Aust. J. Biol. Sci., 1976, **29**, 545–55

Disruption of the Metabolism, Motility and Morphology of Spermatozoa by Injection of α-Chlorohydrin into Rams

P. D. C. Brown-Woodman and I. G. White

Department of Veterinary Physiology, University of Sydney, Sydney, N.S.W. 2006.

Abstract

Ejaculated spermatozoa from rams given intramuscular injections of α-chlorohydrin (25 mg/kg, daily for 5 days) were studied. Respiratory and glycolytic activity of the spermatozoa was almost entirely suppressed within 1 day and motility had decreased within 4 days of the first injection. Morphologically abnormal spermatozoa appeared in ejaculates after 2 weeks. The most common abnormality was an increase in the number of spermatozoa with looped or bent tails. There was little change in the fructose or amino acid concentration of the seminal plasma. All effects of α-chlorohydrin were fully reversible. It is suggested that the initial primary mode of action of α-chlorohydrin is to disrupt the metabolism of spermatozoa in the cauda epididymis.

Introduction

α-Chlorohydrin (3-chloro-1,2-propanediol) has been shown to be an effective antifertility agent when given orally to the rat, ram and boar, and studies in the rat suggest that it may interfere with the metabolic activity of spermatozoa in the male reproductive tract (Samojlik and Chang 1970; Brown and White 1973; Brown-Woodman and White 1975). Although α-chlorohydrin is too toxic to administer to man, investigation of its mode of action in animals may eventually lead to the development of an oral contraceptive for human use. This paper is concerned with the effect of injected α-chlorohydrin on the ejaculated spermatozoa of the ram; the respiratory and other metabolic activity of the spermatozoa have been studied in conjunction with changes in spermatozoan morphology and motility. The fructose concentration of the seminal plasma was also measured since it reflects the secretory activity of the accessory glands and thus indirectly androgen production by the testes (Mann 1964). These studies show that systemically administered α-chlorohydrin can temporarily suppress the metabolism of spermatozoa in the male genital tract and this may account for its antifertility action.

Materials and Methods

Experiment 1

Seven samples of semen (two per week) were obtained from each of five mature Merino rams (54–69 kg body weight) during a control period. The rams were then injected intramuscularly with α-chlorohydrin, 25 mg/kg body weight each day for 5 days. Semen samples were collected twice during the injection period (1 and 4 days after the first injection) and at the time intervals shown in Table 1 after the last injection.

Semen was collected by electroejaculation (Blackshaw 1954); about 0·6 ml of each ejaculate was frozen in dry ice immediately on collection and later thawed and centrifuged at 1200 *g* for 10 min after which the supernatant was analysed for fructose (Mann 1948; White 1959).

A drop (0·05 ml) of semen was added to 1·0 ml of Krebs–Ringer bicarbonate containing fructose (300 mg/100 ml), and spermatozoan motility was scored by the method of Emmens (1947) over a 3-h period. The maximum possible score was 16.

Estimates were made of the number of morphologically normal and abnormal spermatozoa, separate heads and tails, and spermatozoa with cytoplasmic droplet still attached; for this purpose 0·02 ml of semen was diluted to 10 ml with formal saline.

Five volumes of calcium-free Krebs–Ringer phosphate (KRP), pH 7·2 (Umbreit *et al.* 1972), were added to the semen and the suspension centrifuged at 400 *g* for 10 min. The supernatant was removed and the procedure repeated. The sperm plug was then made up to 10 ml with KRP and duplicate sperm counts were made using a haemocytometer. Duplicate 2·0-ml aliquots of the suspension of spermatozoa were added to the outer compartment of Warburg flasks, containing 0·1 μCi D-[U-^{14}C]glucose, 14 μmol D(+) glucose and antibiotics (0·5 mg each of crystalline penicillin G and streptomycin sulphate) in 0·1 ml KRP; CO_2 was collected into 0·1 ml 20% KOH in the centre well. The flasks were incubated at 37°C for 3 h and shaken 60 times per minute with air as gas phase. Oxygen uptake was determined using the direct manometric technique described by Umbreit *et al.* (1972) and readings were taken at 30-min intervals. Duplicate 2·0-ml aliquots of the suspension of spermatozoa were added to 1·0 ml of the labelled diluent for the determination of the initial glucose and lactic acid concentrations. Initial and incubated samples were deproteinized with equal volumes of 5% $ZnSO_4.7H_2O$ and 0·15 M $Ba(OH)_2.8H_2O$. Glucose was measured by the method of Schmidt (1963) and Werner *et al.* (1970) and lactic acid was measured by the method of Barker and Britton (1957).

