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Some preliminary observations of the nephrotoxicity of the male antifertility drug (±)α-chlorohydrin

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(±)α-Chlorohydrin (80 mg kg^{-1}) given by mouth to group-housed adult male Sprague Dawley rats produced an increase in the weight of the kidneys which persisted for at least 7 days, but there were no deaths. This dose administered to Sprague Dawley rats caged singly killed 3 out of 8 animals. The toxicity was studied in more detail using Wistar rats caged singly in metabolic cages. 4 out of 9 animals died with oliguria and anuria after 120 mg kg^{-1} (±)α-chorohydrin, 100 and 120 mg kg^{-1} (in the surviving animals), produced a loss in appetite and body weight, proteinuria, a dose-related diuresis and an increased water intake. Urinary glucose was dramatically elevated after 100 mg kg^{-1} but after 120 mg kg^{-1} the glucosuria was not as marked. By day 7 all parameters were returning to their pre-injection values. A dose of 80 mg kg^{-1} had no effect upon any of the parameters studied. The results are discussed in relation to the basic biochemical mechanism by which the drug exerts its antifertility action, which is achieved at much lower doses.

(±)α-Chlorohydrin is a novel male antifertility drug. Its effects can be attributed to a direct reversible action upon epididymal sperm without affecting spermatogenesis; morphologically mature sperm are produced which are incapable of fertilizing the ovum (Jackson 1975). The desirability of such a male contraceptive has led to extensive research into the pharmacology of (±)α-chlorohydrin; however the impetus was severely reduced when the drug was found to cause death and bone marrow depression in monkeys at doses producing infertility (Kirton et al 1970; Setty et al 1970). Other toxicological properties remain to be described.

In the rat high doses of (±)α-chlorohydrin produce irreversible sterility by the formation of spermatocoeles in the caput epididymis which block the efferent ducts of the testes (Ericsson 1970; Hoffer et al 1973; Cooper et al 1974). Other pathological changes were described by Brown-Woodman & White (1975) who reported that doses of (±)α-chlorohydrin close to the lethal level produced haemolytic gastroenteritis, an increase in adrenal weight, leukocytosis and a decrease in body weight, the latter being confirmed at lower doses by Morris & Jackson (1978). During a study in this laboratory in which hormonal changes after α-chlorohydrin were examined it was found that, as well as changes in the gonadotrophins, luteinizing hormone and follicle-stimulating hormone, the prolactin serum concentration was also elevated (Morris & Jackson 1978). Prolactin is implicated in the control of certain kidney functions (Horrobin 1976) and Jones & Murcott (1976) noted a diuresis which occurred at the same time as increased prolactin output. Some aspects of the nephrotoxicity of the commercially available (±)α-chlorohydrin have therefore been studied. This information may be of use in the development of less toxic derivatives of α-chlorohydrin as recent evidence suggests that the therapeutic ratio of α-chlorohydrin can be improved by the use of the *S*(+) isomer (Jackson & Robinson 1976; Ford et al 1977; Jackson et al 1977).

MATERIALS AND METHODS

Adult male rats (325–410 g) bred in this institution were used, (±)α-chlorohydrin (Koch–Light redistilled) was given either by mouth or i.p. (dose volume 2 ml kg^{-1} in distilled water). The initial studies used Sprague Dawley rats, housed either in groups of 10 or individually and allowed free access to food and water. The metabolic studies used Wistar rats which were housed singly in all glass (Jencons metabowl) or aluminium/glass metabolic cages at 22–24 °C, relative humidity 55% and constant lighting (lights on 05:00–19:00). The rats were allowed free access to 25 g powdered rat pellets (Prepared Rat Diet, Oakes Ltd.) and 100 ml of water daily. After acclimatization to these conditions (never less than two days) rats were given a single i.p. dose of (±)α-chlorohydrin. Control animals received the vehicle alone. A maximum of five animals were studied concurrently, the doses of α-chlorohydrin were admin-

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istered in a random order and at least one cage was assigned to the control group throughout the experimental period. The faeces and urine were collected separately in glass vessels, the urine under toluene. The cages and vessels were cleaned daily after data collection (09:00–12:00). Urine was filtered and stored at −15 °C.

Urine analysis

An equal volume of 10% trichloroacetic acid was added to the filtered urine and the mixture placed on ice for at least 20 min then centrifuged. The supernatant was assayed for glucose by the *o*-toluidine method (Cooper & McDaniel 1970) and the precipitate assayed for protein (Lowry et al 1951). The hexokinase method was used to confirm the elevated glucose concentrations in some selected samples (Bergmeyer 1963). The results were identical to those from the ortho-toluidine method and are not included. The results are expressed as the mean ±s.e. Statistical analyses were performed using the Student's *t*-test by comparing these data with those obtained in the 24 h immediately preceeding the injection.

