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Possible Mechanism of Antifertility Activity of 3-Chloro-1, 2-Propanediol (U-5897) on the Female Genital Tract of *Rattus rattus* Rufescens—A Biochemical and Histophysiological Study

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3-chloro-1, 2-propanediol (U-5897: α-chlorohydrin), an effective antifertility drug caused follicular atresia when administered chronically in female house rats. The luteal cells were shrunken and degenerative.

The uterus was smaller in size and showed poorly developed endometrial gland after α-chlorohydrin treatment.

Depletion of RNA, protein and sialic acid contents of ovary, uterus and vagina reflects an antioestrogenic nature of the compound.

The decrease in glycogen contents and alkaline/acid phosphatase activity of ovary, uterus and vagina after drug administration further points out the antioestrogenic nature of the compound.

Ovariectomy caused reduction in the protein, RNA, sialic acid, alkaline phosphatase and glycogen contents of uterus and vagina.

Oestradiol dipropionate enhanced the growth of the uterus and vagina as well as increased the protein, RNA, sialic acid, alkaline phosphatase and glycogen contents.

Oestradiol dipropionate failed to restore the growth as well as RNA, sialic acid, alkaline phosphatase activity of the uterus and vagina to normal level in spayed animals treated with α-chlorohydrin.

Key Words: α-chlorohydrin, Antioestrogenic, Luteolytic, Follicular atresia, RNA

Introduction

3-chloro-1, 2-Propanediol (U-5897) has been proposed as a male chemosterilant for the control of rat population, since a single administration produced permanent sterility with no diminution in agressiveness or libido (Ericsson 1968). Reports on the effect of α-chlorohydrin on the motility of spermatozoa have also been conflicting. Ericsson and Baker (1970) were unable to detect any change in motility of either uterine or

epididymal spermatozoa in rats given an antifertility dose of α-chlorohydrin. Other workers have observed an inhibitory effect of α-chlorohydrin on the motility of spermatozoa in the rat (Samojlik & Chang 1970, Vickery et al. 1974). Tsunoda and Chang (1976) reported that α-chlorohydrin effects the fertilizing capacity of spermatozoa *in vitro* and *in vivo* in rat.

Literature survey revealed that almost no work has been reported on the possible luteolytic effects of α-chlorohydrin on the female reproductive system of mammals except from this laboratory (Dixit et al. 1974, 1975).

In the present investigation α-chlorohydrin is being used to confirm its luteolytic/antioestrogenic properties in intact and spayed house rats.

Materials and Methods

House rats were kept for two weeks in the laboratory before the commencement of the experiments. Vaginal smears were taken daily for at least two complete estrous cycle. The animals were given rat food (Hindustan Lever Private Ltd.) and water *ad libitum*. Sixty house rats with regular oestrous cycle were taken and divided into groups of ten animals each. The treatment was made as under:

Group A—animals receiving 0.2 ml distilled water each day and served as controls

Group B—α-chlorohydrin (supplied by UPJOHN Company Kalamazoo) (50 mg/body wt./animal) was injected daily for a period of 3 weeks

Group C—Ovariectomized: The ovaries were removed surgically and on day 5 of ovariectomy, each animal received the vehicle alone for a period of 15 days and served as controls for groups D, E and F

Group D—Ovariectomized + α-chlorohydrin (50 mg/kg/day) for 15 days

Group E—Ovariectomized+Oestradiol dipropionate (Ovocyclin: CIBA Ltd., 80 μg/day) for 15 days

Group F—Ovariectomized + Oestradiol dipropionate (80 μg/day) + α-chlorohydrin (50 mg/kg day) for 15 days

All the animals were killed by rapid decapitation 24 hr after the administration of the final dose of α-chlorohydrin. Ovaries, uterus, vagina, adrenals and preputial glands were dissected free of fat and weighed on a torsion balance. Right ovary, right uterine horn and a piece of vagina were fixed in Bouin's fluid and embedded in paraffin wax for histological studies. The left ovary, left unterine horn and remaining part of vagina and adrenal glands were immediately frozen for the estimation of total RNA, protein, sialic acid, glycogen and enzyme phosphatases by the method of Munro and Fleck (1966), Lowry et al. (1951), Warren (1959), Montogomery (1957), Fiske and Subbarow