Experiment 2

Six rams were trained to serve an artificial vagina and 10 ejaculates were obtained from each ram. Semen was collected on Mondays and Thursdays for sperm metabolism and motility studies, and on Tuesdays and Fridays for examination of sperm morphology and analysis of fructose in the seminal plasma. Sperm motility, morphology, metabolic parameters and seminal fructose concentrations were determined as in experiment 1.

Three rams were then injected with α-chlorohydrin as described in experiment 1 and three rams received an equivalent volume of 0·9% sterile saline and thus served as simultaneous controls.

Semen samples were collected 1 and 4 days after the first injection for metabolic and motility studies and 2 and 5 days after the first injection for examination of sperm morphology and analysis of fructose in the seminal plasma. In addition, phospholipid (Poulos *et al.* 1973) and amino acid concentrations (Brown-Woodman and White 1974) of plasma obtained during the pre-injection and subsequent period were determined.

The three rams injected with α-chlorohydrin were killed on the ninth day after the first injection. Fluid was collected from the rete testis and cauda epididymis for sperm counts and amino acid analysis. Testicular and epididymal tissue was taken for histological examination; the tissue was fixed in Stieve's fixative, processed for light microscopy, stained by the Feulgen reaction and counterstained with light green.

Statistical Analysis

Motility data from the control period were analysed for homogeneity and correlation between variates using a program designed for the CSIRO CDC 3200 computer by Dr I. C. A. Martin. An analysis of variance program (VARTN) originally designed for this computer by Claringbold (1957) and modified by Dr I. C. A. Martin was used for analysis of trends during treatment and post-treatment periods.

One estimation of the fructose content of the seminal plasma was missing from the data used to compile Tables 1 and 3 and a value has been calculated using the formula suggested by Snedecor and Cochran (1971).

Analysis of spermatozoan motility data was done by partitioning of Chi-square using a program developed by Claringbold (1961) for use on the CSIRO CDC 3600 computer, Canberra.

Results

Experiment 1

(i) *Oxygen Uptake of Spermatozoa*

The oxygen uptake of spermatozoa from rams prior to injecting α-chlorohydrin was linear over a 3-h incubation period (Fig. 1*a*). An analysis for homogeneity showed that there was no significant difference between rams and the variability in oxygen uptake was usually homogeneous between rams during the control period.

