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Inhibition of Fructolytic Enzymes in Boar Spermatozoa by (S)-α-Chlorohydrin and 1-Chloro-3-hydroxypropanone

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Abstract

When boar spermatozoa were incubated with the (S)-isomer of the male antifertility agent α-chlorohydrin the activity of glyceraldehyde-3-phosphate dehydrogenase was inhibited. The (R)-isomer had no significant effect on the activity of this enzyme whereas (R,S)-3-chlorolactaldehyde caused an inhibition of its activity and also in that of lactate dehydrogenase. The *in vitro* production of (S)-3-chlorolactaldehyde, the active metabolite of (S)-α-chlorohydrin, was attempted by incubating boar spermatozoa with 1-chloro-3-hydroxypropanone. Preliminary results lead us to propose that this compound is converted into (S)-3-chlorolactaldehyde as well as to another metabolite which is an inhibitor of other enzymes within the fructolytic pathway.

Introduction

Of the many non-steroidal chemicals which are known to elicit an antifertility response in the male (Jackson 1966), only one compound has most of the attributes of an ideal male contraceptive. This compound, (S)-α-chlorohydrin [(S)-3-chloropropan-1,2-diol, I (Fig. 1)], affects the metabolic activity of mature spermatozoa by inhibiting the activity of the glycolytic or fructolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) thereby causing a decrease in the fructolytic flux. This limits the capability of the spermatozoa to synthesize ATP so that when

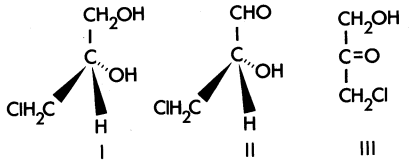

Fig. 1. Structures of (S)-α-chlorohydrin (I), (S)-3-chlorolactaldehyde (II) and 1-chloro-3-hydroxypropanone (III).

they are ejaculated, the affected spermatozoa are unable to sustain their motility and fertilization cannot be achieved (Jones 1983). The isomeric specificity of this inhibitory action has been demonstrated in the mature spermatozoa of the ram (Ford *et al.* 1979), boar (Stevenson and Jones 1982), guinea pig (Jones and Ford 1984) and bull (Jones and du Toit 1985). Furthermore, the renal toxicity associated with the administration of (R,S)-α-chlorohydrin (Jones 1978) has been shown to be due solely to metabolites of the (R)-isomer (Porter and Jones 1982) which has neither an antifertility action *in vivo* nor an anti-fructolytic action *in vitro* (Jones 1978).

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The mechanism proposed for the inhibitory action of (S)-α-chlorohydrin on fructolysis has been deduced from studies using boar spermatozoa (Stevenson and Jones 1985). (S)-α-Chlorohydrin is oxidized *in situ* by an $NADP^+$-dependent dehydrogenase to (S)-3-chlorolactaldehyde (II, Fig. 1) which is the actual inhibitor of glyceraldehyde-3-phosphate dehydrogenase. However, investigations into the manner by which this inhibition occurs have been impaired by the unavailability of (S)-3-chlorolactaldehyde. The racemic mixture has been synthesized (Williams *et al.* 1960) but a study of its action on spermatozoal metabolism involves other effects due to the presence of (R)-3-chlorolactaldehyde and, possibly, to the conversion of this isomer into toxic metabolites (Stevenson and Jones 1984).

In this paper we report on the effects of both (S)-α-chlorohydrin and (R,S)-3-chlorolactaldehyde on the activity of glyceraldehyde-3-phosphate dehydrogenase in boar spermatozoa and our preliminary attempts to overcome the problems associated with (R)-3-chlorolactaldehyde. This has involved the synthesis of 1-chloro-3-hydroxypropanone (III, Fig. 1) and a study of its action on spermatozoal metabolism in the event that this compound could be converted specifically into (S)-3-chlorolactaldehyde by triosephosphate isomerase *in situ*.

Materials and Methods

Collection of Spermatozoa

For each metabolic experiment, entire testis–epididymis complexes were obtained from groups of 15–30 mature boars (*Sus domesticus*) within 1 h of slaughter at the abattoir. Spermatozoa were flushed, at 34°C, from the cauda epididymides (Hutton *et al.* 1980) with the phosphate-buffered saline (PBS) devised by Robinson (1949) but with glucose omitted. The spermatozoal suspension (40–60 ml) was centrifuged at 2500 *g* for 10 min at 20°C. The supernatant solution was removed and the sedimented spermatozoa were resuspended in PBS, centrifuged again at 2500 *g* for 10 min at 20°C and the spermatozoa finally suspended in the same medium at 34°C at a concentration of 2–3 g of sedimented spermatozoa per 10 ml (15–25 mg protein/ml).

