EFFECTS OF α -CHLOROHYDRIN AND GONADECTOMY ON THE ADENOHYPOPHYSIAL CELLS OF MALE RATS AND GERBILS

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Alpha-chlorohydrin (3-chlor-1,2 propanediol) possesses antifertility properties when administered to male rats, hamsters, guinea-pigs, rams and monkeys (Ericsson, 1968; Ericsson & Norland, 1970). The compound is thought to exert its action on the epididymis (Gunn, Gould & Anderson, 1969; Samojlik & Chang, 1970). Coppola (1969) and Gunn *et al.* (1969) suggested that α -chlorohydrin might act as a metabolic antagonist to glycerol in phospholipid metabolism and the compound has also been shown to act as an alkylating agent *in vitro* (Jones, Davies, Edwards & Jackson, 1969).

The effects of α -chlorohydrin on the adeno-hypophysis have not so far been reported. The purpose of the present investigation was to compare the cytological changes occurring in the gonadotrophic cells of the anterior lobe of the pituitary in male rats and gerbils after the administration of α -chlorohydrin, with those changes following bilateral gonadectomy.

The α -chlorohydrin (sp. gr. 1.326) was supplied by Upjohn Company, Kalamazoo, Michigan, in 0.25% aqueous (1.3 g/ml) methylcellulose. A working solution was prepared by diluting the stock solution with distilled water.

Thirty adult male gerbils (*Meriones hurrianae*) and thirty male rats were allotted to six groups of ten each. The drug was given orally, the gerbils receiving 20 mg/kg/day for 50 days and the rats, 25 mg/kg/day for 24 days. An equal number of controls received distilled water. The animals were killed 24 hr after administration of the final dose and various organs were removed, weighed and fixed. Pituitaries were fixed in Halmi's Susa (Susa: saturated picric acid, 9:1 v/v), processed, sectioned at 5 μ m and stained with periodic acid Schiff (PAS)–orange G–methyl blue, and Cleveland-Wolfe trichromic stains. Differential cell counts were made in the sections stained with Cleveland-Wolfe trichromic stain.

The investigations revealed that administration of α -chlorohydrin to adult male gerbils and rats was associated with a decrease in the weights of the testes and accessory sex glands (Table 1) and inhibited spermatogenesis (unpublished observations). A significant decrease in thymus weight was also noted (gerbil, 36.6%; rat, 38.5%), but there was no significant influence on the adrenal and thyroid weights.

Although the general structure of the normal rat pituitaries is well known, that of the gerbil is less familiar. The gland consists of three distinct parts: pars

Table 1. Changes in body weight, and the weights of thyroid, adrenal, thymus, testes and accessory sex glands of male gerbils and rats after oral treatment with α -chlorohydrin

Group	Treatment	Initial body wt (g)	Final body wt (g)	Thyroid wt	Seminal vesicle wt	Ventral prostate wt	Epididymus wt	Adrenal wt	Levator ani wt	Thymus wt	Penis wt	Testes wt
Gerbil	Control	87 $\pm$ 4	96 $\pm$ 7	9.44 $\pm$ 0.74	475 $\pm$ 47	9.60 $\pm$ 0.52	260.4 $\pm$ 10.2	48.3 $\pm$ 5.6	95.8 $\pm$ 4.3	31.6 $\pm$ 2.06	305.8 $\pm$ 18.04	666 $\pm$ 48
	α -Chlorohydrin (total oral dose: 75 mg in 50 days)	74 $\pm$ 3	84 $\pm$ 6	9.50 $\pm$ 1.4 N.S.	191 $\pm$ 26****	5.80 $\pm$ 0.67****	105 $\pm$ 14.8****	49.6 $\pm$ 5.6 N.S.	18.8 $\pm$ 4.7****	19.04 $\pm$ 3.6****	170.7 $\pm$ 26.1****	398 $\pm$ 13****
	40 days post-recovery	74 $\pm$ 7	94 $\pm$ 9	9.50 $\pm$ 1.0 N.S.	457 $\pm$ 33 N.S.	5.3 $\pm$ 1.0****	175.5 $\pm$ 13.0****	43.6 $\pm$ 6.6 N.S.	70.2 $\pm$ 7.8 N.S.	28.5 $\pm$ 1.04 N.S.	293.3 $\pm$ 41.0 N.S.	561 $\pm$ 36 N.S.
Rat	Control	—	257 $\pm$ 13	4.49 $\pm$ 0.24	286 $\pm$ 39	111.2 $\pm$ 7.74	306.5 $\pm$ 11.5	12.6 $\pm$ 2.9	46.7 $\pm$ 2.3	22.1 $\pm$ 1.9	326.6 $\pm$ 27.1	894 $\pm$ 75
	α -Chlorohydrin (total oral dose: 120 mg in 24 days)	—	217 $\pm$ 18	4.60 $\pm$ 0.72 N.S.	141 $\pm$ 19****	36.9 $\pm$ 6.1****	213 $\pm$ 6.6**	13.5 $\pm$ 2.7 N.S.	29.5 $\pm$ 4.5****	13.7 $\pm$ 1.7****	249 $\pm$ 10.2**	452 $\pm$ 36****
	40 days post-recovery	—	211 $\pm$ 13	6.30 $\pm$ 1.5**	319 $\pm$ 29 N.S.	131.1 $\pm$ 21.7 N.S.	344.5 $\pm$ 17.9 N.S.	15.0 $\pm$ 3.2 N.S.	47.1 $\pm$ 2.7 N.S.	—	315.5 $\pm$ 12.7 N.S.	950 $\pm$ 79 N.S.