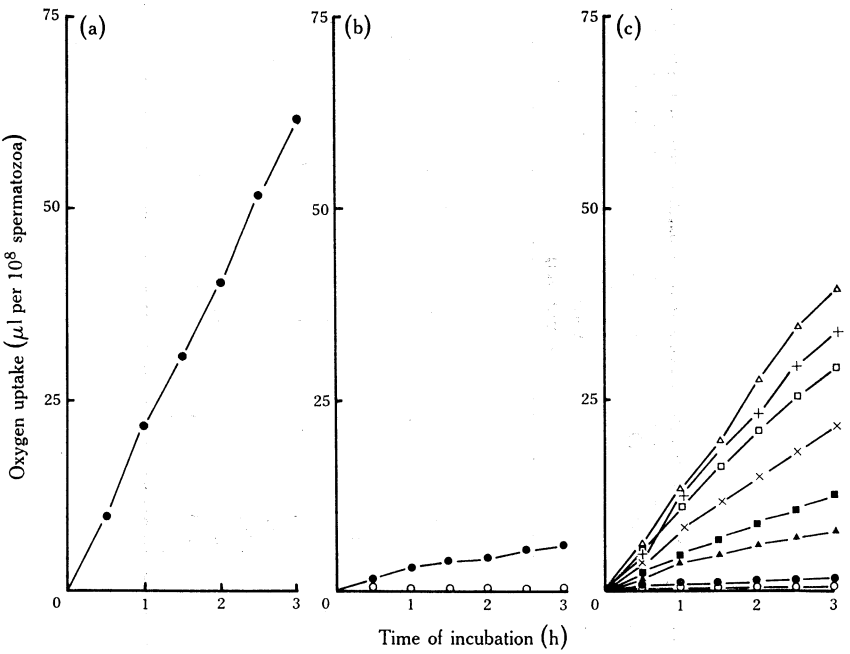

Fig. 1. Suppression and subsequent recovery of oxygen uptake of spermatozoa after injecting α-chlorohydrin (25 mg/kg) into five rams (experiment 1). (*a*) Pre-injection control. (*b*) Injection period. Days after first injection: 1 (●), 4 (○). (*c*) Post-injection period. Days after last injection: 4 (●), 7 (○), 11 (▲), 14 (△), 18 (■), 21 (□), 25 (×), 28 (+).

There was a marked reduction in oxygen uptake of the spermatozoa within 1 day of the first injection of α-chlorohydrin, and 4 days after the first injection oxygen uptake was negligible (Fig. 1*b*). However, the capacity of the spermatozoa to respire recovered in a linear fashion (linear effect $P < 0{\cdot}001$) during the post-injection period of 28 days (Fig. 1*c*) despite day to day fluctuations. Although respiratory activity did not reach values as high as during the pre-injection period, the trend indicated a complete return to normal had it been possible to continue the experiment.

(ii) *Metabolism of Glucose by Spermatozoa*

Very little glucose was utilized or oxidized by spermatozoa and practically no lactic acid formed within 1 day of the first injection of α-chlorohydrin; metabolic activity was almost completely obliterated after 4 days (Table 1). When the injections ceased, these parameters returned towards normal. An analysis of variance showed that the overall recovery was linear.

Table 1. Effect of injecting α-chlorohydrin (25 mg/kg) on the spermatozoa and seminal plasma of five rams (experiment 1)

Period	Days	Sperm metabolism (μmol/10^8 sperm)			Sperm motility score	Days	Seminal plasma fructose (μmol/ml)	Sperm morphology (%)			Abnormal heads or tails
		Glucose utilized	Glucose oxidized	Lactic acid accumulated				Normal	Free heads, free tails	Attached droplet	
Pre-injection (mean of 35)		1·79	0·38	2·23	9·1		10·7	96	0, 0	4	0
After first injection	1	0·14	0·01	0·00	6·9	2	10·2	95	0, 0	5	0
	4	0·02	0·00	0·00	2·5	5	10·2	87	5, 2	4	2
After last injection	4	0·23	0·00	0·00	4·0	5	18·3	77	7, 3	1	12
	7	0·02	0·00	0·01	3·6	8	18·6	61	7, 4	0	28
	11	0·09	0·03	0·22	7·4	12	19·4	57	14, 4	2	23
	14	1·31	0·23	0·81	8·2	15	22·8	27	10, 8	18	37
	18	0·41	0·07	0·39	10·0	19	17·1	77	17, 2	1	3
	21	0·92	0·15	1·03	10·6	22	11·4	52	8, 10	8	22
	25	0·56	0·11	0·79	9·3	26	16·1	71	13, 8	0	8
	28	0·97	0·62	1·11	11·2	29	15·0	80	6, 4	1	9

(iii) *Motility of Spermatozoa*

Spermatozoan motility over a 3-h period was reduced within 1 day of the first injection and further reduced after 4 days (Table 1); motility scores then began to return towards the pre-treatment values; recovery was linear and normal scores were reached about 18 days after the last injection.

