

The presence of glucose increases the lethal effect of α -chlorohydrin on ram and boar spermatozoa *in vitro*

W. C. L. Ford and Anne Harrison

Department of Physiology & Biochemistry, The University, Whiteknights, Reading RG6 2AJ, U.K.

Summary. Ram cauda epididymal spermatozoa were incubated for 10 min at 34°C with or without 1.0 mM-*RS- α* -chlorohydrin before (1) 5 mM-D-glucose or (2) 10 mM-L-lactate plus 1 mM pyruvate or (3) 5 mM-D-glucose plus 10 mM-L-lactate plus 1 mM-pyruvate or (4) no substrate was added. Without α -chlorohydrin, the motility, the ATP concentration and the energy charge of the spermatozoa were maintained for 240 min by substrate combinations 1–3 but with no added substrate (4) the motility declined after 60 min. All the values decreased dramatically after 10 min in spermatozoa exposed to α -chlorohydrin in substrate conditions 1 and 3 (glucose present) but α -chlorohydrin had no significant effect in conditions 2 and 4 (no glucose) except after prolonged incubation. In a dose-response experiment glucose-dependent ATP dissipation began to occur with 0.025 mM-*RS- α* -chlorohydrin. A similar effect was seen in boar spermatozoa exposed to 0.1–5.0 mM- α -chlorohydrin and 5 mM-D-glucose. With boar spermatozoa the presence of 10 mM-L-lactate and 1 mM-pyruvate as well as glucose prevented the loss of ATP.

We conclude that this concerted action of α -chlorohydrin and glucose is probably responsible for the contraceptive action of α -chlorohydrin and propose that it may depend on 'futile substrate cycling' in the glycolytic pathway.

Introduction

Both α -chlorohydrin (see Jones, A.R., 1978, 1983; Lobl, 1980) and the 6-chloro-6-deoxysugars (see Ford, 1982) have a rapid and reversible antifertility action in male animals. The contraceptive effect is believed to result from the inhibition of glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12) in spermatozoa (Brown-Woodman, Mohri, Mohri, Suter & White, 1978; Ford, Harrison & Waites, 1981). When α -chlorohydrin and 6-chloro-6-deoxysugars are administered to male rats their effects on the spermatozoa are identical but only α -chlorohydrin will inhibit glyceraldehyde 3-phosphate dehydrogenase in normal spermatozoa exposed to it *in vitro* (Ford & Waites, 1982). The inhibition of glyceraldehyde 3-phosphate dehydrogenase prevents the spermatozoa from obtaining energy from the metabolism of glucose or fructose and if the inhibited spermatozoa are incubated with these substrates the cells soon become immotile with a concomitant decline in ATP concentration (Brown-Woodman *et al.*, 1978; Ford & Harrison, 1981). However, this may not be sufficient to explain why the spermatozoa are infertile because contraceptive doses of the compounds do not impair the ability of the spermatozoa to utilize non-glycolytic substrates (Brown-Woodman *et al.*, 1978; Ford *et al.*, 1981) and spermatozoa from rats made infertile with 6-chloro-6-deoxyglucose remained motile with a normal ATP concentration when incubated with pyruvate plus lactate (Ford & Harrison, 1981). A rich mixture of substrates including lactate and pyruvate is available in the female reproductive tract (Lutwak-Mann, 1962; Iritani, Gomes & VanDemark, 1969; Brackett & Mastroianni, 1974) together with an adequate supply of oxygen (Mastroianni & Jones, 1965). Therefore the spermatozoa should be equally able to obtain energy from lactate and pyruvate *in*

vivo. The presence of glucose is required for the capacitation of mouse spermatozoa *in vitro* although other substrates can maintain the viability of the cells which remain motile and rapidly capacitate if glucose is added subsequently (Hoppe, 1976; Fraser & Quinn, 1981). Therefore α -chlorohydrin might work by blocking the glucose metabolism associated with capacitation. α -Chlorohydrin at 10 μ M will block the acrosome reaction of hamster spermatozoa (Dravland & Meizel, 1981), but hamster spermatozoa will capacitate without glucose (see Rogers, 1978) whereas mice are unusually resistant to the contraceptive action of α -chlorohydrin (Tsunoda & Chang, 1976). It is difficult to carry out extensive metabolic experiments with rodent spermatozoa because of the comparatively small number of cells available. At the same time α -chlorohydrin has similar effects on metabolism in spermatozoa from rams and boars and from rodents and we decided to take advantage of the large numbers of spermatozoa available from these large animals to design experiments to clarify the effect of different substrate combinations on the viability of spermatozoa treated with α -chlorohydrin. A preliminary account of some of this work has appeared (Ford & Harrison, 1983a).

