-acetamidobenzoyl



7

7a: R^1 = H, R^2 = phenyl
7b: R^1 = methyl, R^2 = ethyl
7c: R^1 = methyl, R^2 = n-pentyl
7d: R^1 = H, R^2 = *p*-tolyl
7e: R^1 = H, R^2 = *p*-chlorophenyl
7f: R^1 = H, R^2 = *p*-methoxyphenyl
7g: R^1 = H, R^2 = *p*-nitrophenyl
7h: R^1 = H, R^2 = styryl
7i: R^1 = methyl, R^2 = phenyl
7j: R^1 = H, R^2 = n-hexyl



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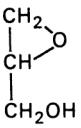
1,2-Cyclohexyl derivative



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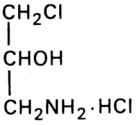
Glycidol
(2,3-epoxypropan-1-ol)



10

1-Amino-3-chloropropan-2-ol hydrochloride

The structures of compounds related to α-chlorohydrin

The bis-acetate (*6a*) and bis-caproate (*6b*) esters showed comparable molar potencies to that of α-chlorohydrin when administered to male rats, as did the bis-palmitate (*6c*) (Rooney and Jackson 1980*a*). Unlike the other two esters, the bis-palmitate was a solid and could be purified by recrystallization but it had the disadvantage of having a molecular weight five times that of α-chlorohydrin. Two other crystalline derivatives, the bis-*m*-nitrobenzoate (*6d*) and mono-*p*-acetamidobenzoate (*6e*), were less toxic to rats than α-chlorohydrin but showed no improved efficacy

(Rooney and Jackson 1980*b*); the bis-*p*-nitrobenzoate (*6f*) was approximately half as active as α-chlorohydrin on a weight basis (Rooney and Jackson 1980*c*). The ketal derivative (*7a*) possessed an antifertility action in male rats whereas the three analogues *7b*, *7c*, and *8* were ineffective (Brown-Woodman *et al.* 1976, 1979, 1982). Compounds *7a*, *7b*, *7c* and *8* reduced the aerobic glycolysis and oxidation of fructose by ejaculated ram sperm (Brown-Woodman *et al.* 1981).

On the assumption that an aromatic group as a substituent on a ketal derivative conferred antifertility activity, Brown-Woodman *et al.* (1982) examined the effect of several of these derivatives in male rats. With the exception of *7d*, all of the compounds *7e–7i* were ineffective in reducing both fertility and sperm motility. In general, these aromatic derivatives were less toxic to the rat than α-chlorohydrin itself.

Due to the stringent structural requirements for antifertility activity, these modifications to the α-chlorohydrin structure were, of necessity, superficial. Increasing the lipophilic nature of the molecule, thereby enhancing its uptake by, and slow release from, adipose tissue probably accounts for a lessening of the toxicity with no concomitant increase in antifertility effectiveness. Evidence for this was proposed by Jones and O'Brien (1980) in a study of the metabolism by the rat of two ketal derivatives (*7c*) and (*7j*) which had previously been shown to have activities in the rat comparable to that of α-chlorohydrin (Banik *et al.* 1972; Hirsch *et al.* 1975). Both compounds produced 3-chlorolactate (*4*), the major oxidative metabolite of α-chlorohydrin, indicating that the ketal derivatives are degraded to α-chlorohydrin. In the same study, the epoxide glycidol, 2,3-epoxypropan-1-ol (*9*), was also shown to be metabolized to 3-chlorolactate. This suggested that the epoxide, which has an antifertility action when very large doses are administered orally to male rats (Jackson *et al.* 1970), is converted to α-chlorohydrin in the acid milieu of the stomach. Thus, glycidol appears to be a latent form of α-chlorohydrin which explains why it has been included in structure–activity studies (Brown-Woodman *et al.* 1979).

