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Teratogenic Effects of Cyclopamine and Jervine in Rats, Mice and Hamsters[1] (38794)

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Certain steroidal alkaloids from *Veratrum californicum*, including cyclopamine and jervine, produce congenital cyclopia and related cephalic terata in domestic livestock (1), chick embryos (2), and rabbits (3). We are testing teratogenicity of structural analogs of these and related alkaloids prepared by organic synthesis. We desire to determine the absolute structural and configurational requirements a steroidal alkaloid teratogen of this type must possess in order to induce deformities. We believe this to be important because steroidal alkaloids of structural similarity to the Veratrum teratogens are found in human foods (the solanum alkaloids of potatoes, tomatoes, egg plant, etc.). Knowledge of structural and configurational requirements (structure-activity relationship) will enable one to predict which, if any, of the known or yet to be isolated alkaloids present a potential hazard. Therefore, for assay we have sought a laboratory animal that is susceptible to the alkaloids, that requires only small doses, that produces a high and reliable incidence of malformations, that has a large litter size, that is easily maintained, and that has a low background level of cephalic malformations. Neither rabbits nor chick embryos meet all these requirements. Preliminary experiments (4) suggested that rats were susceptible and offered some promise, but dose levels were rather high.

This report describes experiments designed to test more thoroughly the teratogenic effect of cyclopamine and jervine in rats as well as in hamsters and mice and to assess how well these species meet the above criteria.

Materials and Methods. Individually housed pregnant Albino rats (Sprague–Dawley derived),[2] Golden hamsters,[2] and Swiss Webster mice[3] were gavaged in the morning with the experimental compounds suspended in water at designated levels and on the designated days of gestation (Tables I–III). Others were gavaged once each day on the designated days with water only and others served as nongavaged controls. Day 1 of gestation was considered to begin at midnight preceding the finding of sperm in the vaginal tract on morning examination. Rats, mice, and hamsters were provided with laboratory chow[4] and water *ad lib.* Rats were killed at day 20, mice at day 19, hamsters at day 15 by chloroform inhalation, and fetuses were examined grossly for cranial or spinal defects. Implantation sites were counted to determine whether there was an unusual number of resorptions from any treatment. They were readily discernible as enlarged segments in the uterine horn of a size nearly 0.5 that of gravid sites at term. Dams found nonpregnant when sacrificed were excluded from data tabulation if examination showed no previous uterine activity.

The water gavaged controls received the same amount of water given the treatment groups (5 cc for rats, 3 cc for hamsters, and 1.5 cc for mice) but without the experimental compounds. In some instances, an inorganic buffer ($CaCO_3$) was administered with the experimental compounds at a level of 15 mg of buffer per mg of experi-

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[2] Simonsen Laboratories.

[3] PelFreeze BioAnimals, Inc.

[4] Purina.

(Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U. S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.)

TABLE I. EFFECT OF JERVINE AND CYCLOPAMINE ON RATS

Group and gestation period	Individual daily dose (mg)	Dose (mg/kg)	No. of dams	Ratio of affected to normal litters	No. of normal offspring	No. of affected offspring	No. of resorptions in litters w/offspring	No. of totally resorbed litters	No. of overdose deaths in dams	Malformations[a]
Jervine:										
6–8	10	40	4	0/4	39	0	2	0	0	—
6–8	20	80	3	0/1	0	0	0	0	2	—
6–9	60	240	1	0	0	0	0	0	1	—
7–11	20	80	2	0	0	0	0	1	1	—
7–10	60	240	3	0	0	0	0	0	3	—
8–12	20	80	8	0/5	22	0	34	2	1	—
8–10	30	120	1	0/1	13	0	0	0	0	—
8–11	60	240	1	0	0	0	0	0	1	—
6	30[b]	120	5	0/5	57	0	1	0	0	—
6	60[b]	240	3	0/3	29	0	6	0	0	—
7	60[b]	240	2	0/2	10	0	6	0	0	—
8	30[b]	120	4	0/4	44	0	1	0	0	—
8	60[b]	240	3	0/3	29	0	6	0	0	—
9	30[b]	120	5	0/5	41	0	5	0	0	—
9	90[b]	360	5	0/1	10	0	0	0	4	—
10	60[b]	240	5	0/1	11	0	0	2	2	—
Cyclopamine:										
6–9	60[b]	240	10	3/5	45	28	0	1	0	16-C, 8-M, 2-H, 1-E, 1-A
6–9	90[b]	360	5	4/5	22	23	8	0	0	23-C
6	60[b]	240	8	0/8	86	0	4	0	0	—
7	60[b]	240	12	0/12	121	0	3	0	0	—
8	90[b]	360	5	0/5	31	0	0	0	0	—
9	90[b]	360	4	0/4	42	0	2	0	0	—
Controls:										
nongavaged			19	0/19	204	0	5	0	0	—
H_2O-gavaged			15	0/15	133	0	2	0	0	—