Materials and Methods

Materials

Testes from sexually mature ram lambs were obtained from Alf Meade Ltd, The Abattoirs, Reading. The slaughtermen were asked to keep organs cool but not to chill them and we attempted to collect them as soon as possible after slaughter. The sperm rich fraction of boar semen was generously donated by the Ministry of Agriculture, Fisheries and Food, Artificial Insemination Centre, Shinfield, Reading, and was brought to the laboratory within 1 h of ejaculation. D-[U- 14 C]glucose and D-[2- 3 H]glucose were purchased from Amersham International p.l.c., Amersham HP7 9LL, U.K. Enzymes and biochemicals were supplied by Sigma London Chemical Co. Ltd, Poole, Dorset BH17 7NH, U.K., or by Boehringer Corp. (London) Ltd, Lewes BN7 1LG, U.K. RS- α -Chlorohydrin was supplied by Koch-Light Ltd, Colnbrook SL3 0BZ, U.K., and was redistilled under reduced pressure before use. Other reagents were obtained from Fisons Scientific Apparatus, Loughborough LE11 0R9, U.K.

Preparation of spermatozoa

Ram. A polyvinylchloride cannula (0.8 mm o.d., 0.5 mm i.d.) was inserted in the vas deferens and a cut was made in the cauda epididymidis. Spermatozoa were expelled by forcing phosphate-buffered physiological saline (PBS) (143 mM-NaCl; 2.7 mM-KCl; 8.1 mM- Na_2HPO_4 ; 1.5 mM- KH_2PO_4 ; 0.8 mM- CaCl_2 ; 0.8 mM- MgCl_2 ; 0.1 mM-EDTA (tetrasodium salt); 15 μ M-bovine serum albumin (Cohn Fraction V); 0.25 mM-penicillin G; 80 μ M-streptomycin sulphate: Ford *et al.*, 1981) down the cannula. The spermatozoa were dispersed in the saline and centrifuged (500 g_{av} , 10 min, room temperature); the pellet was washed once and the spermatozoa were suspended in PBS at a concentration of about 10^8 cells/ml.

Boar. The semen was diluted with a roughly equal volume of PBS and was centrifuged (500 g_{av} , 10 min room temperature). The pellet was washed twice and the spermatozoa were suspended in PBS at a concentration of about 10^8 cells/ml.

The concentration of spermatozoa was determined with a haemocytometer.

Experiment 1: the effect of different substrates on ram spermatozoa treated with 1.0 mM- α -chlorohydrin

Two 24-ml samples, A and B, were removed from the sperm suspension. Sample A was mixed with 1.0 ml 25 mM-RS- α -chlorohydrin and Sample B with 1.0 ml PBS. The two samples were incubated with shaking at 34°C and, after 9 min, a 0.25-ml sample was removed from Sample A for motility measurement. After 10 min, a similar sample was removed from Sample B and 1.0-ml samples were taken from each flask and mixed with 0.50 ml 1.0 M-perchloric acid. At the same time,