Determination of Enzyme Activities

Suspensions of washed spermatozoa in PBS (5 ml) were incubated at 34°C. The substrate was either D-fructose (1 mM) or glycerol (10 mM) and the incubations contained (S)-α-chlorohydrin (0·5 mM) or (R)-α-chlorohydrin (10 mM) or (R,S)-3-chlorolactaldehyde (5 and 10 mM). After 1 h these incubates, together with the appropriate control incubations, were centrifuged at 3000 *g* for 5 min at 4°C. The spermatozoal pellets were resuspended in 1·25 ml sucrose (0·25 M) containing EDTA (1 mM) and mercaptoethanol (1 mM) and the cells were disrupted at 0°C by sonic oscillation (15–20 kHz) in 0·5-min bursts for a total of 5 min (Brown-Woodman *et al.* 1978). The sonified suspensions were centrifuged at 2000 *g* for 10 min at 4°C and samples of the supernatant solutions (0·05 ml) were used for the assay of glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase activities by the methods of Racker (1957) and Czok and Lamprecht (1974), respectively.

Metabolic Studies

For the measurement of metabolically derived $^{14}CO_2$, suspensions of washed spermatozoa, in a final volume of 1 ml, were incubated for varying times at 34°C in conventional Warburg flasks (capacity 27 ml) in the presence of 1-chloro-3-hydroxypropanone, at concentrations indicated in the text, with the following substrates (0·5 μCi, 18·5 kBq): D-[U-^{14}C]fructose (1 mM), [U-^{14}C]pyruvate (2 mM), L-[U-^{14}C]lactate (2 mM), [U-^{14}C]glycerol (2 mM) and L-[U-^{14}C]glycerol-3-phosphate (2 mM). The centre wells contained 0·1 ml of 2 M NaOH and the flasks were shaken at 120 cycles/min with air as the gas phase. The incubations were terminated by the addition of 0·1 ml 3 M $HClO_4$ and the liberated $^{14}CO_2$, trapped in the centre well as $Na_2{}^{14}CO_3$, was assayed by liquid scintillation counting (Dawson 1977). After removal of the deproteinized material by centrifugation at 2500 *g* for 10 min at 4°C, the supernatant solution was neutralized to pH 6·5–7·5 with 3 M KOH by using narrow-range indicator paper. The neutralized solution was adjusted to a final volume of 3·5 ml with purified water and kept

overnight at −20°C. The precipitated $KClO_4$ was removed by centrifugation at 2500 *g* for 10 min and the supernatant solution, stored at −20°C, used for the assay of lactate and fructolytic intermediates.

Assay of Lactate and Fructolytic Intermediates

Lactate (Hohorst 1963), fructose-1,6-bisphosphate, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate (Michal and Beutler 1974) were measured spectrophotometrically with a Varian 634 spectrophotometer by following the change in $A_{340\,nm}$ corresponding to the oxidation or reduction of nicotinamide nucleotides.

Counting of Radioactivity

Liquid scintillation counting was performed in a Beckman LS3800 liquid scintillation counter, cpm being corrected for background and converted to dpm by reference to a quench-correction curve prepared from a ^{14}C-labelled standard. The scintillation fluid (5 ml/vial) consisted of 0·6% (w/v) 2,5-diphenyloxazole (Koch-Light Ltd, Colnbrook, Bucks., U.K.) in toluene : Triton X-100 (1 : 1 v/v).

Materials

(R)- and (S)-α-Chlorohydrin were prepared by the method of Porter and Jones (1982) and (R,S)-3-chlorolactaldehyde according to Williams *et al.* (1960). The synthesis, characterization and stability of 1-chloro-3-hydroxypropanone is described in the Appendix. D-[U-^{14}C]Fructose, L-[U-^{14}C]lactate, [U-^{14}C]pyruvate, [U-^{14}C]glycerol and L-[U-^{14}C]glycerol-3-phosphate were purchased from Amersham International plc, Bucks., U.K. All enzymes, co-enzymes and substrates were obtained from Sigma Chemical Co., St Louis, Missouri, U.S.A. All other chemicals and reagents were of analytical grade and solutions were prepared in water which had been purified by reverse osmosis.

Protein Determination

Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard. Results were calculated using the linear transform equation of Coakley and James (1978).

