Production of (*S*)-3-chlorolactaldehyde from (*S*)- α -chlorohydrin by boar spermatozoa and the inhibition of glyceraldehyde 3-phosphate dehydrogenase *in vitro*

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Summary. The (*S*)-isomer of the male antifertility agent α -chlorohydrin was metabolized by mature boar spermatozoa *in vitro* to (*S*)-3-chlorolactaldehyde. This oxidative process, which did not occur when (*R*)- α -chlorohydrin was offered as a substrate, was catalysed by an NADP⁺-dependent dehydrogenase that converts glycerol to glyceraldehyde. (*S*)-3-chlorolactaldehyde, produced by this metabolic reaction or when added to suspensions of boar spermatozoa, was a specific inhibitor of glyceraldehyde 3-phosphate dehydrogenase as assessed by the accumulation of fructose 1,6-bisphosphate and the triosephosphates. When glycerol and (*S*)- α -chlorohydrin were added concomitantly to boar spermatozoa *in vitro*, the presence of glycerol decreased the degree of inhibition of glyceraldehyde 3-phosphate dehydrogenase. Extracts of glyceraldehyde 3-phosphate dehydrogenase that were obtained from boar spermatozoa incubated with (*S*)- α -chlorohydrin or (*R,S*)-3-chlorolactaldehyde showed significant reductions in their enzymic activity.

Introduction

The action of α -chlorohydrin (3-chloropropan-1,2-diol) as a male antifertility agent has been investigated in many species *in vivo* and with spermatozoa *in vitro* (see Jones, 1978, 1983). The mechanism of its action, which has been deduced from studies involving spermatozoa obtained from the ram (Brown-Woodman, Mohri, Mohri, Suter & White, 1978), boar (Hutton, Dawson & Jones, 1980), rhesus monkey, rat, hamster and man (Ford, Harrison, Takkar & Waites, 1979), involves the inhibition of the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12). This causes a decrease in the glycolytic flux which limits the ability of the spermatozoa to synthesize ATP. Consequently, when they are ejaculated, the affected spermatozoa have diminished motility and fertilization cannot occur.

The (*S*)-enantiomer of α -chlorohydrin (*I*, Text-fig. 1) possesses the antifertility response *in vivo* and the antiglycolytic action *in vitro* whilst the (*R*)-enantiomer is completely ineffective (see Jones,

Text-fig. 1. The structures of (*S*)- α -chlorohydrin (*I*) and (*S*)-3-chlorolactaldehyde (*II*).

1983). The (*S*)-isomer is not the inhibitory compound *per se* but it undergoes oxidation within mature spermatozoa to an active metabolite, (*S*)-3-chlorolactaldehyde (*II*, Text-fig. 1), which possesses the same stereochemistry as (*R*)- or (*D*)-glyceraldehyde-3-phosphate, the substrate for the inhibited enzyme (Stevenson & Jones, 1982).

In this paper we present evidence that this biotransformation within mature boar spermatozoa is performed by an NADP⁺-dependent dehydrogenase that is involved in the metabolism of glycerol. In addition, the activity of glyceraldehyde 3-phosphate dehydrogenase was measured in boar spermatozoa that had been incubated with (*S*)- α -chlorohydrin or (*R,S*)-3-chlorolactaldehyde.

Materials and Methods

Chemicals and substrates. (*R*)- and (*S*)- α -chlorohydrin (Porter & Jones, 1982) and (*R,S*)-[3-³⁶Cl]- α -chlorohydrin (sp. act. 240 μ Ci/mmol) (Jones, 1975) were synthesized to confirmed purity by established procedures. (*R,S*)-3-chlorolactaldehyde was prepared by the method of Williams, Payne, Sullivan & Van Ess (1960). D-[U-¹⁴C]Fructose, L-[U-¹⁴C]lactate, [1-¹⁴C]pyruvate, [U-¹⁴C]glycerol and [U-¹⁴C]glycerol 3-phosphate were purchased from Amersham International plc (Amersham HP7 9LL, U.K.). All enzymes, substrates and co-factors were obtained from Sigma Chemical Co. (St Louis, Missouri 63178, U.S.A.). All other chemicals and reagents were of analytical grade and all solutions were prepared in glass-distilled deionized water.