Another compound with an antifertility response in rats similar to that of α-chlorohydrin is the crystalline hydrochloride salt of 1-amino-3-chloropropan-2-ol (*10*). Although its formula defies those structure–activity requirements defined for α-chlorohydrin, this compound, like glycidol, is a latent form of α-chlorohydrin into which it is metabolized, presumably by monoamine oxidases (Jones *et al.* 1979*c*). While the antifertility response of rats to this amino-analogue compares favourably to that of α-chlorohydrin (Brown-Woodman *et al.* 1976, 1979), it was this compound that added a new dimension to structure–activity studies, the requirement being one of stereochemical specificity. Both α-chlorohydrin and 1-amino-3-chloropropan-2-ol have a chiral carbon atom at C2. Classical chemical syntheses of either compound, therefore, produces a racemic product containing equimolar amounts of the (R)- and (S)-isomers. Due to the presence of the amino group, the amino analogue could be resolved into its (R)- and (S)-isomers and when the antifertility activity of these enantiomers was assessed (Coppola and Saldarini 1974), the (R)-isomer was found to be ineffective whereas the (S)-isomer possessed the antifertility activity. While the effects of (S)-1-amino-3-chloropropan-2-ol on male fertility have not been studied in detail, possibly because of an unfavourable report concerning its neurological toxicity in Rhesus monkeys (Heywood *et al.* 1978), the stereospecificity for antifertility activity that this compound demonstrated prompted the synthesis of the individual isomers of α-chlorohydrin. The first synthesis of (R)- and (S)-α-chlorohydrin (Jackson and Robinson 1976; Jackson *et al.* 1977) has been followed by two

others (Jones 1978; Porter and Jones 1982*a*), all of which commence with stereochemically defined carbohydrates.

Mechanism of the Low-dose Action

Before the separate isomers of α-chlorohydrin had been synthesized and their effects on fertility examined, biochemical investigations were confined to the racemic or (R,S)-mixture of isomers. The pioneering work of Brown-Woodman *et al.* (1978) established that racemic α-chlorohydrin caused a decrease in the glycolytic or fructolytic activity of ejaculated ram sperm *in vitro*. The analysis of glycolytic intermediates in ram sperm that had been incubated with the compound showed that the site of action was primarily an inhibition of glyceraldehyde-3-phosphate dehydrogenase and, to a lesser extent, of triosephosphate isomerase and fructose-bisphosphate aldolase (Mohri *et al.* 1975). Such an effect results in a decrease in substrate-level phosphorylation, the main process by which human sperm generate ATP (Ford and Harrison 1981*a*), thus causing a decrease in their motility when they are ejaculated. Subsequent studies showed that this action of racemic α-chlorohydrin in ram sperm was common to the sperm of other species including that of the rat, hamster and human (Ford *et al.* 1979), the boar (Hutton *et al.* 1979, 1980) and the Rhesus monkey (Ford *et al.* 1979; Ford and Harrison 1980).

The first report of the apparent stereochemical specificity of α-chlorohydrin was by Jackson and Robinson (1976) who synthesized (R)-α-chlorohydrin and demonstrated that it had no antifertility activity in male rats. This was confirmed in the following year when the (S)-isomer was prepared and shown to elicit the antifertility response (Ford *et al.* 1977; Jackson *et al.* 1977). With the individual isomers of α-chlorohydrin available, this stereochemical specificity has been confirmed by *in vitro* studies involving the sperm of a number of species; thus, (S)-α-chlorohydrin inhibits glycolysis in the sperm of the ram (Ford *et al.* 1977), the human (Ford *et al.* 1979), the boar (Jones and Stevenson 1982; Stevenson and Jones 1982*a*, 1982*b*) and the bull (Ford and Waites 1982). The (R)-isomer is ineffective in inhibiting fructolysis in boar sperm *in vitro* (Stevenson and Jones 1982*b*) and glycolysis in rat sperm *in vivo* (Ford *et al.* 1977).

It would be fortuitous, perhaps, if this stereochemical specificity also applied to the toxic actions of racemic α-chlorohydrin; i.e. is the (R)-isomer responsible for the renal toxicity and the development of spermatocoeles? This is partly true since (R)-α-chlorohydrin, or more specifically its oxidative metabolite (R)-3-chlorolactate,* induces diuresis and glucosuria in the rat whereas (S)-α-chlorohydrin does not (Porter and Jones 1982*a*, 1982*b*). However, (S)-α-chlorohydrin is known to cause the development of spermatocoeles in the rat (Jackson *et al.* 1977; Ford and Waites 1982) but it is not known whether the (R)-isomer has this ability or not.

With the knowledge that the active isomer possesses the (S)-configuration and that inhibition of sperm glycolysis *in vitro* is due to a metabolite, the biochemical mechanism of action of α-chlorohydrin has been resolved. In both rat and boar sperm *in vitro*, (R,S)-α-[^{36}Cl]chlorohydrin is metabolized to [3-^{36}Cl]chlorolactaldehyde of unknown configuration (Jones *et al.* 1981). In a subsequent study, (R)-α-[^{36}Cl]chlorohydrin

* In two previous papers (Porter and Jones 1982*a*; Stevenson and Jones 1982*b*) the incorrect R,S designations were assigned to the oxidative metabolites of the isomers of α-chlorohydrin. The correct designations are given in this paper.