Table 1. Activity of glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase isolated from boar spermatozoa which were incubated for 1 h at 34°C in the presence of various inhibitors and substrates

*$P < 0·05$; **$P < 0·02$; ***$P < 0·01$ (Student's *t*-test)

Inhibitor	Substrate	Glyceraldehyde-3-phosphate dehydrogenase activity		Lactate dehydrogenase activity	
		Control[A]	As % of control	Control[A]	As % of control
–	D-Fructose (1 mM)	5·50±0·76	100	30·9±3·4	100
(R)-α-Chlorohydrin (10 mM)	D-Fructose (1 mM)		84±15		95·4±5·3
(S)-α-Chlorohydrin (0·5 mM)	D-Fructose (1 mM)		38·5±9·7*		101±3
–	D-Fructose (1 mM)	5·56±1·07	100	22·9±3·1	100
(R,S)-3-Chlorolactaldehyde (5 mM)	D-Fructose (1 mM)		63·5±5·6**		90·5±6·6
(R,S)-3-Chlorolactaldehyde (10 mM)	D-Fructose (1 mM)		48·1±6·2***		79·3±3·7*
–	Glycerol (10 mM)	6·72±1·16	100	24·9±2·4	100
(S)-α-Chlorohydrin (0·5 mM)	Glycerol (10 mM)		102±4		106±6
(R,S)-3-Chlorolactaldehyde (5 mM)	Glycerol (10 mM)		72·6±4·3		88·1±3·4*

[A] Expressed as nanomoles of substrate oxidized or reduced per minute per milligram protein at 25°C. Values are the mean ± s.e.m. for four experiments.

Results

Inhibition of Glyceraldehyde-3-Phosphate Dehydrogenase by (S)-3-Chlorolactaldehyde

When boar spermatozoa were incubated with D-fructose (1 mM) as the substrate, the presence of (R)-α-chlorohydrin (10 mM) had no significant effect on the activity of glyceraldehyde-3-phosphate dehydrogenase when it was subsequently isolated (Table 1). When the incubation was performed in the presence of (S)-α-chlorohydrin (0·5 mM) the activity of the isolated enzyme was reduced by 60% but this was not evident when the substrate was glycerol (10 mM). Neither (R)- nor (S)-α-chlorohydrin had any appreciable effect on the activity of lactate dehydrogenase. When the incubation was performed with either D-fructose (1 mM) or glycerol (10 mM) as the substrate, the presence of (R,S)-3-chlorolactaldehyde (5 and 10 mM) reduced the activity of glyceraldehyde-3-phosphate dehydrogenase and the higher concentration showed a significant effect on the activity of lactate dehydrogenase.

Inhibition of the Oxidation of Fructose by 1-Chloro-3-hydroxypropanone

Low concentrations of 1-chloro-3-hydroxypropanone inhibited the production of respiratory $^{14}CO_2$ by boar spermatozoa with D-[U-^{14}C]fructose (1 mM) as the substrate (Fig. 2).

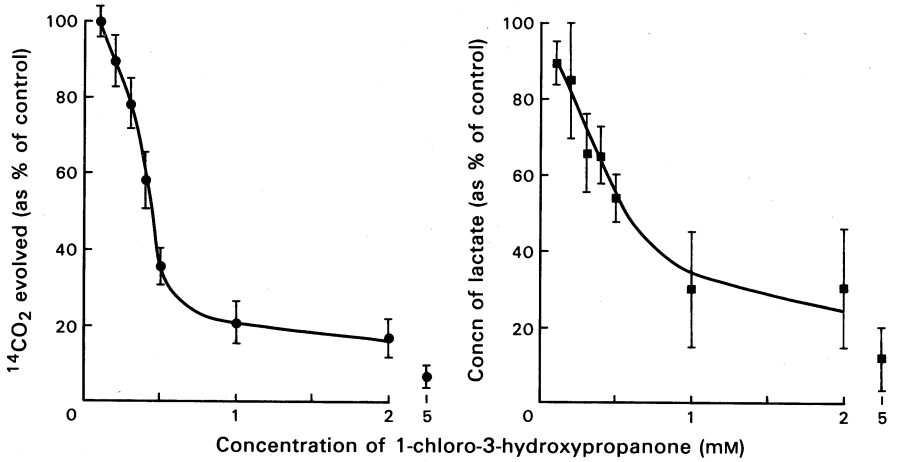

Fig. 2. Effect of increasing concentrations of 1-chloro-3-hydroxypropanone on the oxidation of D-[U-^{14}C]fructose (1 mM) to $^{14}CO_2$ (●) and the concentration of lactate (■) by boar spermatozoa for 1 h at 34°C. Values, given as a percentage of duplicate controls, are the mean ± s.e.m. for four experiments.

Onset of Inhibitory Activity

Inhibition of the oxidation of D-[U-^{14}C]fructose to $^{14}CO_2$ in boar spermatozoa by 1-chloro-3-hydroxypropanone was studied over a period of 1 h. While the inhibitory activity increased with increasing periods of incubation, there was no significant effect at a concentration of 0·25 mM for the first 0·3 h (Fig. 3*a*); after 1 h the oxidation was inhibited by approx. 20%. At a concentration of 0·5 mM, no significant effect was evident for the first 0·15 h but after 1 h the oxidation was inhibited by approx. 70% (Fig. 3*b*).