4.8-ml portions were mixed with 0.20 ml PBS which contained the additions required to produce the following final concentrations: (1) no substrate, (2) 5 mM-D-[U- ^{14}C , 2- ^3H]glucose, (3) 10 mM-L-lactate plus 1 mM-pyruvate, (4) 5 mM-D-[U- ^{14}C , 2- ^3H]glucose plus 10 mM-L-lactate plus 1 mM-pyruvate. The specific activity of the glucose was 0.5 Ci ^3H /mol and 0.05 Ci ^{14}C /mol. Incubation continued at 34°C and 1.0-ml samples were taken from all the flasks 10, 30, 60 and 240 min after the addition of substrate and mixed with 0.50 ml 1.0 M-perchloric acid. Samples for motility measurement (0.25 ml) were removed from the α -chlorohydrin flask with no added substrate (Group 1) after 13, 43 and 225 min and from the corresponding control flask (no α -chlorohydrin) after 28, 58 and 240 min. Similar samples were taken from the other flasks at 1 min (Group 2), 2 min (Group 3) and 3 min (Group 4) after these samples. In the statistical analysis of the motility data, each series of samples taken from the α -chlorohydrin-treated spermatozoa was compared to the series of control samples succeeding it, e.g. the series beginning at 13 min was compared to the control series starting at 28 min. Since sperm motility usually declines during control incubations, this procedure will tend to underestimate the effect of treatment. The acidified samples were centrifuged and neutralized as described previously (Ford *et al.*, 1981).

Experiment 2: the minimum concentration of α -chlorohydrin needed to induce the rapid loss of ATP from ram spermatozoa after the addition of 5 mM-D-glucose

Portions (5 ml) of a suspension of ram spermatozoa were incubated with 0, 0.01, 0.025, 0.05, 0.075 or 0.10 mM-RS- α -chlorohydrin at 34°C for 10 min. Samples (1.0 ml) were removed from each flask and mixed with 0.50 ml 1.0 M-perchloric acid and 5 mM-D-glucose was added to the flasks. Incubation continued at 34°C and 1.0-ml samples were taken 10, 30 and 60 min later.

Experiment 3: the effect of glucose on the loss of ATP in boar spermatozoa treated with α -chlorohydrin

Five 6-ml portions of sperm suspension were incubated at 34°C for 10 min with 0, 0.1, 0.05, 1.0, 2.0 or 5.0 mM-RS- α -chlorohydrin. A 0.5-ml sample was removed from each and mixed with 1.0 ml 0.5 M-perchloric acid and then 1.7-ml portions were taken and mixed with substrate or PBS to give (1) no substrate, (2) 5 mM-D-glucose, (3) 5 mM-D-glucose plus 10 mM-L-lactate plus 1 mM-pyruvate in a total volume of 2.0 ml. Incubation continued at 34°C and 0.5-ml samples were removed from all the flasks 15, 30 and 60 min after the addition of substrate.

The measurement of the motility of the spermatozoa

Motility was measured by a turbidimetric procedure based on that described by Sokoloski, Blasco, Storey & Wolf (1977). A length of polyvinylchloride cannula tubing (2.0 mm o.d., 1.0 mm i.d.) was cemented in the corner of a plastic disposable 3-ml cuvette so that the lower end was about 1 mm above the floor of the cuvette whilst the upper end stood proud of the top of the cuvette by about 1 mm. PBS (1.8 ml) was added to the cuvette which was placed in a Gilford Model 250 spectrophotometer maintained at 34°C by thermostat and the absorbance at 500 nm (A_0) was recorded. A Gilson 'Minipuls II' peristaltic pump was adjusted to deliver 1 ml/min and was attached to a polyvinylchloride cannula which ended in a length of 21-gauge stainless-steel tubing and was sufficient to contain 0.3 ml. Sperm suspension was taken up into the cannula by reversing the pump for 18 sec, the stainless-steel tube was inserted in the plastic guide tube glued to the cuvette and the pump was run forwards for 15 sec to form a layer of 0.25 ml sperm suspension on the floor of the cuvette. Spermatozoa were allowed to swim up into the buffer for 10 min (preliminary experiments indicated that all the motile spermatozoa left the basal layer in this period) and the absorbance (A_1) was measured. The contents of the cuvette were now stirred to bring all the spermatozoa into the suspension and the absorbance was measured again (A_2). The percentage of progressively motile spermatozoa (or the motility index) was taken to be $(A_1 - A_0) \times 100 / (A_2 - A_0)$. The spermato-

zoa would not swim into the top millimeter or so of the buffer and very motile samples sometimes gave results in excess of 100%.

Assay procedures

ATP, ADP, AMP, lactate and pyruvate were measured as described previously (Ford *et al.*, 1981; Ford & Harrison, 1981). Tritiated water was measured after subliming it from a sample of the neutralized perchloric acid extract frozen in the upper compartment of a Thunberg tube. No corrections were applied for isotope discrimination effects.