was shown not to be metabolized by boar sperm to (R)-[^{36}Cl]-3-chlorolactaldehyde (Stevenson and Jones 1982*b*). From this it was deduced that at least in boar sperm (S)-α-chlorohydrin was being converted to (S)-3-chlorolactaldehyde. This metabolite, which has the same absolute stereochemistry as D- or (R)-glyceraldehyde-3-phosphate, the substrate for glyceraldehyde-3-phosphate dehydrogenase, would appear to be the inhibitory metabolite of (S)-α-chlorohydrin and this is supported by mechanistic considerations (Walsh 1979). When the effect of racemic 3-chlorolactaldehyde was examined on boar sperm *in vitro*, fructolysis was inhibited and accumulation of the glycolytic intermediates fructose-1,6-bisphosphate, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate confirmed that inhibition of glyceraldehyde-3-phosphate dehydrogenase had occurred (Stevenson and Jones 1982*b*).

When (R,S)-α-[^{36}Cl]chlorohydrin was incubated with rabbit sperm, there was neither an inhibition of fructolysis nor the production of 3-chlorolactaldehyde (Jones *et al.* 1981). Since α-chlorohydrin is ineffective as an antifertility agent when administered to male rabbits (Back *et al.* 1975), this indicated that species specificity may be due to the ability of mature sperm to produce (S)-3-chlorolactaldehyde. Furthermore, isolated kidney tubules from the rabbit, rat and the boar were incapable of performing this biotransformation and their glycolytic activity was unimpaired in the presence of α-chlorohydrin (Jones *et al.* 1981). It was postulated that the inhibitory activity of α-chlorohydrin within a particular cell depended, therefore, on the ability of that cell to produce (S)-3-chlorolactaldehyde. If the enzyme responsible for this oxidation was localized in the mature sperm of certain species then this would explain why α-chlorohydrin exhibits species specificity as an antifertility agent and why it is not a general inhibitor of glycolysis in all cells.

Chlorinated Sugars

In 1978, the reversible contraceptive action of some 6-chloro-6-deoxy sugars in the male rat was reported by Ford and Waites (1978*a*, 1978*b*). 6-Chloro-6-deoxyglucose and 6′-chloro-6′-deoxysucrose were effective when administered orally; related sugars containing further chlorine atoms had a reduced effect and those with the 1 (or 1′) position blocked were ineffective. The range of chlorinated carbohydrates with antifertility activity has since increased to include 6-chloro-6-deoxyfructose and 6-chloro-6-deoxysucrose (Ford and Waites 1978*b*), 6-chloro-6-deoxygalactose, 6-chloro-6-deoxymannose, 6,6′-dichloro-6,6′-dideoxysucrose and 6-chloro-6-deoxyglucose-1,2,3,4-tetraacetate (Ford 1980) and 6-chloro-6-deoxyglucitol (Ford *et al.* 1981). The type of antifertility action displayed by these sugar derivatives is similar to that of (S)-α-chlorohydrin. Sperm from rats that had been treated with several of the sugars were defective in metabolizing glucose, their glycolytic activity being inhibited at the glyceraldehyde-3-phosphate dehydrogenase stage (Ford and Waites 1980; Ford *et al.* 1981). Sperm motility was decreased as was their content of ATP (Warren *et al.* 1979) and their energy charge potential; their motility, however, was increased when they were exposed to pyruvate and lactate as carbon sources (Ford and Harrison 1981*b*). When rat sperm were incubated in the presence of a high concentration of 6-chloro-6-deoxyglucose, the rate of glycolysis was unaffected (Ford and Waites 1978*a*).

Four other similarities in the antifertility action of these sugars (especially 6-chloro-6-deoxyglucose, which has been the most extensively studied) to that of

(S)-α-chlorohydrin have been noted. First, the development of spermatocoeles in rats when a single large dose was administered (Ford and Waites 1981, 1982). Second, the immediate and reversible phase of infertility brought about by low doses (Ford and Waites 1978*a*; Heitfeld *et al.* 1979; Vickery *et al.* 1979; Warren *et al.* 1979) with no gross or histological effect on the testes or accessory reproductive organs (Vickery *et al.* 1979). Third, the decrease in the ATP content of sperm from rats treated with either (S)-α-chlorohydrin or 6-chloro-6-deoxyglucose, which returns to normal values when treatment ceases (Heitfeld *et al.* 1979) and, finally, inhibition by both compounds of the reabsorption of sodium ions and water in the perfused cauda epididymis of the rat which is completely reversible (Wong *et al.* 1980).