Effect on Fructolytic Intermediates

When key fructolytic intermediates were assayed in the incubations resulting from the onset of inhibitory activity experiments, fructose-1,6-bisphosphate and dihydroxyacetone phosphate were found to have accumulated (Fig. 3). The presence of glyceraldehyde 3-phosphate could not be demonstrated in those incubations

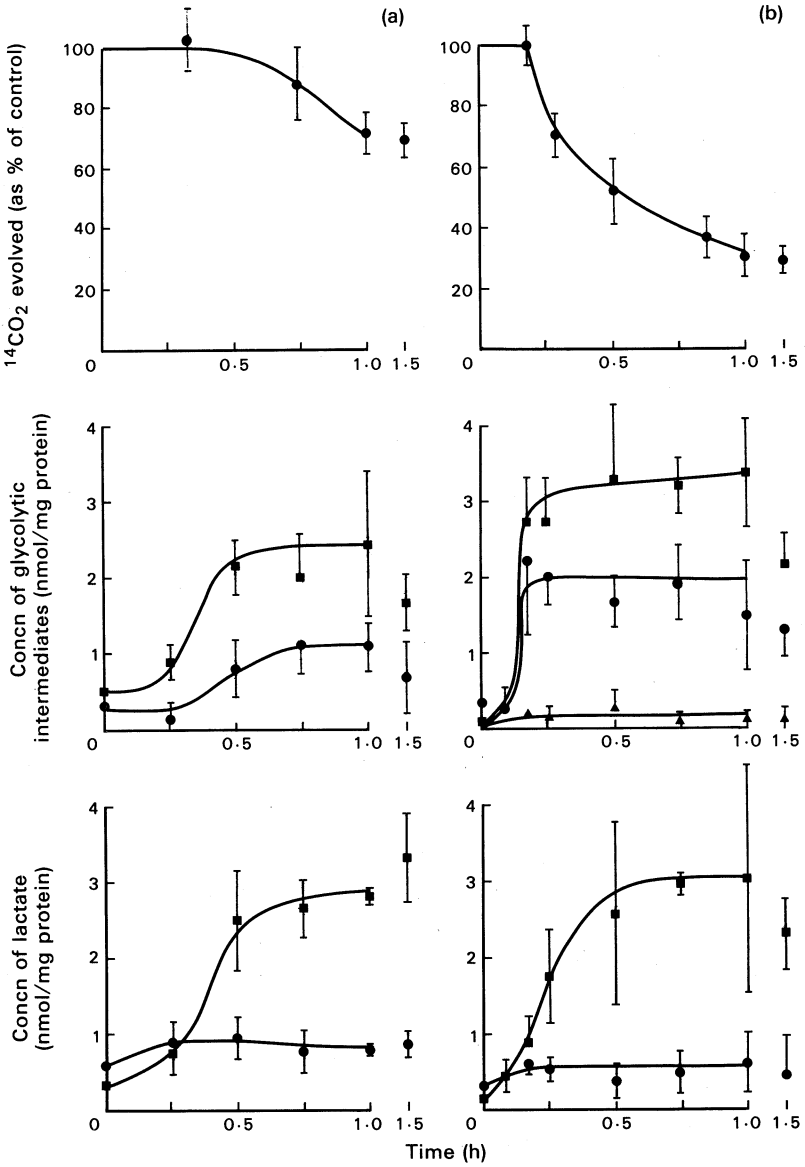

Fig. 3. Effect of 0·25 mM (*a*) and 0·5 mM (*b*) 1-chloro-3-hydroxypropanone on the oxidation of D-[U-^{14}C]fructose (1 mM) to $^{14}CO_2$ by boar spermatozoa for 1 h at 34°C (upper graphs); the accumulation of fructose-1,6-bisphosphate (■), dihydroxyacetone phosphate (●) and glyceraldehyde 3-phosphate (▲) (centre graphs) and the effect on the concentration of lactate (●) relative to control values (■) (lower graphs). Values are given as the mean ± s.e.m. of three experiments.

performed in the presence of 0·25 mM 1-chloro-3-hydroxypropanone but when the concentration of inhibitor was 0·5 mM small amounts of this intermediate could be detected. At both concentrations of inhibitor the increase in the concentrations of fructose-1,6-bisphosphate and dihydroxyacetone phosphate was not immediate but occurred after short delays which paralleled the onset of inhibitory activity.