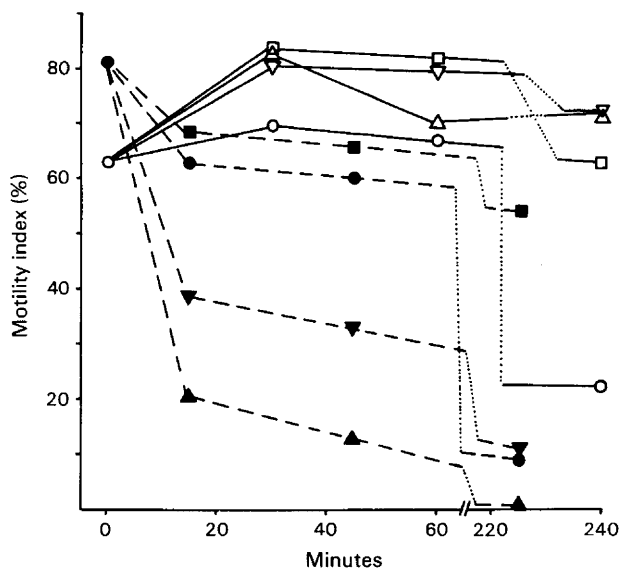
Statistical evaluation of results

The effects of treatments and substrates were analysed by a multifactorial analysis of variance and differences between individual treatments were assessed by a *t* test using the standard errors of difference of the means. The computations were done with a computer program 'GENSTAT' provided by the Department of Applied Statistics, University of Reading. The effect of α -chlorohydrin in the preincubation period before the addition of substrate was tested in a two-way analysis of variance.

Results

Experiment 1: the effects of different substrates on ram spermatozoa treated with 1.0 mM-*RS*- α -chlorohydrin

Motility. The spermatozoa in the initial suspension had a motility index of 65 ± 11.9 (mean \pm s.e.m., $n = 4$). This increased significantly ($P < 0.05$) to 81 ± 10.0 after the 10-min preincubation with 1 mM- α -chlorohydrin but remained essentially unchanged (63 ± 4.8) in the controls. With no added substrate the control spermatozoa maintained their motility for 60 min but after 240 min the motility index had declined to 27 ± 2.8 (Text-fig. 1). When substrates were added the spermatozoa