RESULTS

The paired kidney weights from group-housed Sprague Dawley rats after (±)α-chlorohydrin 80 mg kg^{-1} by mouth are presented in Table 1. Twenty four hours after (±)α-chlorohydrin the kidney weight had significantly increased and remained so for at least 7 days after the drug was administered. The kidneys were of normal coloration, but were tense and in some cases, lobulated. Forty-two days after treatment, kidney weight and appearance were comparable with the control values. No significant changes or trends were recorded for the weight of the liver. There were no deaths in this treatment group, however in another experiment when the rats were housed singly 3 out of 8 rats died. The animals which died became anuric, the kidneys were whitish and furry. In transverse section the white region extended 1–2 mm in from the surface, where after normal coloration was observed. Endocrine and organ weight data from these and other animals have been published (Morris & Jackson 1978; Morris 1979).

In view of these preliminary data, experiments were carried out to examine possible changes in kidney function after (±)α-chlorohydrin. For these experiments Wistar rats were used, as fertility and toxicity data have already been obtained for this strain (Jackson 1975). No significant changes ($P > 0.05$) for any of the parameters studied were observed for the control rats (9 rats for 9 days) the data for which have been omitted from Fig. 1. Pre-injection values for the test rats were also not signifi-

Table 1. Paired kidney weights (g) after a single dose of (±)α-chlorohydrin (80 mg kg^{-1} by mouth) administered to adult Sprague Dawley rats (n = 5) mean ± s.e.m.

Days after dose	Treated Kidney weight g	Control Kidney weight g
1	1·445 ± 0·019*	1·29 ± 0·024
2	1·538 ± 0·042*	1·184 ± 0·015
7	1·694 ± 0·123*	1·228 ± 0·247
42	1·375 ± 0·119	1·465 ± 0·064

Significance of difference treated vs control. Student's *t*-test (unpaired) *$P < 0.01$.

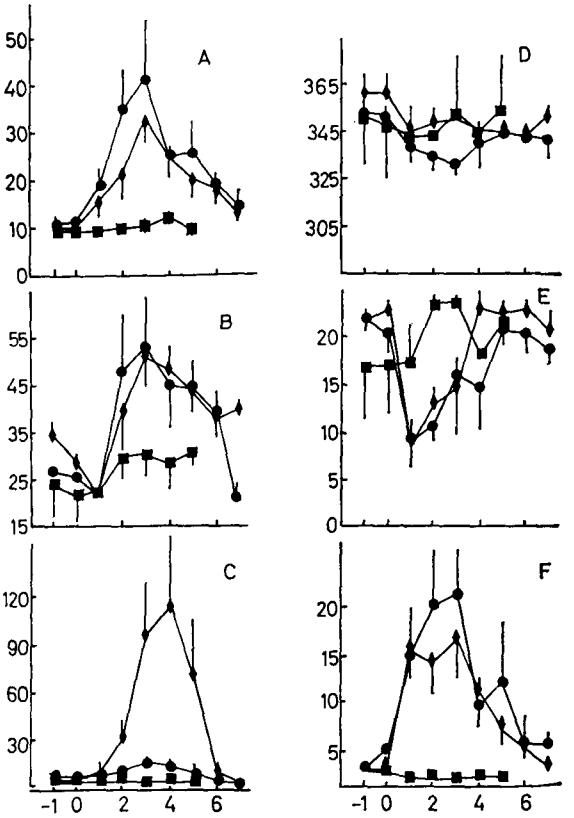

FIG. 1. The effects of (±)α-chlorohydrin (mg kg^{-1}) ■ 80 mg, ◆ 100 and ● 120 administered intraperitoneally to adult male Wistar rats. Ordinate: A. Urine volume (ml). B. Water intake (ml). C. Total urinary glucose (mg). D. Body weight (g). E. Food intake (g) and F. Total urinary protein (mg) measured at 24h intervals. Abscissa: Days of treatment. Mean ± s.e. n = 4–6. In some instances the symbol is larger than the s.e.