Metabolic studies. For each experiment, spermatozoa were obtained from each cauda epididymidis of 15–20 mature boars (*Sus scrofa*) within 1.5 h of slaughter at the abattoir. Suspensions of washed spermatozoa were prepared at a concentration of 2–3 g/10 ml (20–35 mg protein/ml or $\sim 10^8$ cells/ml) in modified phosphate-buffered saline (PBS) (Stevenson & Jones, 1982). Only those samples observed by microscopic examination to contain >90% motile spermatozoa were used in the metabolic studies. Incubations were performed in stoppered Warburg flasks shaken at 120 cycles/min at 34°C for 1 h, each incubate containing washed spermatozoa (0.9 ml), (*S*)- α -chlorohydrin, (*R,S*)-3-chlorolactaldehyde or PBS (0.05 ml) and radioactive substrate (50 nCi, 0.05 ml) at the concentrations indicated in Table 1. Metabolically-derived ¹⁴CO₂ was collected (Dawson, 1977) and assayed in a Beckman model LB3800 liquid scintillation counter (Stevenson & Jones, 1982). Neutralized and deproteinized incubation solutions (Stevenson & Jones, 1982) were used for the assay of fructose 1,6-bisphosphate, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate by the method of Michal & Beutler (1975). Protein was estimated (Lowry, Rosebrough, Farr & Randall, 1951) using bovine serum albumin as a standard and the results were calculated using the linear transform equation of Coakley & James (1978).

Spectrophotometric studies. Enzyme activities were assayed in a Varian 634 spectrophotometer by following the change in absorbance at 340 nm corresponding to the oxidation or reduction of nicotinamide coenzymes. The conditions used were those of O'Brien & Schofield (1980) (for aldose reductase activity) and Kormann, Hurst & Flynn (1972) (for NADP⁺-dependent dehydrogenase activity).

Isolation of aldose reductase(s) from boar spermatozoa. Samples of boar spermatozoa (10–30 g) were collected in and washed with 20 mM-sodium phosphate buffer (pH 7.4) containing 5 mM-mercaptoethanol. The sedimented spermatozoa were suspended in 4 volumes of distilled water and disrupted by sonic oscillation (20 kHz for 10 min at 4°C). This suspension was processed by the method of O'Brien & Schofield (1980) to the stage of 60% (NH₄)₂SO₄ saturation, the precipitate was dissolved in 10 mM-sodium phosphate (pH 7.2) and this solution was used for spectrophotometric studies.

Isolation of an NADP⁺-dependent dehydrogenase from boar spermatozoa. Suspensions of boar spermatozoa in PBS were disrupted by sonic oscillation and processed by the method of Kormann

et al. (1972) to the stage of 80% $(\text{NH}_4)_2\text{SO}_4$ saturation. The precipitate was dissolved in 50 mM-sodium phosphate (pH 7.0) and dialysed against 100 volumes of 10 mM-sodium phosphate (pH 7.0) at 4–6°C for 40 h. The dialysate was concentrated to 50% volume by ultrafiltration and used in the spectrophotometric studies. Reaction flasks containing the dialysate (3 ml), 100 mM-sodium phosphate (pH 7.0, 3.75 ml), NADP^+ (0.4 mM) and $[\text{U-}^{14}\text{C}]$ glycerol (100 mM) or (R,S) - $[3\text{-}^{36}\text{Cl}]\alpha$ -chlorohydrin (100 mM) in a total volume of 7 ml were incubated at 34°C. After 4 h, 2,4-dinitrophenylhydrazine reagent (2 ml) was added to each flask. $[\text{U-}^{14}\text{C}]$ Glyceraldehyde or $[3\text{-}^{36}\text{Cl}]\text{chlorolactaldehyde}$ (of unknown configuration) were isolated from the incubates as their 2,4-dinitrophenylhydrazones (Jones, Stevenson, Hutton & Dawson, 1981).