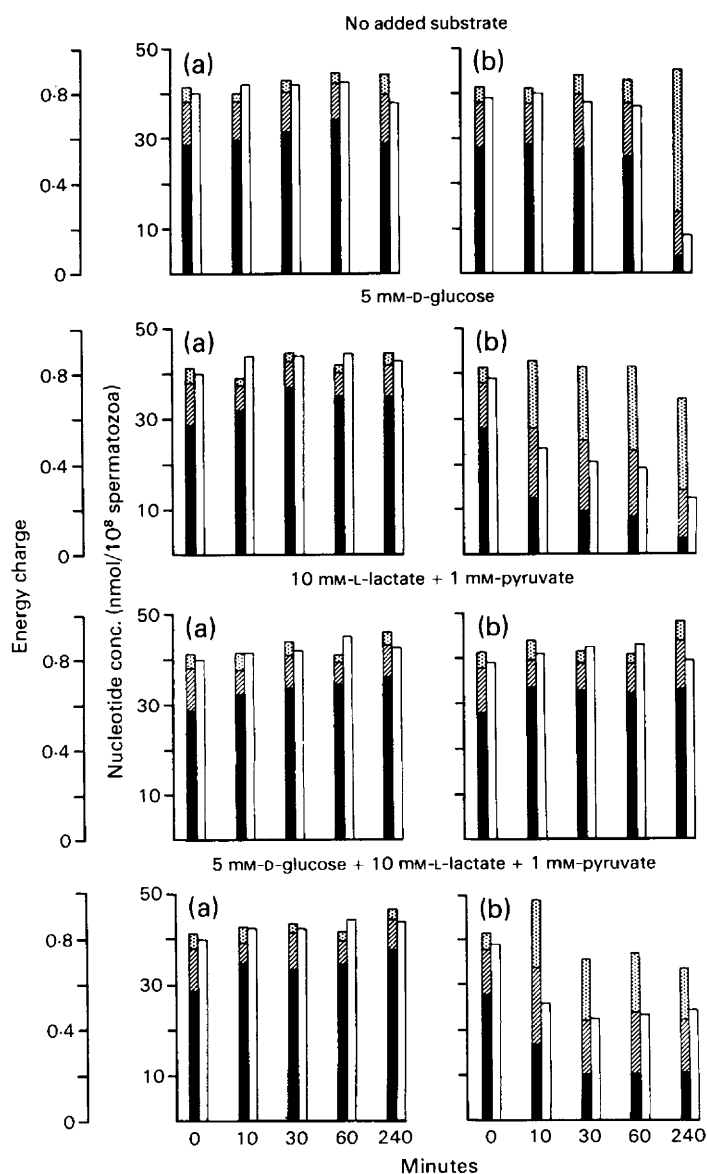


Text-fig. 1. The motility index (for definition see text) of ram cauda epididymal spermatozoa washed and suspended in PBS and preincubated for 10 min at 34°C with 1 mM-*RS*- α -chlorohydrin (●, ▲, ■, ▼) or with no additions (○, △, □, ▽) before the substrates were added and the incubation continued for a further 4 h. No added substrate (○, ●); 5 mM-D-glucose (△, ▲); 10 mM-L-lactate plus 1 mM-pyruvate (□, ■); 5 mM-glucose plus 10 mM-L-lactate plus 1 mM-pyruvate (▽, ▼). Values are means of 4 experiments.

were more motile and they maintained their activity for 240 min (Text-fig. 1). The analysis of variance showed that the effect of substrate overall was significant ($P < 0.001$) but the differences at 30 or 60 min taken by themselves were of doubtful significance ($0.05 < P < 0.10$). When no substrate was added to spermatozoa treated with 1.0 mM- α -chlorohydrin, the motility index was 60 ± 9.8 after 45 min but only 9 ± 3.8 after 225 min and when 10 mM-L-lactate plus 1 mM-pyruvate was added the motility index was 66 ± 6.8 after 45 min and 54 ± 3.3 after 225 min (Text-fig. 1). In these 2 conditions the motility at 15 or 45 min of the spermatozoa exposed to α -chlorohydrin was not significantly different from that of control spermatozoa measured 15 min later, but when the 225/240 min data were included a significant deleterious effect of α -chlorohydrin was revealed ($P < 0.05$, no substrate; $P < 0.01$, lactate + pyruvate). When 5.0 mM-D-glucose was added the motility index declined to 20 ± 2.8 after 15 min and with 5.0 mM-D-glucose plus 10 mM-L-lactate plus 1 mM-pyruvate the motility index was 39 ± 4.2 at this time (Text-fig. 1). The interaction between α -chlorohydrin and glucose responsible for this decline produced a highly significant ($P < 0.001$) interaction sum of squares between α -chlorohydrin and substrate in the analysis of variance.

Adenine nucleotide concentrations. The concentrations of ATP, ADP and AMP in control spermatozoa changed only slightly during incubation for 240 min under any of the substrate conditions and the only significant difference ($P < 0.01$) between the groups was a higher concentration of ADP when no substrate was added. The energy charge (Atkinson & Walton, 1967) remained about 0.80 throughout (Text-fig. 2). The nature of the substrate provided had a large effect in the spermatozoa treated with 1 mM- α -chlorohydrin. With 10 mM-lactate plus 1 mM-pyruvate the adenine nucleotide concentrations remained similar to those in control spermatozoa throughout the experiment. The same was true with no added substrate for the first 60 min but then the concentration of ATP in the treated spermatozoa fell markedly by 240 min ($P < 0.01$) with a concomitant increase in AMP ($P < 0.001$) and a decrease in the energy charge (Text-fig. 2). By contrast, in the incubations with 5 mM-D-glucose or with 5 mM-D-glucose plus 10 mM-L-lactate plus 1 mM-pyruvate, the ATP concentration fell by about 60% after only 10 min and remained low for the remainder of the incubation. There was a corresponding increase in the concentrations of ADP and of AMP so that the total adenine nucleotide concentration remained constant for 60 min although it did decline slightly after 240 min. The energy charge declined dramatically in concert with the ATP concentration (Text-fig. 2). The interaction between α -chlorohydrin treatment and the effect of substrate was significant ($P < 0.001$).