cantly different from the control values. The results after the administration of 80, 100 and 120 mg kg^{-1} of (±)α-chlorohydrin to Wistar rats housed singly in metabolic cages are presented in Fig. 1. No deaths were recorded after 80 or 100 mg kg^{-1}. However, after 120 mg kg^{-1} 4 out of 9 rats died in the dark period between days 3 and 6 after treatment with intermittent oliguria and anuria. The data from the animals that died have not been included in Fig. 1. (±)α-Chlorohydrin at doses of 100 and 120 mg kg^{-1}, but not at 80 mg kg^{-1}, caused an immediate fall in body weight ($P < 0{\cdot}01$) which lasted for at least 5 days after treatment. Similarly at 100 and 120 mg kg^{-1}, but not 80 mg kg^{-1}, the drug caused a fall in food intake ($P < 0{\cdot}001$) which returned to pretreatment levels after 4 and 5 days respectively. The two higher doses also caused an increased water intake, not detectable on day 1 after treatment, but which reached a maximum on day 3 after treatment ($P < 0{\cdot}01$). After 7 days water intake had returned to pretreatment values in those rats given 120 mg kg^{-1} but was still elevated in rats given 100 mg kg^{-1}.

One day after 100 or 120 mg kg^{-1} doses the urine volume had increased, and the diuresis was dose related, reaching a maximum 4 days after treatment, with values more than 4 times the pre-injection levels ($P < 0{\cdot}01$). The data for the 120 mg kg^{-1} dose are from a selected population of rats in which the urine volume increased after treatment, 4 other rats became anuric after this dose and died 3–6 days later. The rate of urine production returned to pretreatment values after approximately one week. Total urinary glucose was unaltered after the 80 mg kg^{-1} dose. At 100 mg kg^{-1} the drug produced marked glucosuria, increasing 180-fold from $0{\cdot}611 \pm 0{\cdot}123$ mg to 113 ± 45 mg ($P < 0{\cdot}001$). In contrast to this severe glucosuria, 120 mg kg^{-1} produced only a 20-fold increase in glucose output ($P < 0{\cdot}01$). Total urinary glucose intake returned to pretreatment values after 6 days. After 100 mg kg^{-1} ($P < 0{\cdot}01$), total urinary protein was elevated to a maximum 1 day after treatment and after the 120 mg kg^{-1} dose rose further to a maximum 3 days after treatment ($P < 0{\cdot}01$). Values then fell to approach control figures by 7 days. Urine Na^+, K^+ and Cl^- ions were also measured but there were no significant changes whether the results were expressed as a urinary total or as a concentration.

Spermatocoeles were found at autopsy in all (±)α-chlorohydrin-treated animals.

DISCUSSION

The experiments demonstrate that high doses of the antifertility drug (±)α-chlorohydrin, as well as producing the general toxicological phenomena of loss of appetite and body weight, also appears to impair kidney functions. Kidney enlargement, anuria, polyuria, proteinuria and glucosuria were all recorded after 100 and 120 mg kg^{-1}. 80 mg kg^{-1} had no effect. The polyuria is unlikely to be due to a specific action of (±)α-chlorohydrin upon the tubular resorption of sodium, for example, because of the concurrent finding of protein in the urine which suggests that the glomerular and/or tubular functions have been severely disarranged. Glucosuria could suggest proximal tubule damage, however, in view of (±)α-chlorohydrin's other biochemical actions (see later), this may only reflect a systemic action. Higher doses of (±)α-chlorohydrin which were lethal cause anuria as does mercury, and could possibly be due to shedding of the tubular lining or back diffusion of the filtrate through the damaged tubular epithelium (Foulkes & Hammond 1975). However it is not possible with the present observations to give a detailed account of the mechanisms involved in the renal toxicity.

The doses of (±)α-chlorohydrin we used are many times higher than the dose required to produce reversible infertility so that these toxic reactions may not be related to the mechanism by which infertility is brought about (Ericsson & Norland 1970). However, the changes in urinary glucose are worth consideration in view of recent reports that (±)α-chlorohydrin exerts its low dose antifertility effects by inhibiting glucose metabolism in the sperm itself. (±)α-Chlorohydrin in vitro or in vivo inhibits glycolysis by epididymal and testicular sperm of ram and rat (Mohri et al 1975; Edwards et al 1976). In addition, the recent availability of the separate isomers of (±)α-chlorohydrin has demonstrated that the $S(+)$ isomer is both more potent in vitro in inhibiting sperm glycolysis and in vivo as an antifertility agent than the $R(-)$ (Ford et al 1977). In vitro (±)α-chlorohydrin only slowly inhibits the glycolysis of sperm. However, both the phosphorylated derivative, the active metabolite of (±)α-chlorohydrin, and the more potent $S(+)$ isomer, competitively inhibit the purified enzyme glyceraldehyde-3-phosphate dehydrogenase prepared from rabbit muscle (Mashford & Jones 1978; Fitzpatrick et al 1978). As the rabbit is not susceptible to the antifertility effects of (±)α-chlorohydrin (Samojlik & Chang 1970), the biochemical data would suggest that a specific action upon the spermatozoal enzymes cannot be proposed and that more general disturbances of glucose metabolism may be expected. The