Assay of glyceraldehyde 3-phosphate dehydrogenase activity. Suspensions of washed boar spermatozoa (5 ml) were incubated at 34°C. The substrates were either D-fructose (1 mM) or glycerol (10 mM) and the incubates contained (S)- α -chlorohydrin (0.5 mM), (R)- α -chlorohydrin (10 mM) or (R,S)-3-chlorolactaldehyde (5 mM). After 1 h, these incubations, together with the appropriate control incubations, were centrifuged at 3000 g for 5 min at 4°C. The sperm pellets were suspended in 1.25 ml sucrose (0.25 M) containing EDTA (1 mM) and disrupted by sonic oscillation (20 kHz) at 4°C in 0.5-min bursts for a total of 5 min. The suspensions were centrifuged at 20 000 g for 10 min at 4°C and the supernatant solutions were used for assays of glyceraldehyde 3-phosphate dehydrogenase activity by the method of Velick (1955).

Results

Inhibition of the oxidation of substrates to CO_2

The effects of (S)- α -chlorohydrin or (R,S)-3-chlorolactaldehyde on the oxidation of various ^{14}C -labelled substrates to $^{14}\text{CO}_2$ by boar spermatozoa are summarized in Table 1. A high concentration (5 mM) of (S)- α -chlorohydrin had no effect on the oxidative metabolism of lactate or pyruvate but

Table 1. The effect of (S)- α -chlorohydrin (S α Cl) and (R,S)-3-chlorolactaldehyde (RS3CL) on the oxidative metabolism of various ^{14}C -labelled substrates to $^{14}\text{CO}_2$ by boar spermatozoa for 1 h at 34°C

Substrate		Inhibitor (mM)		CO_2 produced (% of control)†
^{14}C -labelled	Unlabelled	S α Cl	RS3CL	
Lactate (2 mM)	—	5	0	121 $\pm$ 7.8
Pyruvate (2 mM)	—	5	0	99.3 $\pm$ 5.5
Fructose (1 mM)	—	0.5	0	14.8 $\pm$ 6.0***
Fructose (1 mM)	—	0	5	41.8 $\pm$ 9.3*
Glycerol (2 mM)	—	0.5	0	120 $\pm$ 10.5
Glycerol (2 mM)	—	0	5	12.4 $\pm$ 1.8**
Fructose (1 mM)	Glycerol (1 mM)	0.5	0	87.5 $\pm$ 5.8
Fructose (1 mM)	Glycerol (10 mM)	0.5	0	91.7 $\pm$ 2.6
Fructose (1 mM)	Glycerol (1 mM)	0	5	28.2 $\pm$ 4.1***
Fructose (1 mM)	Glycerol (10 mM)	0	5	22.4 $\pm$ 1.3***
Glycerol 3-phosphate (2 mM)	—	0.5	0	19.4 $\pm$ 2.5***
Glycerol 3-phosphate (2 mM)	—	0	5	10.3 $\pm$ 0.24***
Glycerol 3-phosphate (2 mM)	Glycerol (1 mM)	0.5	0	93.6 $\pm$ 12.7
Glycerol 3-phosphate (2 mM)	Glycerol (10 mM)	0.5	0	121 $\pm$ 15.2
Glycerol 3-phosphate (2 mM)	Glycerol (1 mM)	0	5	9.3 $\pm$ 0.51***
Glycerol 3-phosphate (2 mM)	Glycerol (10 mM)	0	5	6.2 $\pm$ 0.56***

Values are mean $\pm$ s.e.m. for 4 separate experiments.

† The results for each particular substrate were compared to control incubations containing that substrate with an equivalent volume of PBS replacing the solution of inhibitor.

* $P < 0.02$, ** $P < 0.01$, *** $P < 0.001$ (Student's *t* test).

0.5 mM-(*S*)- α -chlorohydrin inhibited the oxidation of fructose and glycerol 3-phosphate. The inhibition was reduced when 1 mM unlabelled glycerol was present and was abolished by 10 mM-glycerol. When 5 mM-(*R,S*)-3-chlorolactaldehyde was present, the oxidation of fructose, glycerol and glycerol 3-phosphate was inhibited. However, the presence of 10 mM unlabelled glycerol did not overcome the inhibition of the oxidation of fructose or glycerol 3-phosphate.