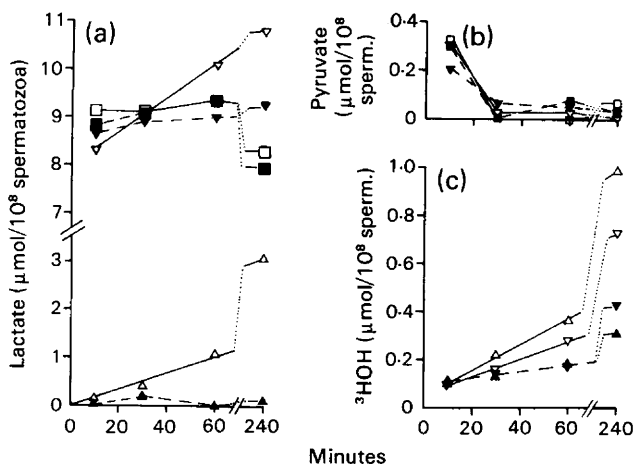
Metabolism of the substrates. No lactate was produced in the absence of added substrate. Control spermatozoa produced about 1 μ mol lactate/ 10^8 spermatozoa from 5 mM-glucose in the first 60 min of the incubation and had produced about 3 μ mol lactate/ 10^8 spermatozoa after 4 h. In the presence of 5 mM-D-glucose plus 10 mM-L-lactate plus 1 mM-pyruvate about 1.8 μ mol of additional lactate/ 10^8 spermatozoa was formed in the first 60 min but only a further 0.6 μ mol lactate/ 10^8 spermatozoa was produced in the next 3 h. The production of lactate was nearly completely inhibited by α -chlorohydrin treatment (Text-fig. 3a). In the incubations with 10 mM-L-lactate plus 1 mM-pyruvate the concentration of lactate did not change significantly during the first 60 min of incubation and about 1 μ mol lactate/ 10^8 spermatozoa was consumed in the subsequent 3 h by both control and α -chlorohydrin-treated spermatozoa, but α -chlorohydrin-treated spermatozoa incubated with glucose, lactate and pyruvate consumed no lactate (Text-fig. 3a). When pyruvate was present in the substrate mixture it was consumed rapidly and this was not affected by α -chlorohydrin (Text-fig. 3b). The release of ^3HOH from [$2\text{-}^3\text{H}$]glucose was only sufficient to account for 60–70% of the lactate production of control spermatozoa and it was slightly decreased when the substrate mixture contained lactate and pyruvate as well as glucose. The apparent shortfall in ^3HOH release can probably be explained by tritium retention in fructose 6-phosphate and isotope discrimination (see Katz & Rognstad, 1976). ^3HOH release after the first 10 min of the incubation was markedly but not completely inhibited by α -chlorohydrin and with α -chlorohydrin treatment the presence of pyruvate and lactate enhanced ^3HOH release in the later stages of the incubation (Text-fig. 3c).



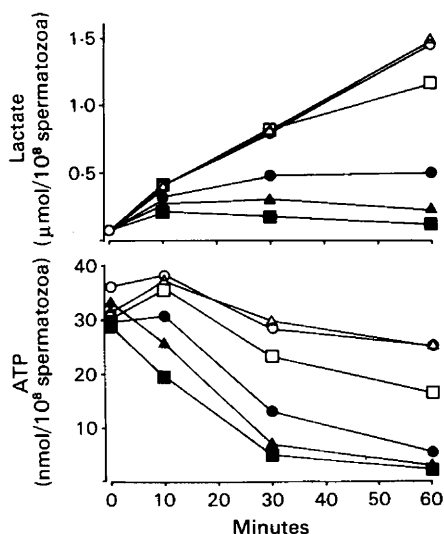
Text-fig. 2. The concentrations of ATP (■), ADP (▨), and AMP (□) and the energy charge $(\text{ATP} + \frac{1}{2}\text{ADP})/(\text{ATP} + \text{ADP} + \text{AMP})$ (□) in the spermatozoa described in Text-fig. 1. (a) Controls, (b) 1 mM-*RS-α*-chlorohydrin. Values are means of 3 experiments.