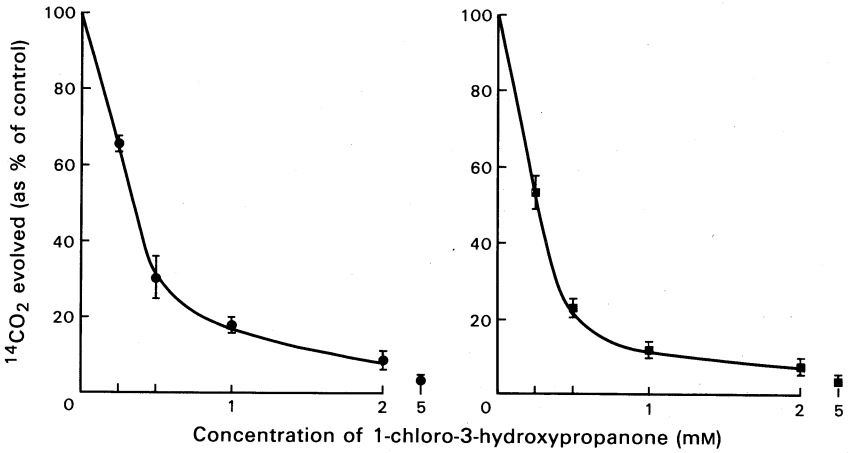

Fig. 4. Effect of increasing concentrations of 1-chloro-3-hydroxypropanone on the oxidation of [U-^{14}C]glycerol (2 mM) (●) and L-[U-^{14}C]glycerol-3-phosphate (2 mM) (■) to $^{14}CO_2$ by boar spermatozoa for 1 h at 34°C. Values, given as a percentage of duplicate controls, are the mean ± s.e.m. of four experiments.

In the absence of 1-chloro-3-hydroxypropanone, lactate was found to increase in concentration (Fig. 3). However, in the presence of the compound there was no accumulation of lactate, the concentration of which remained stable during the incubation period.

Table 2. Effect of increasing concentrations of 1-chloro-3-hydroxypropanone on the oxidation of [U-^{14}C]pyruvate (2 mM) and L-[U-^{14}C]-lactate (2 mM) to $^{14}CO_2$ by boar spermatozoa for 1 h at 34°C

Concn of 1-chloro-3-hydroxypropanone in incubation medium (mM)	CO_2 produced (as % of control) with substrates: Pyruvate	Lactate
0·1	113·5 ± 10·9	106·5 ± 5·6
0·5	101·5 ± 5·1	108·0 ± 14·2
1·0	57·2 ± 3·2	61·0 ± 8·7
2·0	31·0 ± 2·3	30·8 ± 3·4

Inhibition of the Oxidation of Glycerol and Glycerol-3-Phosphate

Low concentrations of 1-chloro-3-hydroxypropanone inhibited the production of respiratory $^{14}CO_2$ by boar spermatozoa with [U-^{14}C]glycerol (2 mM) or L-[U-^{14}C]-glycerol-3-phosphate (2 mM) as substrates (Fig. 4). While it was not feasible to assay key fructolytic intermediates when glycerol-3-phosphate was the substrate (Michal

and Beutler 1974), it was possible when glycerol was used as the substrate. In this case, fructose-1,6-bisphosphate, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate could not be detected.

Inhibition of the Oxidation of Lactate and Pyruvate

Concentrations of 1-chloro-3-hydroxypropanone up to 0·5 mM had no effect on the production of respiratory $^{14}CO_2$ by boar spermatozoa with L-[U^{14}C]lactate (2 mM) or [U-^{14}C]pyruvate (2 mM) as substrates. However, inhibition of the oxidation of both substrates occurred at higher concentrations of the inhibitor (1 and 2 mM) to similar extents (Table 2).

Discussion

The action of (S)-α-chlorohydrin in boar spermatozoa is an inhibition of the reaction involving glyceraldehyde-3-phosphate dehydrogenase which results in an increase in the amounts of fructose-1,6-bisphosphate, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate and a depletion of those intermediates beyond the formation of 1,3-bisphosphoglycerate, including lactate (Stevenson and Jones 1982). This inhibitory action does not involve (S)-α-chlorohydrin *per se* but requires the oxidation of (S)-α-chlorohydrin to (S)-3-chlorolactaldehyde, the actual inhibitory metabolite, which has the same stereochemistry as that of (R)-glyceraldehyde 3-phosphate, the substrate for the inhibited enzyme. Furthermore, the oxidation of (S)-α-chlorohydrin is catalysed by an $NADP^+$-dependent dehydrogenase which is involved in the oxidative metabolism of glycerol (Stevenson and Jones 1985).

Investigations into the manner by which glyceraldehyde-3-phosphate dehydrogenase is inhibited have been hindered by the unavailability of synthetic (S)-3-chlorolactaldehyde. Experiments which employ (R,S)-3-chlorolactaldehyde are complicated by the fact that the (R)-isomer, or its metabolites, has other inhibitory actions on metabolic processes within boar spermatozoa (Stevenson and Jones 1984). Nevertheless, in the present study the inhibition of glyceraldehyde-3-phosphate dehydrogenase has been confirmed (Table 1). When boar spermatozoa were incubated in the presence of (S)-α-chlorohydrin or (R,S)-3-chlorolactaldehyde, the activity of the isolated enzyme was decreased. (R)-α-Chlorohydrin had no inhibitory effect nor did (S)-α-chlorohydrin when glycerol was used as a carbon source since its presence does not permit the oxidation of (S)-α-chlorohydrin to (S)-3-chlorolactaldehyde. Whereas (S)-α-chlorohydrin had no effect on the activity of lactate dehydrogenase (the activity of which was assessed as a 'control' enzyme), this was always decreased when (R)-3-chlorolactaldehyde was present in the incubations.