Figures 1–6 *1*, Microphotograph of an ovum from the normal cycling house rat. HE (× 100); *2*, Ovum after α-chlorohydrin treatment. Note the atretic changes in a mature ovum. HE(× 100); *3*, Section of uterine horn from a normal cycling house rat. The uterine glands are seen full of secretory material. HE (× 100); *4*, Uterus after α-chlorohydrin treatment. Note the regression of the uterine glands. HE (× 100); *5*, Section of vagina of cycling house rat (estrous cycle). Intense mucification and Keratinization of the epithelium is conspicuous. HE (× 100); *6*, Vagina after α-chlorohydrin treatment. Note the vacuolization of the epithelium with a pronounced passage of leucocyte. in the lumen. HE (× 100)


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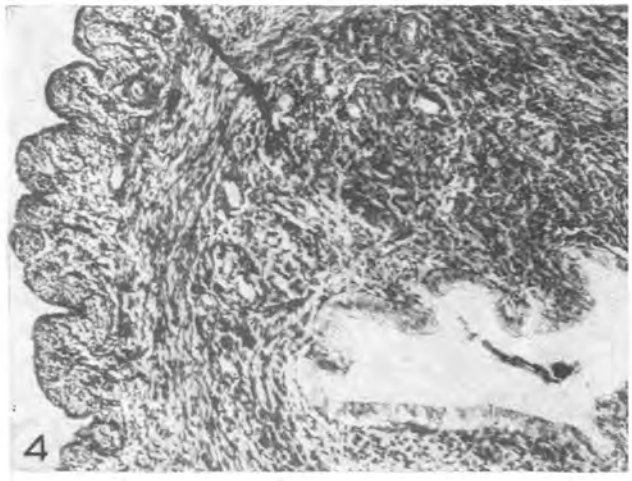
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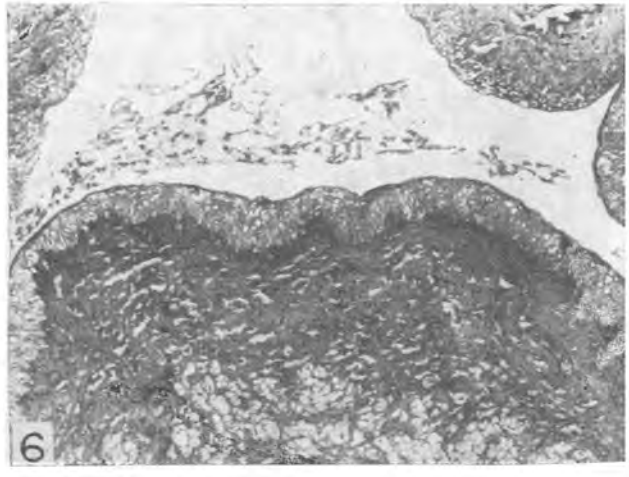
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(1925). The measurement of the diameter of 50 corpora lutea was carried out on four sections from each ovary with camera lucida drawing at 80×; averaged and expressed in terms of means of corpora lutea diameter. The standard error of the average values was calculated. Students' '*t*' test was applied in comparing means.

Observations

Body weight: Administration of α-chlorohydrin in intact (Group B) and ovariectomized (Group C) animals caused a small change in body weight. Combined treatment of α-chlorohydrin and oestradiol in ovariectomized (Group F) animals brings about insignificant reduction in the body weight. However, administration of oestradiol in ovariectomized (Group E) animals resulted in an increase in the body weight.

Organ weight: The relative weights of ovaries, uterus, vagina, and preputial glands were drastically reduced after α-chlorohydrin treatment (50 mg/kg body wt/day for 21 days), when compared with control (Group A) animals. The weight of adrenal glands was not changed after α-chlorohydrin treatment.