(iv) *Fructose Concentration of the Seminal Plasma*

Although the fructose concentration of the seminal plasma appeared to rise during the recovery period (Table 1), the differences were not statistically significant.

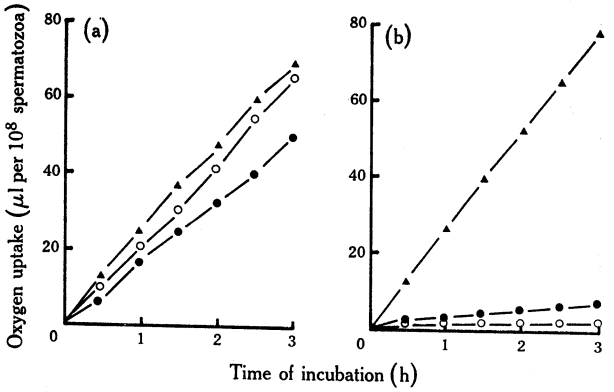

Fig. 2. Suppression of oxygen uptake of spermatozoa after injecting α-chlorohydrin (25 mg/kg) into rams (experiment 2). ▲ Pre-injection period. ● 1 day after first injection. ○ 4 days after first injection.

(v) *Spermatozoan Morphology*

Few abnormal spermatozoa were found in the pre-injection semen samples and only 4% of the spermatozoa still had the cytoplasmic droplet attached (Table 1). One day after the first injection there was no change in the percentage of abnormal spermatozoa but by 4 days after the first injection the number of abnormal forms in the ejaculate began to increase. Spermatozoan abnormalities reached a maximum 14 days after the last injection with a return towards normal by the end of the observation period. The most common abnormality was a looped or bent tail but there was also an increase in the number of spermatozoa without tails and, 14 days after the last injection, an increase in the number of spermatozoa with attached cytoplasmic droplets.

No change occurred in the total number of spermatozoa during the injection or post-injection periods nor in the volume of the ejaculate.

Experiment 2

In this experiment, simultaneous control rams were injected with 0·9% saline to eliminate any possibility that the effects observed in experiment 1 were due to temporary fluctuations rather than to α-chlorohydrin.

(i) *Metabolism of Spermatozoa*

The oxygen uptake and other metabolic activity (i.e. glucose utilization and oxidation and lactic acid accumulation) of spermatozoa was again greatly reduced 1 and 4 days after injecting α-chlorohydrin into three rams (Fig. 2 and Table 2). All metabolic parameters of spermatozoa from the control rams remained fairly constant throughout the experimental period.

Table 2. Effect of injecting α-chlorohydrin (25 mg/kg) on the sperm of the ram (experiment 2)

Group	Period	Days	Sperm metabolism (μmol/10^8 sperm)			Sperm motility scores	Days	Sperm morphology (%)				
			Glucose utilized	Glucose oxidized	Lactic acid accumulated			Normal	Free heads, free tails	Attached droplet	Abnormal tails	Abnormal heads
Control	Pre-injection		1·73	0·37	1·54	11·0		71	1, 1	1	23	3
($n = 3$)	After first	1	1·55	0·23	1·66	8·7	2	72	3, 2	0	19	4
	injection	4	1·55	0·31	1·49	11·2	5	81	0, 0	0	17	2
α-Chloro-	Pre-injection		2·01	0·46	1·62	11·7		70	1, 0	1	26	2
hydrin	After first	1	0·07	0·00	0·01	7·0	2	72	1, 0	1	26	0
($n = 3$)	injection	4	0·10	0·01	0·00	7·0	5	75	1, 0	0	23	1
	Cauda epi-didymis[A]		–	–	–	–		37	0, 1	12	–	1
	Rete testis[A]		–	–	–	–		12	1, 0	85	2	0

[A] At slaughter (9 days).