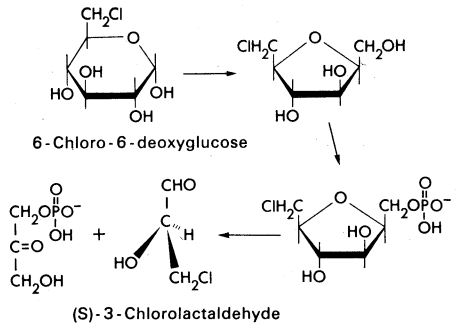


Fig. 2. A possible scheme for the production of (S)-3-chlorolactaldehyde from 6-chloro-6-deoxyglucose either by glycolysis or a related metabolic pathway.

Two essential requirements for antifertility activity are that the chlorinated sugars must possess a 6- (or 6′-) chlorine atom (Ford and Waites 1978*a*) and that they must be activated by a metabolic process (Ford 1980). Indeed, [^{36}Cl]-6-deoxyglucose undergoes rapid dechlorination when injected into rats with radioactivity achieving plateau levels in blood and urine after 5 days (Ford and Waites, 1978*a*). Assuming that these sugars either enter glycolysis or are metabolized by a similar degradative pathway, 6-chloro-6-deoxyglucose, for example, could eventually be converted by aldolase or a similar hexose-cleaving enzyme into (S)-3-chlorolactaldehyde, the active metabolite of (S)-α-chlorohydrin (Fig. 2). While this is feasible and has been proposed (Ford 1982*a*; Ford and Waites 1982), it remains to be confirmed. Evidence is accumulating to suggest that (S)-α-chlorohydrin and 6-chloro-6-deoxyglucose share a common biochemical mechanism for their antifertility action.

The production of (S)-3-chlorolactaldehyde from (S)-α-chlorohydrin has been proposed to require a specific enzyme which may be confined to the mature sperm of certain species (Stevenson and Jones 1982*b*) but the conversion of 6-chloro-6-deoxyglucose to this aldehyde may be able to be performed in a number of glycolytic cells. Exposure of rabbit sperm to 6-chloro-6-deoxyglucose resulted in an accelerated loss of their motility (Vickery *et al.* 1979) indicating that although (S)-α-chlorohydrin is not metabolized by rabbit sperm, this sugar may be able to be degraded. The fertility of male mice is unimpaired by high doses of either (S)-α-chlorohydrin or 6-chloro-6-deoxyglucose (Jacobs and Ford 1981) and both compounds are neurotoxic to mice (Ford and Waites 1982) and marmosets (Jacobs and Duchen 1980; Jacobs and Ford 1981). The neurotoxicity was similar in both species, the sites of the central

nervous system lesions in mice corresponding closely to regions of high glucose utilization (Jacobs and Ford 1981).

The specificity of a chlorine atom in α-chlorohydrin as a requirement for reversible antifertility activity has been well documented; α-bromohydrin and α-iodohydrin are inactive (Banik *et al.* 1972). This requirement appears to hold true for the sugar derivatives as a single high dose of 6-fluoro-6-deoxyglucose did not produce a rapid and reversible antifertility effect in the rat (Ford 1982*b*), but did have an effect on the testicular phase of spermatogenesis causing damage to the seminiferous tubules and testicular atrophy.

Conclusion

While (S)-α-chlorohydrin and the chlorinated sugars may never become acceptable as antifertility agents for the human male, continued research into their mechanism of action within mature sperm could highlight specific cellular processes which may be exploited by other, less toxic, chemicals. Very few studies with α-chlorohydrin have so far been performed using human sperm but it appears that human sperm are not as susceptible to the antiglycolytic effects as are the sperm of other species (Hommonai *et al.* 1975; Chulavatnatol *et al.* 1977; Ford *et al.* 1979).

The toxicity of α-chlorohydrin in humans is unknown. Nevertheless, there is evidence that humans are ingesting compounds that are being metabolized to α-chlorohydrin. Mono- and di-esters of α-chlorohydrin have been identified in protein hydrolysates used in dehydrated soups and as food flavourings (Velisek *et al.* 1980; Silhankova *et al.* 1982) and fatty acid chlorohydrins have been characterized as lipid components of an edible jellyfish (Phylum Cnidaria, Class Scyphozoa) (White and Hager 1977). The registration of soil fumigants and nematocides containing 1,2-dibromo-3-chloropropane, which is metabolized by the rat to α-chlorohydrin (Jones *et al.* 1979*a*), has been banned by the U.S.A. Environmental Protection Agency (Campt 1977). This was a result of an investigation which found that the compound may 'damage human reproductive functions, and may cause sterility in males'.

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