In ovariectomy, the weight of uterus, vagina, preputial glands and adrenal glands was reduced significantly ($P<0.001$). α-chlorohydrin treatment (50 mg/kg b. wt/day) in ovariectomized animals brings about a further reduction in the weight of uterus, vagina and preputial glands whereas an increase in adrenal gland weight was recorded (table 1). Oestradiol dipropionate (80 μg/day for a period of 15 day) enhanced the growth of uterus vagina and adrenal glands in ovariectomized house rats (Group E) ($P<0.001$). α-chlorohydrin (6 mg) and oestradiol (80 μg) when given simultaneously did not cause any increase in the growth of the uterus, vagina and preputial glands. The organ weights were maintained at the level of ovariectomized Group-C animals.

Histological changes

In α-chlorohydrin-treated house rats, follicular atresia were conspicuous (figures 1 & 2). The uterine endometrium was reduced. The uterine glands were regressed and showed no secretion (figures 3 & 4).

In α-chlorohydrin-treated ovariectomized animals, the utrine changes were similar as seen in case of intact α-chlorohydrin-treated animals. In oestrogen-treated spayed animals, the uterus was enlarged and the uterine glands showed the presence of secretory material, whereas the uteri of oestrogen + α-chlorohydrin-treated animals, or (Group F) were reduced. The uterine glands were regressed.

Vagina: The vagina was lined with highly mucinous zone of cells overlying a multilayered epithelium. The epithelium consisted of five to six cell layers. After α-chlorohydrin treatment, the mucin was sloughed and there was a pronounced passage of leucocytes into the vaginal lumen (figures 5 & 6).

In oestrogen-treated ovariectomized animals, the vagina showed well-defined keratinization. The vagina of α-chlorohydrin and oestradiol dipropionate-treated animlas showed sloughed mucin. The leucocytes were present in the vaginal smear.

Biochemical changes

Protein and RNA: The protein and RNA contents of ovary, uterus, vagina and preputial glands were reduced after α-chlorohydrin-treatment in comparison of control (Group-A) group animals (table 2, $P<0.001$).

α-chlorohydrin treatment in spayed house rats caused a reduction in the protein and RNA contents of uterus, vagina and preputial glands. This reduction was highly significant in uterus and vagina ($P<0.001$)

Table 1 *Efects of α-chlorohydrin administration on the body weight and weights of ovary, uterus, vagina, adrenal and preputial glands of house rats (Animals examined=10)*

Treatment	Initial body wt. (g)	Final body wt. (g)	Ovary	Uterus	Vagina	Preputials	Adrenals	Corpora lutea diam. (in μm)
					mg/g			
Control (G–A)	127±8	125±2	35.9±6.5	151.3±6.8	147±9.5	201±37	62±1.53	0.608±0.035
α-Chlorohydrin (G–B) (150 mg)	114±5	112±1	12.7±0.8*	79±20.8*	87.5±2.8*	90±15*	78±4.3+	0.285±0.025*
Ovariectomy (G–C)	119±5	120±7	—	48.9±06.2*	85.5±3.9*	74.0±13.3*	49.3±2.7*	—
Ovariectomy+ α-Chlorohydrin (75 mg) (G–D)	110±7	111±9	—	47.7±15.2†	76.5±3.3+	62.7±3†	58.7±4†	—
Ovariectomy+ Oestradiol dipropionate (1.2 mg)	115±8	144±11	—	266.3±11.8**	168.2±8.3**	61.3±6.3†	65.5±1.3++	—
Ovariectomy+ α-Chlorohydrin (75 mg) +Oestradiol dipropionate (1.2 mg) (G–F)	116±6	115±8	—	43.6±8.3†	84.3±6.6†	58.3±2.5†	78.6±8.9**	—

* $P<0.001$ compared with control (G–A)
** $P<0.001$ compared with (G–C) group
\+ $P<0.01$ compared with (G–C) group
++ $P<0.01$ compared with Ovariectomy (G–C) group
\+ Non significant compared with (G–C) group
All figures: Mean±S.E.M.