(ii) *Seminal Plasma*

By collecting semen with an artificial vagina rather than by electroejaculation, it was hoped to reduce the variability in the fructose concentration of the seminal plasma found in experiment 1. Although this was not achieved the increase in the fructose concentration of the seminal plasma 5 days after the last injection of α-chlorohydrin did prove statistically significant (Table 3).

There were no statistically significant changes in the amino acid concentration of the seminal plasma following injection of α-chlorohydrin (Table 3).

The only change in phospholipid profile following injection of α-chlorohydrin was a slight increase in the ethanolamine plasmalogen component of the spermatozoa and seminal plasma. However, this is based on a comparison of only one analysis during the injection period with the control.

(iii) *Motility of Spermatozoa*

Although there appeared to be some drop in the motility of spermatozoa after injecting α-chlorohydrin (Table 2), this was not statistically significant. However, observations could only be made on two rams since one lost libido after the third injection of α-chlorohydrin and did not ejaculate again until the injections were stopped.

(iv) *Spermatozoan Morphology*

Over the 4-day period of investigation, no alterations occurred in spermatozoa morphology after the injection of α-chlorohydrin (Table 2). As expected there was a high percentage of spermatozoa with attached cytoplasmic droplets in the rete testis fluid collected at slaughter but no abnormalities were seen.

(v) *Histology*

Histological studies of various parts of the epididymis and testis showed no evidence of a lesion in the epididymis or any abnormality due to α-chlorohydrin at the dose rate administered.

(vi) *General*

The wool, especially 'belly wool', appeared to be more easily pulled out of the rams injected with α-chlorohydrin. Libido was lost to a variable extent as judged by failure of trained rams to serve an artificial vagina.

Discussion

Since spermatozoa take at least 2 weeks to pass through the epididymis of the ram (Dawson 1958) and severe metabolic defects were evident in ejaculated spermatozoa within 1 day of injecting α-chlorohydrin, these studies indicate that the cauda epididymis is the most likely site of initial action of the drug. It may be noted in this regard that the cauda epididymis of the rat concentrates α-chlorohydrin from the blood (Crabo and Appelgren 1972; Edwards *et al.* 1975). Our conclusion is also supported by the observations that spermatozoa recovered from the cauda epididymis of the boar are infertile within 7 h of the animals being fed α-chlorohydrin (Johnson and Pursel 1973) and by the fact that a marked decrease in fertility occurs within 3 days of the daily injection of α-chlorohydrin in the hamster (Lubicz-Nawrocki and Chang 1974).

Table 3. Fructose (μmol/ml) and amino acid (mM) levels in seminal plasma before and after injecting α-chlorohydrin (25 mg/kg) into three rams (experiment 2)

Values given are means ± s.d.

Group	Period	Days	Fructose	Lysine	Arginine	Glycine	Alanine	Serine	Glutamic acid	Aspartic acid
Control	Pre-injection		27·4	0·62±0·22	0·65±0·25	2·04±0·39	1·88±0·05	3·17±0·08	13·18±3·42	1·92±0·41
	After first	2	29·8	—	—	—	—	—	—	—
	injection	5	28·9	—	—	—	—	—	—	—
		9	—	1·31±0·38	1·31±0·27	2·19±0·59	1·67±0·17	9·11±0·50	5·91±1·46	0·25±0·13
Injected	Pre-injection		19·4	0·31±0·13	0·50±0·32	2·14±0·81	1·36±0·14	2·09±0·50	10·40±0·25	1·36±0·12
	After first	2	21·8	—	—	—	—	—	—	—
	injection	5	38·5	—	—	—	—	—	—	—
		9[A]	—	1·59	1·16	2·59	1·41	2·88	4·57	0·54

[A] Two rams only.