Accumulation of glycolytic intermediates

In the particular incubations in which an inhibition was demonstrated (Table 1) there was an accumulation of fructose 1,6-bisphosphate, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate compared to the matching controls. This accords with an action involving the inhibition of glyceraldehyde 3-phosphate dehydrogenase (Table 2).

Table 2. Amounts of fructose 1,6-bisphosphate (FBP), dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (G3P) in boar spermatozoa incubated for 1 h at 34°C in the presence or absence of 0.5 mM-(*S*)- α -chlorohydrin (S α Cl) or 5 mM-(*R,S*)-3-chlorolactaldehyde (RS3CL)

Substrate	Inhibitor (mM)		Amount of glycolytic intermediate (pmol/mg protein)		
	S α Cl	RS3CL	FBP	DHAP	G3P
Fructose (1 mM)	0	0	3.1 $\pm$ 3.0	60.5 $\pm$ 3.1	n.d.
	0.5	0	5330 $\pm$ 1160	4020 $\pm$ 640	558 $\pm$ 140
	0	5	2350 $\pm$ 237	1610 $\pm$ 96	195 $\pm$ 40
Glycerol (2 mM)	0	0	63.3 $\pm$ 2.85	164 $\pm$ 22.4	n.d.
	0.5	0	110 $\pm$ 40	116 $\pm$ 31	n.d.
	0	5	409 $\pm$ 174	264 $\pm$ 63.4	26.1 $\pm$ 15.7
Glycerol 3-phosphate (2 mM)	0	0	40.4 $\pm$ 15.6	141 $\pm$ 36	27.7 $\pm$ 13.7
	0.5	0	135 $\pm$ 63.9	206 $\pm$ 14.5	144 $\pm$ 38
	0	5	198 $\pm$ 83.4	290 $\pm$ 50.6	118 $\pm$ 5.61

Values are mean $\pm$ s.e.m. for 4 separate experiments.
n.d. = not detectable.

Preincubation of spermatozoa with (S)- α -chlorohydrin

The oxidation of fructose was inhibited by 85% when 0.5 mM-(*S*)- α -chlorohydrin was added at the same time as the substrate but by 95% when the (*S*)- α -chlorohydrin was added 2.5 min before the fructose (Table 3). (*S*)- α -chlorohydrin (0.5 mM) had no effect on the oxidation of glycerol when the two substances were added simultaneously but when the spermatozoa were preincubated with 0.5 mM-(*S*)- α -chlorohydrin for 2.5 min the oxidation of glycerol was inhibited by almost 90%.

Table 3. Effect of preincubation time with (*S*)- α -chlorohydrin (0.5 mM) on the oxidation of D-[U-¹⁴C]fructose (1 mM) and [U-¹⁴C]glycerol (2 mM) to ¹⁴CO₂ by boar spermatozoa for 1 h at 34°C

Substrate	CO ₂ evolved (% of control) after preincubation for:			
	0 min	2.5 min	5 min	10 min
Fructose	14.8 $\pm$ 6.0*	6.64 $\pm$ 1.43*	5.49 $\pm$ 1.56*	4.78 $\pm$ 1.24*
Glycerol	104 $\pm$ 8.85	12.7 $\pm$ 0.001*	8.14 $\pm$ 0.525*	7.70 $\pm$ 0.001*

Values are mean $\pm$ s.e.m. for 4 separate experiments.

* $P < 0.001$ (Student's *t* test).

Oxidation of glycerol and (S)- α -chlorohydrin

When 1 mM-glycerol or 1 mM-(S)- α -chlorohydrin were offered as substrates, the extract of aldose reductase(s) from boar spermatozoa did not show any appreciable activity as assessed by the inability to produce NADH. When 1 mM-(R,S)-glyceraldehyde or 1 mM-(R,S)-3-chlorolactaldehyde was added to the reaction cuvettes containing the extract, exogenous NADH was oxidized to NAD⁺. The rate of this oxidation was increased by addition of SO₄²⁻ which is a known stimulator of aldose reductase activity (Hers, 1962).