Table 2 *Changes in total Protein, RNA, Sialic acid, Glycogen and phosphatase enzyme activity of ovary, uterus, vagina and preputial glands of house rats after α-chlorohydrin treatment*

Treatment	Name of tissue	Protein	RNA	Sialic acid	Glycogen	Alkaline phosphatases β unit	Acid Phosphatases β unit
Control (G–A)	Ovary	205±4.6	8.6±0.03	3.7±0.2	—	9.8±0.8	4.6±0.3
	Uterus	190±2.8	6.9±0.5	0.5±0.2	4.3±0.1	10.7±1.3	3.1±0.7
	Vagina	216±2.4	7±0.01	2.9±0.0	3.8±0.1	9.6±0.2	3.4±0.1
	Preputial	241±7.3	12.3±0.3	—	—	—	—
α-Chlorohydrin (150 mg) (G–B)	Ovary	105±1.7*	4.0±0.9*	1.7±0.2*	—	6.8±0.2+	2.1±0.4*
	Uterus	127±4*	5.5±0.3*	1.6±0.3*	2.9±0.2*	7.2±0.5+	2.5±0.8+
	Vagina	135±3.8*	5.1±0.2*	1.7±0.1*	2.6±0.3*	6.8±0.1*	2.6±0.3+
	Preputial	220±1.3†	6.28±0.45*	—	—	—	—
Ovariectomy (G–C)	Uterus	168±38†	5.4±0.3*	2±0.9*	2.6±0.3*	6.7±0.2*	—
	Vagina	205±33†	5.6±0.4+	2.15±0.1*	2.4±0.5*	5.5±0.5*	—
	Preputial	230±10†	4.8±0.3*	—	—	—	—
Ovariectomy+ α-Chlorohydrin (75 mg) (G–D)	Uterus	115±0.9**	4.2±0.2**	1.6±0.2††	2.3±0.4++	4.7±0.3**	—
	Vagina	105±0.2**	4.1±0.2**	1.85±0.2††	1.9±4.4++	4.3±0.1**	—
	Preputial	182±1++	4.1±0.3++	—	—	—	—
Ovariectomy+ Oestradiol dipropionate (1.2 mg) (G–E)	Uterus	252±19**	6.8±0.1**	25±0.1††	3.8±0.4**	10.5±0.4**	—
	Vagina	280±23**	6.3±0.1++	2.65±0.1††	2.9±0 4††	9.5±0.4++	—
	Preputial	200±19††	7.0±0.2**	—	—	—	—
Ovariectomy+ α-Chlorohydrin (75 mg)+	Uterus	125±43†	4.3±0.4++	1.9±0.3††	2.6±0.5††	4.3±0.4**	—
	Vagina	157±14++	4.3±0.3++	1.8±0.4††	2±0.4††	4.9±0.5††	—
	Preputial	178±5++	4.0±0.1++	—	—	—	—

Biochemical estimations: Mean of six determination

+P<0.001 compared with control (G–A)
†P<0.01 compared with control (G–A)
†Non significant compared with control (G–A)
**P<0.001 compared with ovariectomy (G–C) group
++P<0.01 compared with ovariectomy (G–C) group
††Non significant compared with ovariectomy (G–C) group
All figures: Mean ±S.E.M.

(table 2) when compared with ovariectomized controls. Oestradiol dipropionate administration, the protein and RNA contents of uterus and vagina increased in ovariectomized animals ($P<0.001$, table 2), whereas they showed no change in the preputial gland.

α-chlorohydrin inhibited the action of oestradiol dipropionate when administered simultaneously in ovariectomized animals was shown by the reduced levels of protein and RNA in the uterus, vagina and preputial glands (table 2, Group F).

Sialic acid: α-chlorohydrin-treatment caused a reduction in sialic acid contents of ovary, uterus and vagina ($P<0.001$, table 2).

α-chlorohydrin also brings about a reduction in the sialic acid contents of uterus, vagina and preputial glands of ovariectomized house rats.