Investigations into the manner by which spermatozoal glyceraldehyde-3-phosphate dehydrogenase is inhibited led us to consider the synthesis of 1-chloro-3-hydroxypropanone for two reasons. The first was based on an observation by Brown-Woodman *et al.* (1978) who reported that when ejaculated ram spermatozoa were incubated with (R,S)-α-chlorohydrin, the activities of three fructolytic enzymes were shown to be reduced. As these enzymes were glyceraldehyde-3-phosphate dehydrogenase, triosephosphate isomerase and fructose-1,6-bisphosphate aldolase, and with our present knowledge that the former is inhibited by (S)-3-chlorolactaldehyde, it seemed possible that another metabolite could be effecting the activity of the other two enzymes; the likely candidate being 1-chloro-3-hydroxy-

propanone. The second reason was derived from a study in which 3-chlorolactaldehyde (of unknown configuration) was identified as a metabolite of the anti-fructolytic compound 6-chloro-6-deoxyglucose (Ford 1982) in guinea pig spermatozoa (Jones and Ford 1984). As this implied that the chloro-sugar was being degraded within the spermatozoa either by fructolysis or a similar metabolic pathway, it led us to speculate that spermatozoal triosephosphate isomerase could be capable of converting 1-chloro-3-hydroxypropanone into 3-chlorolactaldehyde, specifically of the (S)-configuration. From this it also follows that the reversible nature of the triose-phosphate isomerase-catalysed reaction could produce 1-chloro-3-hydroxypropanone from (S)-3-chlorolactaldehyde which may account for the effect of (R,S)-α-chlorohydrin on triosephosphate isomerase and fructose-1,6-bisphosphate aldolase (Brown-Woodman *et al.* 1978).

When boar spermatozoa were incubated in the presence of 1-chloro-3-hydroxy-propanone the oxidative metabolism of fructose was inhibited but, as with the response to (S)-α-chlorohydrin (Stevenson and Jones 1982), a period of incubation was necessary before this action was evident. Estimations of key fructolytic intermediates revealed a similar response to that caused by (S)-α-chlorohydrin; fructose-1,6-bisphosphate and dihydroxyacetone phosphate had accumulated and there was a concomitant decrease in the concentration of lactate (Fig. 3). Such an effect indicated that 1-chloro-3-hydroxypropanone had inhibited the pathway beyond the formation of the triosephosphates and prior to the production of pyruvate, presumably at the reaction catalysed by glyceraldehyde-3-phosphate dehydrogenase. However, the fructolytic intermediates accumulated rapidly and remained at this level for the duration of the incubation period. This suggested that more than one enzyme was being inhibited because the specific inhibition of glyceraldehyde-3-phosphate dehydrogenase would be expected to lead to the continuing accumulation of the triosephosphates and, consequently, of fructose-1,6-bisphosphate. Evidence supporting this emerged from experiments in which the substrate for the spermatozoa was glycerol. 1-Chloro-3-hydroxypropanone inhibited the oxidative metabolism of glycerol in a concentration-dependent manner (Fig. 4) but when the incubation suspensions were assayed, the triosephosphates and fructose-1,6-bisphosphate were not detected.

Several explanations for these results can be offered but these are conjecture at this stage. The delay in the onset of inhibitory activity towards the oxidation of fructose may correspond to the conversion of the compound to (S)-3-chlorolactaldehyde. As well as this, it seems probable that 1-chloro-3-hydroxypropanone is being converted into another metabolite such as 1-chloro-3-hydroxypropanone phosphate. This phosphate, which would possess the structure necessary in an inhibitor of triosephosphate isomerase and fructose-1,6-bisphosphate aldolase, has been shown to inhibit triosephosphate isomerase in microsomal preparations of preputial gland tumours (Wylke and Snyder 1969), and the iodo-analogue irreversibly inactivates the commercially available enzyme (Hartman 1968). A phosphorylated derivative of 1-chloro-3-hydroxypropanone may also interfere with glycerol-3-phosphate dehydrogenase which would account for the absence of dihydroxyacetone phosphate in spermatozoal incubates when glycerol was the carbon source. Further studies involving the incubation of radioactive 1-chloro-3-hydroxypropanone with boar spermatozoa will be required to examine the validity of these hypotheses.