Experiment 2: the effect of α-chlorohydrin concentration

There was no effect on lactate production from 5 mM-D-glucose by the spermatozoa or on the concentration of ATP in the cells treated by 0.01 mM- α -chlorohydrin, but 0.025 mM-*RS-α*-chlorohydrin caused a small but significant ($P < 0.01$) inhibition of lactate production in the final 30 min of the incubation and a small fall in ATP concentration compared to control spermatozoa ($P < 0.01$). The intensity of both of these effects increased with higher α -chlorohydrin concentration and lactate production after the first 10-min incubation was completely inhibited by 0.075 or by 0.10 mM-*RS-α*-chlorohydrin (Text-fig. 4).



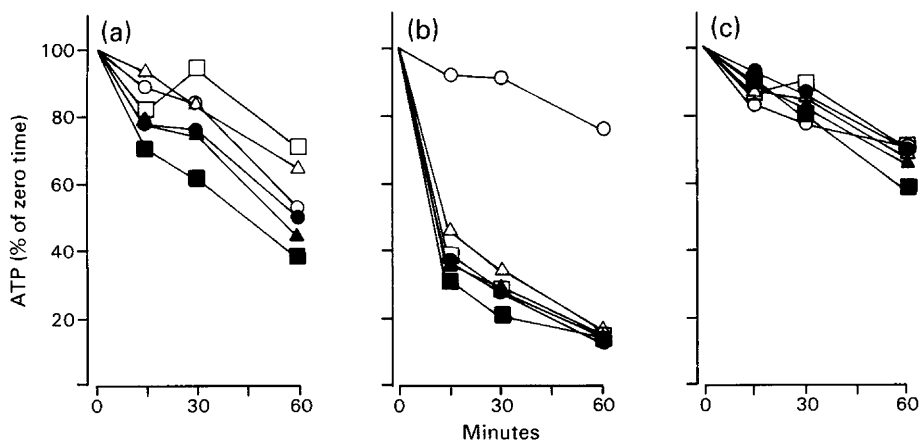
Text-fig. 3. The concentrations of (a) lactate and (b) pyruvate, and (c) the release of ^3HOH from D-[2- ^3H]glucose in the experiment described in Text-fig. 1. $\blacksquare, \blacktriangledown, \blacktriangle$: 1 mM-*RS*- α -chlorohydrin; $\square, \triangledown, \triangle$: controls; $\triangle, \blacktriangle$: 5 mM-D-glucose; \square, \blacksquare : 10 mM-L-lactate plus 1 mM-pyruvate; $\triangledown, \blacktriangledown$: 5 mM-D-glucose plus 10 mM-L-lactate plus 1 mM-pyruvate. Values are means of 3 experiments.



Text-fig. 4. The concentrations of lactate and ATP in ram epididymal spermatozoa, washed and suspended in PBS buffer and incubated for 10 min at 34°C with 0 (\circ), 0.01 (\triangle), 0.025 (\square), 0.05 (\bullet), 0.75 (\blacktriangle) or 0.10 (\blacksquare) mM-*RS*- α -chlorohydrin before 5 mM-D-glucose was added at 0 min on the time-scale shown. Values are means of 3 experiments.

Experiment 3: the effect of α -chlorohydrin and glucose on boar spermatozoa

The initial condition of the spermatozoa was very variable and all the results have been expressed as a percentage of the ATP concentration at zero time which was 18.9 ± 0.58 , 9.9 ± 0.42 and 8.4 ± 0.33 nmol/ 10^8 spermatozoa in the 3 replicates respectively. In the absence of added substrate the ATP concentration in the spermatozoa declined by about 40% during incubation for 1 h



Text-fig. 5. The concentration of ATP as a percentage of its initial value in boar ejaculated spermatozoa washed and suspended in PBS buffer and preincubated for 10 min at 34°C with 0 (○), 0.1 (△), 0.5 (□), 1.0 (●), 2.0 (▲) or 5.0 (■) mM-*RS*- α -chlorohydrin before the addition of no further substrate (a) or 5 mM-D-glucose (b) or 5 mM-D-glucose plus 10 mM-L-lactate plus 1 mM-pyruvate (c). Values are means of 3 experiments.

