superoxide and singlet oxygen[13,14]. As these activated oxygen species are continuously generated during normal aerobic metabolism[15], it seems possible that the toxic action of D_2O on aerobically living cells may be partly based on the potentiation of oxygen toxicity. It is interesting to note that activated oxygen species have been implicated in the generation of 'spontaneous' chromosomal breakage in Fanconi's anemia[16] as well as in Bloom syndrome[17]. The increased sensitivity of Fanconi's anemia lymphocytes to the chromosome-breaking effect of D_2O, as indicated by our experiments, may thus suggest the existence of an oxygen-dependent mechanism for D_2O (geno)toxicity.

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Formation of the active antifertility metabolite of (S)-α-chlorohydrin in boar sperm

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Summary. The male antifertility agent (S)-α-chlorohydrin (**I**) is metabolized by boar sperm to (S)-3-chlorolactaldehyde (**II**) by an enzyme that is involved in the oxidation of glycerol to glyceraldehyde. The presence of glycerol decreases the activity of this enzyme towards (S)-α-chlorohydrin in vitro thereby preventing the formation of (S)-3-chlorolactaldehyde, an inhibitor of glyceraldehyde 3-phosphate dehydrogenase in boar sperm.

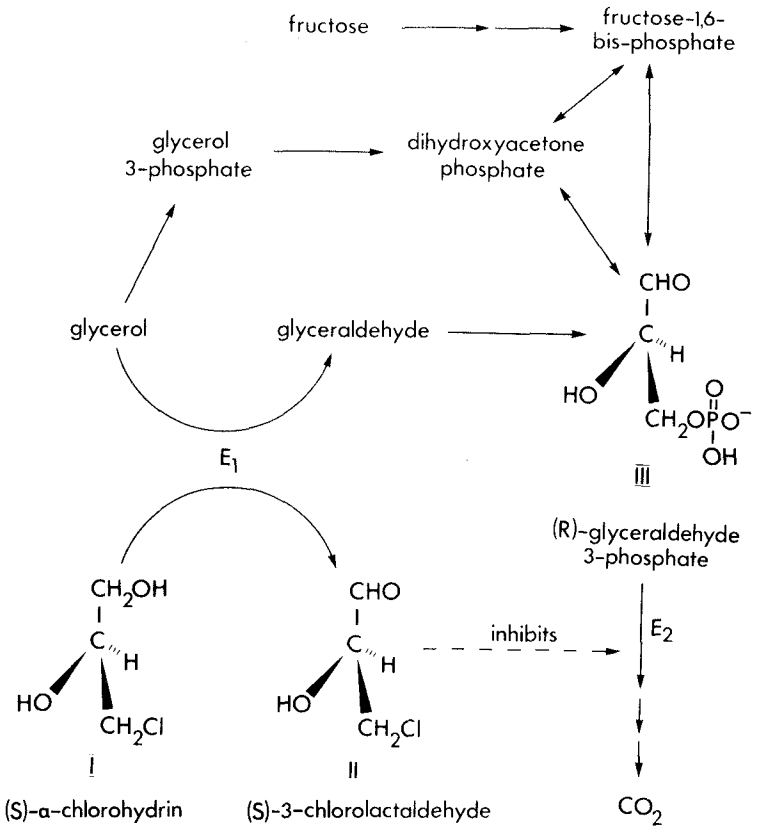
(R,S)-α-Chlorohydrin (3-chloropropan-1,2-diol, **I**) is an antifertility agent that inhibits glycolysis in mature sperm of the ram, guinea-pig, hamster, boar, rat, rhesus monkey and human[2,3]. When the syntheses of the separate isomers of α-chlorohydrin were achieved, this action was shown to be due solely to the (S)-isomer both in vivo[4] and in vitro[5], the site of action involving the inhibition of glyceraldehyde 3-phosphate dehydrogenase[3,5,6]. As the action of (S)-α-chlorohydrin in vitro was not immediate but was evident only after a period of incubation with mature sperm, it was suggested that a metabolite was the actual inhibitory compound[7]. Subsequently, this metabolite was detected in incubates of boar sperm[8] and identified as (S)-3-chlorolactaldehyde (**II**)[9] which is stereochemically identical to (R)-glyceraldehyde-3-phosphate (**III**), the substrate for glyceraldehyde 3-phosphate dehydrogenase[5]. We now present evidence that the formation of this inhibitory metabolite in boar sperm requires an enzyme that is involved in the oxidative metabolism of glycerol.