susceptibility of sperm to (±)α-chlorohydrin probably arises from the localization of the drug within the epididymis as shown by Crabo & Appelgren (1972). These observations would support the results from the 100 mg kg^{-1} dose in which the glycolytic enzymes of the body were inhibited and the excess substrate in the blood excreted into the urine. However it is difficult to explain the reversal of the glucosuria at the higher dose (120 mg kg^{-1}) by this mechanism. Brown-Woodman & White (1975) demonstrated that at doses of (±)α-chlorohydrin which were equivalent to the highest dose used here (approximate LD50) glycolysis was stimulated in rat kidney slices. The increased utilization of glucose by the kidney may then explain why the urinary glucose fell.

In conclusion it is suggested that the elevated urinary glucose is not related to the toxic action of (±)α-chlorohydrin that results in kidney failure which proceeds, via polyuria and proteinuria at the lower doses, to anuria. The glucosuria may reflect the mechanism by which the (±)α-chlorohydrins bring about the low-dose antifertility effect and, if this is so, it may not be possible to separate the glucosuria from the antifertility effect. Such a connection would seriously impair the development of the drug as a male contraceptive.

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REFERENCES

Bergmeyer, H. V. (1963) Methods of Enzymic Analysis Academic Press: New York

Brown-Woodman, P. D. C., White, I. G. (1975) Contraception 11: 69–77

Cooper E. R. A., Jones, A. R., Jackson, H. (1974) J. Reprod. Fertil. 38: 379–386

Cooper, G. R., McDaniel, V. (1970). in: MacDonald, R. M. (ed.) Standard Methods of Clinical Chemistry, Vol. 6. Academic Press, New York, pp. 159–170

Crabo, B., Appelgren, L. E. (1972) J. Reprod. Fertil. 30: 161

Edwards, E. M., Dacheux, J. L., Waites, G. M. H. (1976) Ibid. 48: 265–270

Ericsson, R. J. (1970) Ibid. 22: 213–222

Ericsson, R. J., Norland, J. F. (1970) Excerpta Med. Int. Congr. Ser. 210: 174

Fitzpatrick, R. W., Jackson, H., Dickinson, N. A. (1978) Contraception 18: 477–484

Ford, W. C. L., Harrison, A., Waites, G. M. H. (1977) J. Reprod. Fertil. 51: 105–109

Foulkes, E. C., Hammonds, P. B. (1975) in: Casarett, L. J., Doull, J. (eds) Toxicology The Basic Science of Poisons (eds) Macmillan Publishing Co. Inc., New York, pp 190–200

Hoffer, A. P., Hamilton, D. W., Fawcett, D. W. (1973) Anat. Rec. 175: 203–230

Horrobin, D. F. (1976) Prolactin. Eden Press: Montreal

Jackson, H. (1975) Clin. Endocrinol. Metab. 4: 643–663

Jackson, H., Robinson, B. (1976) Chem. Biol. Interact 13: 193–197

Jackson, H., Rooney, F. R., Fitzpatrick, R. W., Gibson, K. H. (1977) Ibid. 17: 117–120

Jones, A. R., Murcott, C. (1976) Experientia 32: 1135–1136

Kirton, K. T., Ericsson, R. J., Ray, J. A., Forbes, A. D. (1970) J. Reprod. Fertil. 21: 275–278

Lowry, D. H., Rosenbrough, N. J., Farr, A. L., Randall, R. (1951). J. Biol. Chem. 193: 265–275

Mashford, P. M., Jones, A. R. (1978) Experientia, 34: 1267–1268

Mohri, H., Suter, D. A. I., Brown-Woodman, P. D. C., White, I. G., Ridley, D. D. (1975) Nature (London) 255: 75–77

Morris, I. D. (1979) J. Reprod. Fertil. in the press

Morris, I. D., Jackson, C. M. (1978) Int. Androl. 1: 86–95

Samojlik, E., Chang, M. C. (1970) Biol. Reprod. 2: 299–304

Setty, B., Kar, A. B., Roy, S. K., Chowdhury, S. R. (1970) Contraception 1: 279–289