but this decline was only slightly accelerated by 5 mM-*RS*- α -chlorohydrin. When 5 mM-D-glucose was added the ATP concentration in spermatozoa with no α -chlorohydrin declined more slowly and fell by only 25% after 1 h incubation, but all the concentrations of α -chlorohydrin produced a rapid loss of ATP in the first 15 min. When 5 mM-D-glucose plus 10 mM-L-lactate plus 1 mM-pyruvate was present, α -chlorohydrin had no effect on the ATP concentration which declined by about 30% during the incubation (Text-fig. 5).

Discussion

These data demonstrate that the addition of 5 mM-D-glucose increases the spermicidal effect of α -chlorohydrin to a dramatic extent. In the absence of glucose, the motility and ATP concentration of spermatozoa exposed to α -chlorohydrin was little different from the controls except after quite extended periods of incubation, but when 5 mM-glucose was present these values declined markedly after only 10 min. This effect of glucose was blocked by 10 mM-L-lactate plus 1 mM-pyruvate in boar spermatozoa but not in ram spermatozoa.

If the inhibition of the glycolytic pathway by α -chlorohydrin simply blocked glucose metabolism so that the spermatozoa were unable to obtain energy from it, then α -chlorohydrin-treated spermatozoa with 5 mM-D-glucose would be equivalent to control spermatozoa with no substrate and α -chlorohydrin-treated spermatozoa with 5 mM-D-glucose plus 10 mM-lactate plus 1 mM-pyruvate would be equivalent to control spermatozoa with 10 mM-L-lactate plus 1 mM-pyruvate. Since this was not the case, the actions of α -chlorohydrin and glucose must combine together to produce an unknown but greatly deleterious effect on the spermatozoa. One possibility is that a high rate of 'ATP dissipation' could be set up through 'futile substrate cycling' (see Katz & Rognstad, 1976) in the glycolytic pathway. The low flux through the pathway combined with the high concentration of some glycolytic intermediates (Brown-Woodman *et al.*, 1978; see Ford, 1982) in spermatozoa incubated with glucose or fructose and α -chlorohydrin would favour a high rate of 'futile cycling' in bull spermatozoa (Hammerstedt & Lardy, 1983). This speculation is supported by the observation that the release of ^3HOH from D-[2- ^3H]glucose was inhibited less completely than the production of lactate (Text-fig. 3). This would be expected if there was cycling between glucose

and glucose 6-phosphate (which is in rapid equilibrium with fructose 6-phosphate) through hexokinase and an enzyme with glucose 6-phosphatase activity. Glucose 6-phosphatase and fructose 1,6-bisphosphatase activity are both present in ram spermatozoa (W. C. L. Ford & A. Harrison, unpublished observations).

The glucose-induced ATP loss can occur with low α -chlorohydrin concentrations (0.01–0.025 mM) and it is very probable that it is involved in the contraceptive action of α -chlorohydrin *in vivo*. Fructose will also precipitate a rapid loss of ATP in α -chlorohydrin treated spermatozoa (W. C. L. Ford & A. Harrison, unpublished observations). Glyceraldehyde 3-phosphate dehydrogenase in spermatozoa from animals treated with 6-chloro-6-deoxysugars or with α -chlorohydrin will be inhibited as they pass through the cauda epididymidis (Ford & Harrison, 1983b) but this will have little effect on the motility of or ATP concentration in the spermatozoa because of the very low concentration of glucose or of fructose in the duct (Jones, R., 1978). However, upon ejaculation the spermatozoa are exposed to a high concentration of fructose in seminal plasma and this will quickly result in a decline of the ATP concentration to an extent where the viability of the cells cannot be maintained. This hypothesis is supported by observations on spermatozoa from the cauda epididymidis of rats treated with 6-chloro-6-deoxyglucose. The spermatozoa were as motile as those of the controls immediately after collection but soon became immotile in the presence of glucose although they maintained their activity in the presence of pyruvate plus lactate (Ford & Harrison, 1981).

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