The dehydrogenase extract was able to oxidize both glycerol and (S)- α -chlorohydrin when NADP⁺ was present but not when the co-factor was NAD⁺. There was no activity with either co-factor when (R)- α -chlorohydrin was added to the dehydrogenase preparation.

Table 4. The activity of glyceraldehyde 3-phosphate dehydrogenase isolated from boar spermatozoa incubated for 1 h at 34°C in the presence of (S)- α -chlorohydrin or (R,S)-3-chlorolactaldehyde with fructose (1 mM) or glycerol (10 mM) as the substrate

Substrate	Inhibitor	nmol substrate oxidized/min/mg protein at 25°C (% of control)
Fructose	—	100†
	(S)- α -chlorohydrin (0.5 mM)	38.5 $\pm$ 9.74*
	(R)- α -chlorohydrin (10 mM)	83.5 $\pm$ 14.8
	(R,S)-3-chlorolactaldehyde (5 mM)	63.5 $\pm$ 5.59**
Glycerol	—	100‡
	(S)- α -chlorohydrin (0.5 mM)	102 $\pm$ 4.14
	(R,S)-3-chlorolactaldehyde (5 mM)	72.6 $\pm$ 4.29***

Values are the mean $\pm$ s.e.m. of 4 separate experiments.

† Control value 5.56 $\pm$ 1.07 nmol substrate oxidized/min/mg protein.

‡ Control value 6.72 $\pm$ 1.16 nmol substrate oxidized/min/mg protein.

* $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$ (Student's t test).

Activity of sperm glyceraldehyde 3-phosphate dehydrogenase

Boar spermatozoa were incubated for 1 h at 34°C with fructose (1 mM) or glycerol (10 mM) as substrates. When the spermatozoa were disrupted by sonic oscillation, the activities of glyceraldehyde 3-phosphate dehydrogenase were 5.56 $\pm$ 1.07 and 6.72 $\pm$ 1.16 nmol glyceraldehyde 3-phosphate oxidized/min/mg protein, respectively, at pH 8.5 and 25°C. With fructose as the substrate, the presence of 0.5 mM-(S)- α -chlorohydrin in the incubation medium reduced the activity of the enzyme by ~60%, whereas the presence of 10 mM-(R)- α -chlorohydrin had no significant effect (Table 4). With glycerol as the substrate, 0.5 mM-(S)- α -chlorohydrin had no significant effect on the activity of glyceraldehyde 3-phosphate dehydrogenase. In the presence of 5 mM-(R,S)-3-chlorolactaldehyde the activity of the enzyme was reduced by ~35% (fructose as substrate) and ~28% (glycerol as substrate).

Discussion

Boar spermatozoa are capable of converting (S)- α -chlorohydrin into (S)-3-chlorolactaldehyde and this metabolite inhibits glyceraldehyde 3-phosphate dehydrogenase (Stevenson & Jones, 1982). The conversion of (S)- α -chlorohydrin into (S)-3-chlorolactaldehyde was confirmed by comparing the

effects of (*S*)- α -chlorohydrin and (*R,S*)-3-chlorolactaldehyde on the oxidative metabolism by boar spermatozoa of specific glycolytic substrates to CO₂. (*S*)- α -Chlorohydrin inhibited the oxidation of both fructose and glycerol 3-phosphate but had no effect on the oxidation of either substrate when glycerol was present. The oxidation of glycerol was unaffected by (*S*)- α -chlorohydrin but the oxidation of all three substrates was inhibited by (*R,S*)-3-chlorolactaldehyde either alone or, in the case of fructose and glycerol 3-phosphate, in the presence of glycerol. These observations suggested that the presence of glycerol, but not the presence of either of its metabolites (glycerol 3-phosphate or dihydroxyacetone phosphate), was preventing the formation of (*S*)-3-chlorolactaldehyde from (*S*)- α -chlorohydrin. This was confirmed by preincubating spermatozoa with (*S*)- α -chlorohydrin for short periods before the glycerol was added: there was considerable inhibition of the oxidative metabolism of glycerol which was increased as the preincubation time was increased.