The normal composition of fluid in the lumen of the epididymis is dependent upon an intact epithelium which has the capacity for both resorption and secretion. Although changes in ion concentration can, to some extent, modify the metabolism of spermatozoa (Lodge *et al.* 1963; Dott and White 1964; Wallace and Wales 1964), it seems unlikely that such alteration in the composition of the epididymal plasma could explain the rapid and drastic disruption of sperm metabolism that follows the administration of α-chlorohydrin; in any case since the amino acid concentration of the seminal plasma did not change significantly it seems probable that there was little interference with epididymal constituents. The lack of an effect of α-chlorohydrin on the fructose content of the semen, also reported by Kreider and Dutt (1970), indicates that α-chlorohydrin does not reduce the androgen status of the ram.

We suggest that the effect is due to α-chlorohydrin, or a metabolite such as the phosphorylated form (Mohri *et al.* 1975), acting directly upon spermatozoa in the lumen of the cauda epididymis. Recent work in this laboratory has shown that α-chlorohydrin inhibits the glycolysis of ram spermatozoa *in vitro* by interfering with glyceraldehyde-3-phosphate dehydrogenase (Brown-Woodman *et al.* 1975; Suter *et al.* 1975). This would explain the drastically reduced glucose utilization in the present experiments and the almost complete absence of lactic acid accumulation. It would also account for the profoundly decreased oxygen consumption of the spermatozoa from α-chlorohydrin-treated rams observed here and also briefly reported by Kreider and Dutt (1972). Similar results have been obtained with the rat (Samojlik and Chang 1970; Brown-Woodman and White 1975). The observations that spermatozoa from α-chlorohydrin-treated monkeys apparently showed an increased oxygen uptake (Setty *et al.* 1970) is, therefore, very puzzling.

Spermatozoan motility was reduced by 4 days after the first injection of α-chlorohydrin and this confirms findings in other studies in the ram (Brown-Woodman *et al.* 1974; Kreider and Dutt 1970) and boar (Johnson and Pursel 1973). The decline and recovery of motility followed a similar pattern to metabolic activity and fertility (Brown-Woodman *et al.* 1974). It is, therefore, suggested that α-chlorohydrin disrupts the metabolism of spermatozoa to such an extent that insufficient energy is available for critical processes such as motility in the female tract and that this renders the spermatozoa infertile.

The volume of the ejaculate and the number of spermatozoa were not altered by administration of α-chlorohydrin. However, there was an increase in the number of abnormal spermatozoa in the ejaculate and particularly in the number of looped or bent tails. The appearance of abnormal spermatozoa in the ejaculate occurred considerably later than disruption of metabolic activity and a peak was reached about 14 days after the last injection of α-chlorohydrin. This corresponds approximately with the time required for the transport of spermatozoa through the epididymis of the ram (i.e. 13–15 days) (Ortavant 1958) and indicates that the site of morphological damage to spermatozoa is more proximal in the epididymis than the site of metabolic disruption. It is possible that 1–2 weeks following the administration of α-chlorohydrin the composition of the epididymal plasma alters in such a way as to cause an increase in the number of abnormal spermatozoa in the ejaculate, since chemical changes in the epididymis are apparently correlated with increased numbers of free heads and abnormal tails in the lower part of the duct (Swanson and Boyd 1962; Gustafsson 1966).

In rats receiving five times the minimal effective dose of α-chlorohydrin, i.e. daily doses of 35 mg/kg body weight, or one 45 mg/kg dose, orally or subcutaneously, spermatocoeles and sperm granulomata occur (Ericsson 1970; Ericsson and Baker 1970). However, there was no histological evidence of such a lesion in the epididymis of the rams in these experiments.

Kreider and Dutt (1970) stated that libido of rams was not adversely affected by α-chlorohydrin but in our experiments all rams lost some libido as judged by failure to serve an artificial vagina, but this was quickly recovered once the injections ceased. The difference could be due to the fact that the above workers gave α-chlorohydrin orally (25 mg/kg) and some of the drug may have been lost in the rumen. The α-chlorohydrin injected intramuscularly in our experiments would be expected to produce a higher effective dose.