Washed boar sperm[10] were incubated in phosphate-buffered saline in Warburg flasks at 34 °C for 1 h with uniformly-labeled ^{14}C-substrates and the metabolically-derived $^{14}CO_2$ collected and assayed by standard procedures[11]. (S)-α-Chlorohydrin[12] (0.5 mM) inhibited the production of $^{14}CO_2$ by 90% when ^{14}C-fructose (1 mM) was the substrate but had no effect when ^{14}C-glycerol (2 mM) was the substrate. With ^{14}C-glycerol (2 mM) and (R,S)-3-chlorolactaldehyde (5 mM)[13], however, there was an 88% inhibition in $^{14}CO_2$ production. The oxidation of ^{14}C-glycerol-3-phosphate (2 mM) to $^{14}CO_2$ was similarly inhibited by (S)-α-chlorohydrin (0.5 mM) to the extent of 86% confirming that the presence of glycerol, but not of any of its metabolites on the pathway glycerol → glycerol-3-phosphate → dihydroxyacetone phosphate, was apparently preventing the oxidation of (S)-α-chlorohydrin to (S)-3-chlorolactaldehyde.

Two further experiments substantiated the involvement of glycerol metabolism in the oxidation of (S)-α-chlorohydrin. First, using ^{14}C-fructose (1 mM) as substrate, the inhibitory effect of (S)-α-chlorohydrin (0.5 mM) on $^{14}CO_2$ production was reduced from 90% to 12% when 1 mM glycerol was present and abolished in the presence of 10 mM glycerol. Second, when the sperm suspension was pre-incubated with (S)-α-chlorohydrin (0.5 mM) before ^{14}C-glycerol (2 mM) was added, there was inhibition of $^{14}CO_2$ production which increased with increasing time of pre-incubation.

These results can be accommodated by the scheme shown in the figure in which we propose that a pathway exists for the oxidation of glycerol to glyceraldehyde. In the absence of exogenous glycerol, (S)-α-chlorohydrin is converted by enzyme E_1 to (S)-3-chlorolactaldehyde which inhibits glyceraldehyde 3-phosphate dehydrogenase (E_2). In the presence of exogenous glycerol, the oxidative metabolite is not produced and there is no inhibition of E_2. However, when exogenous (R,S)-3-chlorolactaldehyde is added, E_2 is inhibited[15] thus preventing the oxidative metabolism of ^{14}C-fructose, -glycerol or -glycerol-3-phosphate to $^{14}CO_2$.

As to the identity of enzyme E_1, 2 candidates have been considered. Firstly, aldose reductases are known to interconvert a number of aldehydes and primary alcohols but



The proposed scheme for the metabolism of (S)-α-chlorohydrin in boar sperm. Enzyme E_1 oxidizes (S)-α-chlorohydrin (**I**) to (S)-3-chlorolactaldehyde (**II**) which inhibits glyceraldehyde 3-phosphate dehydrogenase (E_2).

the reaction is overwhelmingly in favor of alcohol formation[16]. Secondly, there are certain dehydrogenases that oxidise glycerol to glyceraldehyde and which are usually $NADP^+$-dependent[17]. In the present study extracts of boar sperm that had been disrupted by sonic oscillation have been demonstrated to be capable of oxidising both glycerol and (S)-α-chlorohydrin when $NADP^+$ is added. When (R,S)-[^{36}Cl]-α-chlorohydrin[18] was used as a substrate and 2,4-dinitrophenylhydrazine reagent[19] was added to the reaction cuvette, [^{36}Cl]-3-chlorolactaldehyde of unknown configuration was isolated and identified as its 2,4-dinitrophenylhydrazone derivative[8].

Even though the nature of this enzyme has not been established, its presence in the mature sperm of certain species may be responsible for the species-specificity of (S)-α-chlorohydrin as a male antifertility agent. For example, α-chlorohydrin is effective in the rat and the boar but not in the rabbit and it is metabolized to 3-chlorolactaldehyde by rat and boar sperm but not by rabbit sperm[8]. The characterization of this enzyme from boar sperm is in progress.

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