Values are expressed as Means $\pm$ S.E. and those for all the organ weights are as mg/100 g body wt. The mean body weight and weights of the various organs were based on ten determinations. ** $P < 0.01$ compared with controls. **** $P < 0.001$ compared with controls. N.S., non-significant compared with controls.

anterior, pars intermedia and pars nervosa. The median eminence forms the main component of the stalk with the pars tuberalis. In the pars anterior, six chromophil cell types: erythrosinophil or lactotroph (LTH-cell); orangeophil or somatotroph (STH-cell); FSH-gonadotroph; LH-gonadotroph; thyrotroph and ACTH cells, have been distinguished (Kaul & Ramaswami, 1973).

In rat and gerbil, the largest number of PAS-positive cells were found at the periphery of the gland, particularly in the region adjacent to the pars intermedia. The cytoplasm of these cells stained deeply with Cleveland-Wolfe aniline blue. The nuclei showed a distinct chromatin framework with a well-developed Golgi body. The PAS-Orange G-methyl blue staining technique showed that PAS-purple cells were more numerous than PAS-red cells. The former have been identified as delta-basiphils (FSH-cells) in both rats (Lakshman & Isaac, 1973) and gerbils (Kaul & Ramaswami, 1973). Treatment with α -chlorohydrin brought about remarkable changes in the delta-basiphils. The cell cytoplasm showed fine granulation and vacuolization. Castration vacuoles appeared in many delta-basiphils of the anterior lobe.

Table 2. Pituitary cytology of the intact, castrated and α -chlorohydrin-treated gerbils

Group	Treatment	Basiphils/ median sagittal section	Acidophils/ median sagittal section	Chromophobes/ median sagittal section	Total no. of cells counted
1	Control	444 $\pm$ 56* (15.5 $\pm$ 1.5%)	1022 $\pm$ 143 (35.7 $\pm$ 2.3%)	1401 $\pm$ 117 (48.8 $\pm$ 2.7%)	14335
2	Castration (30 days)	930 $\pm$ 67 (28.6 $\pm$ 0.5%)	815 $\pm$ 80 (24.8 $\pm$ 3.9%)	1540 $\pm$ 89 (46.6 $\pm$ 1.8%)	16425
3	α -Chlorohydrin (75 mg orally)	741 $\pm$ 44 (21.5 $\pm$ 2.6%)	964 $\pm$ 29 (28.0 $\pm$ 5.2%)	1736 $\pm$ 59 (50.5 $\pm$ 5.7%)	17205

* Mean $\pm$ S.E.

It is well known that gonadectomy leads to hypertrophy and hyperplasia of the castration cells (Mietkiewski, Malendowicz & Lukaszyk, 1969). On the 30th postoperative day, a large number of castration cells were noticed in the gerbils and rats. These cells had characteristic ring-like vacuoles of various sizes and the Golgi bodies were hypertrophied.

In the pars anterior of the gerbil pituitary, there were few basiphils, many acidophils and a considerable number of chromophobes (Table 2). Treatment with α -chlorohydrin brought about an increase in the percentage of basiphils from 15.5% (Group 1; Table 2) to 21.5% (Group 3). The percentage of acidophils dropped significantly ($P < 0.01$), but the percentage of chromophobes in Groups 1 and 3 remained unchanged (Table 2).

In castrated gerbils, the basiphilic cell percentage increased very greatly (28.6%).

The results of the present investigation revealed that α -chlorohydrin exerts its effects on the androgen-dependent structures such as seminal vesicles, ventral prostate, epididymides and perineal complex (including the levator ani muscles,

penis and bulb of the penis). These effects are reversible followed by a period of 40 days recovery in rats and gerbils (Table 1).

The compound brings about transient changes resembling those of castration in the anterior lobe of the pituitary of male rats and gerbils. In the treated gerbil, there was a marked decrease in the number of acidophils (Table 2), which have been shown to be the source of growth hormone and of prolactin in the adenohypophysis (Purves, 1961). A rise in the activity of the gonadotrophic cells following α -chlorohydrin administration is quite conclusive in the increased basophilic cell percentage (15.5% to 21.5%).

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