It remains to be seen whether lower doses of α-chlorohydrin will produce similar temporary suppression of the metabolism of spermatozoa in the reproductive tract of the ram without any adverse side effects. However, these experiments clearly establish a metabolic basis for the antifertility action of α-chlorohydrin on spermatozoa.

Acknowledgments

We wish to thank Professor C. W. Emmens for his interest and advice through the study, Dr I. C. A. Martin for assistance with the statistics, Mrs A. Darin-Bennett for phospholipid analyses and Dr R. Clarke for examination of wool follicles. The work has been supported financially by the World Health Organization. We are indebted to the Upjohn Company, Kalamazoo, for the gift of α-chlorohydrin.

References

Barker, J. N., and Britton, H. C. (1957). The enzymic estimation of L(+) lactic acid. *J. Physiol. (London)* **138**, 3–4p.

Blackshaw, A. W. (1954). A bipolar electrode for the production of ejaculation in sheep. *Aust. Vet. J.* **30**, 249–50.

Brown, P. D., and White, I. G. (1973). Studies on the male antifertility drug 3-chloro-1,2-propanediol. *J. Reprod. Fertil.* **32**, 337–8.

Brown-Woodman, P. D. C., Salamon, S., and White, I. G. (1974). Effect of α-chlorohydrin on the fertility of rams. *Acta Europaea Fertilitatis* **5**, 193–7.

Brown-Woodman, P. D. C., and White, I. G. (1974). Amino acid composition of semen and the secretions of the male reproductive tract. *Aust. J. Biol. Sci.* **27**, 415–22.

Brown-Woodman, P. D. C., and White, I. G. (1975). Effect of α-chlorohydrin on rat cauda epididymis and on general physiological status of the animals. *Contraception* **11**, 69–78.

Brown-Woodman, P. D. C., White, I. G., and Salamon, S. (1975). Effect of α-chlorohydrin on the fertility of rams and on the metabolism of spermatozoa *in vitro*. *J. Reprod. Fertil.* **43**, 381.

Claringbold, P. J. (1957). The automatic design and analysis of biological experiments. Proc. Conf. Automatic Computation Weapons Res. Establ., Salisbury, South Aust.

Claringbold, P. J. (1961). The use of orthogonal polynomials in the partition of Chi-square. *Aust. J. Statist.* **3**, 48–63.

Crabo, B., and Appelgren, L.-E. (1972). Distribution of [^{14}C]α-chlorohydrin in mice and rats. *J. Reprod. Fertil.* **30**, 161–3.

Dawson, R. M. C. (1958). The labelling of ram semen *in vitro* with radioactive phosphate and (carboxy = ^{14}C) stearic acid. *Biochem. J.* **68**, 512–19.

Dott, H. M., and White, I. G. (1964). The effect of potassium on ram spermatozoa studied by flow dialysis technique. *J. Reprod. Fertil.* **7**, 127–38.

Edwards, E. M., Jones, A. R., and Waites, G. M. H. (1975). The entry of α-chlorohydrin into body fluids of male rats and its effect upon the incorporation of glycerol into lipids. *J. Reprod. Fertil.* **43**, 225–32.

Emmens, C. W. (1947). The motility and viability of rabbit spermatozoa at different hydrogen ion concentrations. *J. Physiol. (London)* **106**, 471–81.

Ericsson, R. J. (1970). Male antifertility compounds: U-5897 as a rat chemosterilant. *J. Reprod. Fertil.* **22**, 213–22.

Ericsson, R. J., and Baker, V. F. (1970). Male antifertility compounds: biological properties of U-5897 and U-15, 646. *J. Reprod. Fertil.* **21**, 267–73.

Gustafsson, B. (1966). Luminal contents of the bovine epididymis under conditions of reduced spermatogenesis, luminal blockage and certain sperm abnormalities. *Acta Vet. Scand. Suppl.* **17**, 1–80.