[a] C = cebocephalic, M = microphthalmic, H = hydrocephalic, E = exencephalic, A = anencephalic
[b] Sample and buffer.

mental compound. At the outset of experimental feeding, the rats averaged 250 g, the mice 28 g, and the hamsters 120 g.

The two experimental compounds, jervine and cyclopamine, were isolated as previously described (5, 6) although some of the jervine was obtained commercially.[5]

Results and Discussion. The data presented in Table I show that the Albino rat (Sprague–Dawley derived) fetus was susceptible to the teratogen cyclopamine but resistant to jervine. Doses of jervine sufficiently high to produce a few deaths in dams and a significantly higher resorption incidence did not cause terata. Cyclopamine dosed on days 6–9 at levels of 60 mg/day produced malformed offspring without producing deaths in dams or an increased resorption incidence. But efforts to produce terata on single-day dosings were unsuccessful. Cebocephaly was the principal malformation produced on 6- to 9-day dosings with cyclopamine.

The data presented in Table II show that the Swiss Webster mouse fetus was rather resistant to the teratogens jervine and cyclopamine. A few exencephalics were found (8 from a total of 421 fetuses in treated groups = 1.9%). One was found in controls (1 of

[5] S. B. Penick and Co.

TABLE II. EFFECT OF JERVINE AND CYCLOPAMINE ON MICE.

Group and gestation period	Individual daily dose (mg)	Dose (mg/kg)	No. of dams	Ratio of affected to normal litters	No. of normal offspring	No. of affected offspring	No. of resorptions in litters w/offspring	No. of totally resorbed litters	No. of overdose deaths in dams	Malformations[a]
Jervine:										
7	5[b]	180	6	0/3	26	0	0	0	3	—
6–9	10[b]	360	8	0	0	0	0	0	8	—
7–9	5[b]	180	4	0/1	6	0	3	1	2	—
7	5	180	6	0/3	29	0	0	0	3	—
7	10	360	9	0/1	14	0	0	1	7	—
8	5[b]	180	5	0/2	17	0	0	1	2	—
9	5[b]	180	5	2/5	60	3	0	0	0	3-E
9	5	180	8	0/4	46	0	0	0	4	—
8	5	180	7	1/5	43	1	0	0	2	1-E
Cyclopamine:										
7	5[b]	180	3	0/1	10	0	0	0	2	—
6–7	10[b]	360	3	0	0	0	0	0	3	—
6–8	10[b]	360	4	0/2	26	0	0	0	2	—
7–9	10	360	7	0/1	12	0	0	0	6	—
7	5	180	6	0/2	23	0	0	0	4	—
7	10[b]	360	4	1/2	25	1	0	0	2	1-E
8	5	180	6	1/1	11	1	0	1	4	1-E
9	5	180	11	2/7	73	2	0	0	4	2-E
Controls:										
nongavaged			14	0/14	148	1	2	0	0	1-E
H_2O-gavaged			13	0/13	127	0	2	0	0	—

[a] E = exencephalic.
[b] Sample and buffer.

276 = 0.4%). The exencephalics in treated groups may have been fortuitous because this strain of mice has a spontaneous incidence of exencephaly of about 0.5%. The dose levels were high enough to cause a high proportion of deaths in dams, but resorptions and terata were rare.