It would be fortuitous if the inhibitory action of 1-chloro-3-hydroxypropanone on spermatozoal metabolism was confined to the stage of fructolysis involving the production of the triosephosphates and had no effect on the metabolism of pyruvate and lactate as does (R)-3-chlorolactaldehyde (Stevenson and Jones 1984). At a concentration of 0·5 mM this is so with the oxidative metabolism of fructose, glycerol and glycerol-3-phosphate each being inhibited by approximately 65% and that of lactate and pyruvate being unaffected. Above this concentration the oxidative metabolism of both lactate and pyruvate was impeded but at this stage it is not known whether this is a general action on cellular metabolism or whether it is specific to the metabolic activity of spermatozoa. This and other aspects of the anti-fructolytic action of 1-chloro-3-hydroxypropanone are under investigation.

Acknowledgment

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Appendix

Synthesis and Characterization of 1-Chloro-3-hydroxypropanone

The synthesis of 1-chloro-3-hydroxypropanone has been described twice in the literature. Smirnoff (1904) reported an unstable, crystalline solid, m.p. 74°C, reputed to give a stable aqueous solution at a concentration of 0·4–0·5%. Williams (1969) prepared an oil, unstable at room temperature, but which at 0°C slowly yielded a crystalline solid, m.p. 71–2°C, thought to be a dimer of 1-chloro-3-hydroxypropanone.

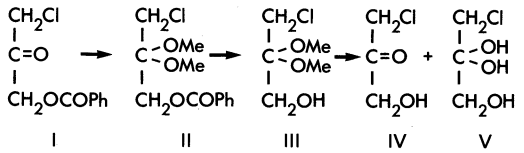

Fig. 5. Synthesis of 1-chloro-3-hydroxypropanone (IV) and its hydrate (V).

The strategy of the present synthesis was to prepare by an unambiguous route a stable, water-soluble precursor from which 1-chloro-3-hydroxypropanone could be generated in aqueous solution. The scheme eventually chosen was the conversion of racemic 3-chloropropane-1,2-diol to 1-*O*-benzoyl-3-chloropropane-1,2-diol followed by oxidation to 1-*O*-benzoyl-3-chloropropanone (I, Fig. 5) according to

the procedure of Hartman (1970). The carbonyl group was protected as the dimethyl ketal (II) and the benzoyl group removed to give the desired precursor, 1-chloro-3-hydroxypropanone dimethyl ketal (III). Mild acid hydrolysis of III at room temperature produced an equilibrium mixture of 1-chloro-3-hydroxypropanone (IV) and its hydrate (V) in approximately equal proportions. The solution was neutralized with solid sodium bicarbonate and suitable dilutions were prepared immediately for the metabolic studies.

Experimental

T.l.c. was performed on Merck pre-coated F_{254} silica gel G plastic sheets (0·2 mm thick) which were developed in diethyl ether: light petroleum (b.p. 30–60°) (1 : 1, v/v). Detection was either by u.v. light (for I and II) or by spraying with 2,4-dinitrophenylhydrazine reagent (for III). ^1H and ^{13}C n.m.r. spectra were measured using a Varian XL-400 spectrometer with chemical shifts referenced to internal TMS for $CDCl_3$ solutions and internal dioxane for D_2O solutions.

1-O-Benzoyl-3-chloropropanone dimethyl ketal (II)

1-*O*-Benzoyl-3-chloropropanone (I, 31 g) (Hartman 1970) was dissolved in a mixture of methanol (170 ml), trimethylorthoformate (260 ml) and H_2SO_4 (18 M, 2·6 ml) and kept at room temperature. T.l.c. indicated the loss of I (R_F 0·55) and the appearance of II (R_F 0·79) which was complete in 20 h. The solution was neutralized with solid K_2CO_3 (5 g), filtered, and the solvents removed at 40°C under vacuum. The residue, in chloroform (200 ml), was washed once with ice-cold saturated $NaHCO_3$ (450 ml), the chloroform phase dried (Na_2SO_4) and the chloroform removed under vacuum. The residue was distilled and redistilled to give II (28·8 g, 76%), b.p. 140–2°C/2 mm, as a colourless, viscous oil. (Found: C, 56·0; H, 5·9. $C_{12}H_{15}O_4Cl$ requires C, 55·7; H, 5·8%). The mass spectrum showed no M; *m/z* 227/9 (M − OCH_3), 209 (M − CH_2Cl), base peak at 123/5 (M − C_6H_5CO − OCH_3), 105 (C_6H_5CO) and 77 (C_6H_5). ^1H n.m.r.: δ ($CDCl_3$) 3·32, s, (2 × OCH_3); 3·68, s, (CH_2Cl); 4·48, s, (CH_2OCOPh); 7·47, m, (2 × *m*-ArH); 7·60, m, (*p*-ArH); 8·06, m, (2 × *o*-ArH).