Oestradiol dipropionate treatment enhanced the sialic acid contents of uterus and vagina of ovariectomized animals (table 2).

α-chlorohydrin and oestradiol, when administered simultaneously did not increase the sialic acid contents of uterus and vagina of ovariectomized animals.

Alkaline phosphatase: The alkaline phosphatase activity in α-chlorohydrin treatment was low in the ovary, uterus, vagina ($P<0.01$).

α-chlorohydrin treatment in ovariectomized animals decreased enzyme activity in the uterus and vagina, whereas oestradiol dipropionate increased it.

α-chlorohydrin and oestradiol, when administered together also resulted in a decrease of the alkaline phosphatase activity of uterus and vagina (table 2).

Acid phosphatase: α-chlorohydrin-treatment caused a reduction in the acid phosphatase enzyme activity of uterus and vagina ($P<0.01$).

Glycogen: α-chlorohydrin reduce the glycogen contents of uterus and vagina ($P<0.001$, table 2). In ovariectomized (Group C) the glycogen contents of uterus and vagina were significantly low ($P<0.01$, table 2). α-chlorohydrin administration in ovariectomized animals caused a further reduction in the glycogen contents of uterus and vagina ($P<0.001$, table 2). Oestradiol dipropionate increased the glycogen contents of uterus whereas no change was noticed in the vagina of ovariectomized animals. Oestradiol dipropionate did not increase the glycogen content of the uterus in the presence of α-chlorohydrin.

Discussion

α-chlorohydrin is an effective antifertility agent, which appears to be devoid of oestrogenic activity (Stacy et al. 1975). Histological changes throw some light on the probable mode of action of α-chlorohydrin in the reproductive tract of female house rat. The follicular atresia were conspicuous. The luteal cells were regressed. The regression was of similar nature as seen in PGF-2 α treatment (Stacy et al. 1975). The uterus of α-chlorohydrin-treated house rat was smaller in size and showed poorly developed endometrial glands. Vaginal smears were of diestrous type.

Ovariectomy caused a reduction in the weight of uterus and vagina (Freudenberger & Hashimoto 1939) whereas oestrogen treatment increased the weight of uterus and vagina and also brings about Keratinization in ovariectomized house rats (Drill 1966).

Dixit et al. (1974) demonstrated thepresence of antioestrogenic activity of α-chlorohydrin in gerbils (*Meriones hurrianae*). By using spayed house rats, the antioestrogenic activity of α-chlorohydrin was demonstrated on the basis of inhibition of uterine weight increase (Astwood Test) in presence of oestradiol dipropionate.

Reduction in RNA and protein contents of the female genital tract of α-chlorohydrin-treated rats suggests an inhibition of oestrogen production (Drasher 1952, Mohla & Prasad 1969).

Changes in the sialic acid level in the vagina are dependent on ovarian hormone (Galleti & Gardir 1973). An antioestrogenic action of α-chlorohydrin is reflected in a decreased sialic acid concentration in the vagina of treated house rats. In the present investigation oestradiol-dipropionate caused an increase in the vaginal sialic acid in ovariectomized house rats which is similar to the findings of Coppola and Ball (1966).

Oestrogens specifically increase the glycogen contents of uterus in rat (Walas 1952). The decrease in glycogen contents of α-chlorohydrin-treated uterus confirms the antioestrogenic nature of the drug. Gregoire et al. (1967) reported that the glycogen content of vagina was not affected by the adminstration of oestradiol. In contrast to this, α-chlorohydrin reduced glycogen contents of vagina in house rats.

Atkinson and Engle (1947) reported that oestrogen enhanced the alkaline phosphatase of the uterus in rat. Whereas spaying depletes it (Manning et al. 1967). Depletion in alkaline phosphatase activity was confirmed in spayed house rats. Manning et al. (1967) also reported that oestrogen enhanced acid phosphatase activity of spayed uterus. The acid phosphatase activity was reduced in α-chlorohydrin treated house rats further reflects the antioestrogenic nature of the compound.

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