Johnson, L. A., and Pursel, V. G. (1973). Alpha-chlorohydrin: studies on the mechanism of action in male swine. *J. Anim. Sci.* **37**, 1207–11.

Kreider, J. L., and Dutt, R. H. (1970). Induction of temporary infertility in rams with an orally administered chlorohydrin. *J. Anim. Sci.* **31**, 95–8.

Kreider, J. L., and Dutt, R. H. (1972). Effect of 3-chloro-1,2-propanediol (U-5897) on accessory gland secretions of rams. *J. Anim. Sci.* **35**, 246 (Abstr.).

Lodge, J. R., Salisbury, G. W., Schmidt, R. P., and Graves, C. N. (1963). Effect of phosphate and related co-factors on the metabolism of bovine spermatozoa. *J. Dairy Sci.* **46**, 473–8.

Lubicz-Nawrocki, C. M., and Chang, M. C. (1974). The onset and duration of infertility in hamsters treated with α-chlorohydrin. *J. Reprod. Fertil.* **39**, 291–5.

Mann, T. (1948). Fructose content and fructolysis in semen. Practical application in the evaluation of semen quality. *J. Agric. Sci.* **38**, 323–31.

Mann, T. (1964). 'The Biochemistry of Semen and of the Male Reproductive Tract'. (Methuen: London.)

Mohri, H., Suter, D. A. I., Brown-Woodman, P. D. C., White, I. G., and Ridley, D. D. (1975). Identification of the biochemical lesion produced by the male antifertility agent α-chlorohydrin in spermatozoa. *Nature (London)* **255**, 75–6.

Ortavant, R. (1958). Le cycle spermatogéneitique chez le belier. D. Sc. Thesis, Paris.

Poulos, A., Voglmayr, J. K., and White, I. G. (1973). Phospholipid changes in spermatozoa during passage through the genital tract of the bull. *Biochem. Biophys. Acta* **306**, 194–202.

Samojlik, E., and Chang, M. C. (1970). Antifertility activity of 3-chloro-1,2-propanediol (U-5897) on rats. *Biol. Reprod.* **2**, 299–304.

Schmidt, F. H. (1963). Enzymatische Methoden zur Bestimmung von Blut-und Harnzucker unter Berücksichtigung von Vergleichsuntersuchungen mit klassischen Methoden. *Internist* **4**, 554–9.

Setty, B. S., Kar, A. B., Roy, S. K., and Chowdhury, S. R. (1970). Studies with sub-toxic doses of α-chlorohydrin in the male monkey (*Macaca mulatta*). *Contraception* **1**, 279.

Snedecor, G. W., and Cochran, W. G. (1971). 'Statistical Methods'. 6th Edn. p.317. (Iowa State University Press.)

Suter, D. A. I., Brown-Woodman, P. D. C., Mohri, H., and White, I. G. (1975). The molecular site of action of the anti-fertility agent α-chlorohydrin in ram spermatozoa. *J. Reprod. Fertil.* **43**, 382–3.

Swanson, E. W., and Boyd, L. J. (1962). Factors affecting coiled-tail spermatozoa in the bull. *Am. J. Vet. Res.* **23**, 300–9.

Umbreit, W. W., Burris, R. H., and Stauffer, J. F. (1972). 'Manometric and Biochemical Techniques'. (Burgess Publishing Co.: Minneapolis.)

Wallace, J. C., and Wales, R. G. (1964). The effect of ions on the metabolism of ejaculated and epididymal ram spermatozoa. *J. Reprod. Fertil.* **8**, 187–203.

Werner, W., Rey, H. G., and Wielinger, H. (1970). On the properties of a new chromogen for the determination of glucose in the blood according to the GOD/POD method. *Z. Anal. Chem.* **252**, 224–8.

White, I. G. (1959). Studies on the estimation of glycerol, fructose and lactic acid with particular reference to semen. *Aust. J. Exp. Biol. Med. Sci.* **37**, 441–50.

Manuscript received 6 December 1975