1-Chloro-3-hydroxypropanone dimethyl ketal (III)

To a solution of II (25·8 g) in methanol (280 ml) was added 4 M aqueous NaOH (35 ml) and the solution was kept at room temperature. T.l.c. indicated the disappearance of II and the appearance of III (R_F 0·35) which was complete in 6 h. The methanol was removed under vacuum and the residue was extracted with ether (5 × 50 ml). The combined ether extracts were dried (Na_2SO_4) and the ether removed to yield III (12·9 g, 84%), b.p. 73–4°C/1·5 mm. (Found: C, 38·7; H, 7·1. $C_5H_{11}O_3Cl$ requires C, 38·8; H, 7·2%). The mass spectrum showed no M; *m/z* base peak 123/5 (M − OCH_3), 105 (M − CH_2Cl) and 91/3 (M − CH_3OH − OCH_3). ^1H n.m.r.: δ ($CDCl_3$) 2·3–2·45, broad, (OH); 3·30, s, (2 × OCH_3); 3·61, s, (CH_2Cl); 3·71, broad s, (CH_2OH). δ (D_2O) 3·23, s, (2 × OCH_3); 3·61, s, (CH_2OH + CH_2Cl). ^{13}C n.m.r.: δ (D_2O) 41·3, tt, (CH_2Cl), *J* 152·4, 2·6 Hz; 49·3, q, (OCH_3), *J* 144·2 Hz; 57·9, tt, (CH_2OH), *J* 144·4, 2·3 Hz; 102·3, tt, (quaternary C), *J* 7·5, 3·7 Hz.

1-Chloro-3-hydroxypropanone (IV)

1-Chloro-3-hydroxypropanone dimethyl ketal (III) (50–100 mg) was dissolved in 1 M HCl (1 ml) and kept at room temperature for 1 h. The solution of IV, the hydrate V and methanol was neutralized (solid $NaHCO_3$) using narrow-range indicator paper, adjusted to 0·5 M with water and aliquots diluted with PBS before their addition to spermatozoal incubations. The products of the hydrolysis were characterized by both ^1H and ^{13}C n.m.r. spectroscopy. To III (155 mg) in D_2O (840 μl) in an n.m.r. tube was added 10 M DCl (10 μl) and the solution mixed to give III (1 M) in DCl (0·1 M). Constant monitoring of the reaction revealed the disappearance of III and the appearance of the three products:

(i) *1-Chloro-3-hydroxypropanone (IV):* ^1H n.m.r.: δ 4·39, s, (CH_2Cl), correlates with δ 46·9 (^{13}C); 4·42, s, (CH_2OH), correlates with δ 66·6 (^{13}C). ^{13}C n.m.r.: δ 46·9, t, (CH_2Cl), *J* 150·4 Hz; 66·6, t, (CH_2OH), *J* 143·5 Hz; 206·2, m, (carbonyl C). Calculated values for direct proton–carbon couplings for $-CH_2Cl$ and $-CH_2OH$ are 151 and 142 Hz, respectively (Pretsch *et al.* 1983).

(ii) *1-Chloro-3-hydroxypropanone hydrate (V):* ^1H n.m.r.: δ 3·56, s, (CH_2OH), correlates with δ 64·9 (^{13}C); 3·60, s, (CH_2Cl), correlates with δ 47·8 (^{13}C). ^{13}C n.m.r.: δ 47·8, tt, (CH_2Cl), *J* 151·2, 3·0 Hz; 64·9, tt, (CH_2OH), *J* 143·7, 1·9 Hz; 95·4, m, (quaternary C).

(iii) *Methanol:* ^{1}H n.m.r.: δ 3·28, s. ^{13}C n.m.r.: δ 49·8, q, J 142·3 Hz.

The extent of the hydrolysis was approximately 20% in 1 h, 60% in 5 h and was complete within 24 h. When the hydrolysis was performed under identical conditions but in the presence of 1 M DCl, the reaction was complete within 1 h. Thus these conditions were employed for the preparation of an aqueous solution of IV for the biological studies. The products of the hydrolysis slowly decomposed in acid solution, as assessed by the loss of the $-CH_2Cl$ resonances. When neutralized with $NaHCO_3$, the relative amounts of IV and V were unaffected but the rate of their decomposition was accelerated.

Stability and Biological Activity of 1-Chloro-3-hydroxypropanone in Solution

For the biological experiments, the hydrolysis of III was performed during the preparation of the spermatozoal suspensions and dilutions of the neutralized hydrolysate were used immediately in the incubations. Samples of the neutralized hydrolysate which were kept at 4°C showed a progressive loss in biological activity and became ineffective within 1 week. Both the dimethyl ketal (III) and methanol were ineffective in inhibiting the oxidative metabolism of the substrates employed when added to incubations at a concentration of 10 mM.