The established pathway for the metabolism of glycerol by spermatozoa is its conversion by glycerol kinase to glycerol 3-phosphate which then produces dihydroxyacetone phosphate by an FAD-linked glycerol 3-phosphate dehydrogenase (Schenkman, Richart & Westerfeld, 1965; Ford, 1981). However, as the activity of glycerol kinase is low in boar spermatozoa (Mohri & Masaki, 1967), there may be an alternative pathway involving the oxidation of glycerol to glyceraldehyde which could then produce glyceraldehyde 3-phosphate. Our results can be explained if such a pathway operated in boar spermatozoa and if the enzyme responsible was able to convert (*S*)- α -chlorohydrin to (*S*)-3-chlorolactaldehyde. There are three classes of enzymes that are capable of interconverting aldehydes and primary alcohols; aldose reductases, NAD⁺-dependent alcohol dehydrogenases and NADP⁺-dependent glycerol dehydrogenases. Our preparation of aldose reductase(s) from boar spermatozoa (O'Brien & Schofield, 1980) used glyceraldehyde and (*R,S*)-3-chlorolactaldehyde as substrates but not glycerol or (*S*)- α -chlorohydrin. This is characteristic of this class of enzyme in which the reaction is overwhelmingly in favour of alcohol formation (Flynn, 1982). However, when boar spermatozoa were processed to yield a fraction of high dehydrogenase activity (Kormann *et al.*, 1972), the oxidation of glycerol and (*S*)- α -chlorohydrin proceeded in the presence of exogenous NADP⁺ but not in the presence of NAD⁺. Addition of 2,4-dinitrophenylhydrazine reagent to these NADP⁺-catalysed reactions, in which [U-¹⁴C]glycerol and [3-³⁶Cl]-(*R,S*)- α -chlorohydrin were the substrates, enabled the isolation of radioactive 2,4-dinitrophenylhydrazone derivatives which were identified as those of, respectively, glyceraldehyde and 3-chlorolactaldehyde of unknown configuration. Since there was no reaction when (*R*)- α -chlorohydrin was offered as a substrate we propose that this conversion is specific for (*S*)- α -chlorohydrin and that the product is (*S*)-3-chlorolactaldehyde.

Therefore, an NADP⁺-dependent enzyme, which may be a glycerol dehydrogenase (Kormann *et al.*, 1972), which is capable of oxidizing glycerol to glyceraldehyde and (*S*)- α -chlorohydrin to (*S*)-3-chlorolactaldehyde, is present in boar spermatozoa. The involvement of glycerol in the antifertility activity of α -chlorohydrin has been observed on many occasions. Coppola (1969) was the first to describe α -chlorohydrin as a "metabolic antagonist" of glycerol while others suggested that it was a "toxic form of glycerol" (Gunn, Gould & Anderson, 1969; Brown-Woodman & White, 1976). Metabolic studies with ram spermatozoa *in vitro* led Brown-Woodman *et al.* (1978) to comment that "the relatively high concentration of α -chlorohydrin required to inhibit the metabolism of glycerol was completely unexpected" while Edwards, Dacheaux & Waites (1976) reported that "the presence of glycerol apparently protects spermatozoa from this damage [inhibition of glycolysis by α -chlorohydrin], which suggests that some derivative of α -chlorohydrin is formed by spermatozoa and is the active agent in the inhibition of glycolysis". With the demonstration that an NADP⁺-dependent enzyme is involved in the production of (*S*)-3-chlorolactaldehyde, these observations can now be explained. Glycerol and (*S*)- α -chlorohydrin can probably compete as substrates for this enzyme so that the presence of glycerol would suppress the amount of (*S*)-3-chlorolactaldehyde which could be produced to a level below that which is necessary to inhibit glyceraldehyde 3-phosphate dehydrogenase.

The second process that is involved in the anti-glycolytic action of (*S*)- α -chlorohydrin is the

inhibition of glyceraldehyde 3-phosphate dehydrogenase by (S)-3-chlorolactaldehyde. While metabolic studies have shown that the accumulation of the triose-phosphates and fructose 1,6-bisphosphate is indicative of the inhibition of this enzyme, no direct evidence has so far been produced. The activity of glyceraldehyde 3-phosphate dehydrogenase was determined in extracts of boar spermatozoa that had been incubated with fructose or glycerol as substrates. When the incubations with fructose were performed in the presence of (S)- α -chlorohydrin, the activity of the enzyme was significantly reduced although there was no effect when (R)- α -chlorohydrin was present in the incubate in higher concentrations. This can be interpreted as an inhibition of the enzyme by (S)-3-chlorolactaldehyde which was produced by the spermatozoa from the (S)- α -chlorohydrin. With glycerol as the carbon source, however, the presence of (S)- α -chlorohydrin had no effect on the activity of glyceraldehyde 3-phosphate dehydrogenase since the presence of glycerol would decrease the amount of (S)-3-chlorolactaldehyde that would be produced by the NADP⁺-dependent dehydrogenase. Confirmation of these proposals was obtained when the incubations were performed in the presence of (R,S)-3-chlorolactaldehyde: for each substrate, the activity of glyceraldehyde 3-phosphate dehydrogenase was significantly reduced.

A much higher concentration of exogenous (R,S)-3-chlorolactaldehyde is required to inhibit sperm glyceraldehyde 3-phosphate dehydrogenase compared to that which is produced endogenously from (S)- α -chlorohydrin. This may be due to there being a limitation on the passage of (S)-3-chlorolactaldehyde across the sperm membrane. Evidence for this has been obtained in a previous study which involved the incubation of boar spermatozoa with (R,S)-[3-³⁶Cl]- α -chlorohydrin (Stevenson & Jones, 1982). (S)-[3-³⁶Cl]Chlorolactaldehyde was not detected in the supernatant solution but was present only in the supernatant solution derived from the spermatozoa after they had been disrupted by sonic oscillation. These observations have been proposed as

Text-fig. 2. The proposed mechanism for the antiglycolytic action of (S)- α -chlorohydrin within mature boar spermatozoa. The compound (I) is oxidized to (S)-3-chlorolactaldehyde (II) by an NADP⁺-dependent dehydrogenase which converts glycerol to glyceraldehyde. The metabolite (II) inhibits glyceraldehyde 3-phosphate dehydrogenase, resulting in a decrease in the glycolytic flux and the accumulation of glyceraldehyde 3-phosphate, dihydroxyacetone phosphate and fructose 1,6-bisphosphate.

indicating that the passage of (S)-3-chlorolactaldehyde across the sperm membrane is not as facile as is the case with the isomers of α -chlorohydrin which, when incubated with boar spermatozoa, rapidly enter the cells (Stevenson & Jones, 1982).

We propose, therefore, that the antiglycolytic activity of (S)- α -chlorohydrin in boar spermatozoa *in vitro* is the result of two distinct events. First, the oxidation of the compound to (S)-3-chlorolactaldehyde by an NADP⁺-dependent dehydrogenase and second, inhibition of glyceraldehyde 3-phosphate dehydrogenase by this specific metabolite (Text-fig. 2). The species-specificity of (S)- α -chlorohydrin as an antifertility agent may be due to one or both events not being able to occur within the spermatozoa of a particular species. For example, the fertility of male rabbits is unaffected by administration of high doses of (R,S)- α -chlorohydrin (Ericsson, 1970; Samojlik & Chang, 1970) and this could be due to the inability of rabbit spermatozoa to oxidize readily (S)- α -chlorohydrin to (S)-3-chlorolactaldehyde (Ford & Jones, 1983). When rabbit spermatozoa were incubated in the presence of low concentrations of (R,S)-3-chlorolactaldehyde, the glycolytic flux was decreased due to the specific inhibition of glyceraldehyde 3-phosphate dehydrogenase (Ford & Jones, 1983).

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