The data presented in Table III show that the Golden hamster fetus was very susceptible to the teratogenic effect of both jervine and cyclopamine. An effective teratogenic dose for a 120 g hamster was 20 mg (170 mg/kg) of jervine or 30 mg (250 mg/kg) of cyclopamine (the latter w/buffer). At these dose levels, a significant increase in resorptions occurred over those of controls and some of the dams died (up to 50%) particularly when multiple-day doses were given. The day of insult proved to be day 7 of gestation. Single or double globe cyclopia commonly results in domestic livestock and rabbits from cyclopamine ingestion but was not found in the hamster offspring. Cebocephaly with or without a cranial bleb was the principal deformity. Harelip was common and there were a few exencephalics. Most harelip individuals also had cleft palates. As had been found earlier to be the case in rabbits (3), the use of $CaCO_3$ as a buffering medium with the cyclopamine was helpful to avoid acid aromatization to the inactive veratramine by the acid of the stomach. Terata were produced by cyclopamine with or without buffer, but the incidence was much higher when buffer was used (see 7-day 20 mg groups). No difference was noted in presence or absence of buffer with jervine, and none was expected because jervine is not particularly acid labile. From the data of Tables I and II no conclusions can be drawn regarding the usefulness of buffering in rats and mice.

We have found only two drawbacks in using hamsters as an animal assay of

TABLE III. EFFECT OF JERVINE AND CYCLOPAMINE ON HAMSTERS

Group and gestation period	Individual daily dose (mg)	Dose (mg/kg)	No. of dams	Ratio of affected to normal litters	No. of normal offspring	No. of affected offspring	No. of resorptions in litters w/offspring	No. of totally resorbed litters	No. of overdose deaths in dams	Malformations[a]
Jervine:										
5–8[b]	30	250	7	0/0	0	0	0	3	4	—
6–9	20	170	6	2/2	0	13	8	2	2	13-C
6–7	20	170	6	4/4	3	18	29	0	2	12-C, 3-C/CB, 3-HL
7–8	20	170	7	3/3	0	14	21	0	4	7-C, 2-C/CB, 3-HL, 2-E
6	20	170	4	0/4	31	0	10	0	0	—
7	20	170	8	7/7	26	49	14	0	1	18-C, 5-C/CB, 18-HL, 8-E
8	20	170	6	5/5	10	22	19	1	0	9-C, 5-C/CB, 6-HL, 2-E
9	20	170	4	0/4	47	0	1	0	0	—
7[b]	20	170	6	6/6	6	21	31	0	0	11-C, 9-HL, 1-HL/CB
7	10	85	6	1/5	49	2	0	0	1	2-HL
7	5	42	3	0/3	27	0	2	0	0	—
Cyclopamine:										
7	30	250	8	1/1	6	3	3	1	6	1-C, 2-HL
7[b]	30	250	6	2/3	11	19	7	0	3	14-C, 5-HL
7[b]	20	170	9	4/4	9	28	14	0	5	3-C/E, 12-C, 1-C/CB, 12-HL
7	20	170	5	1/4	45	1	8	0	4	1-HL
7	10	85	5	0/5	48	0	4	0	0	—
Controls:										
nongavaged			7	0/7	78	0	3	0	0	—
H_2O-gavaged			5	0/5	54	0	1	0	0	—

[a] C = cebocephalic, C/CB = cebocephalic with cranial bleb, HL = harelip, E = exencephalic, HL/CB = harelip with cranial bleb.

[b] Sample and buffer.

steroidal alkaloid teratogens. First their breeding pattern is sensitive to ambient temperature fluctuations. Second, fetuses are susceptible to a congenital hemorrhagic necrosis of the central nervous system, evidently a result of an infectious process (7). The infection briefly and spontaneously appeared and disappeared in our colony prior to the inception of these studies (7). The same disease was reported earlier by Kilham and Margolis (8) in hamsters obtained from three suppliers. Although easily recognized (7, 8) and unlikely to be confused with other terata, the incidence was high enough in their experience and in ours briefly to disrupt other studies.

Aside from these drawbacks, hamsters appear to meet very adequately the criteria listed above for a laboratory animal assay of steroidal alkaloid teratogens. They are easy to maintain. They require but a single small dose (20 mg/animal or about 170 mg/kg) of teratogen to induce malformations. Incidence of terata is high among litters (up to 100%), and high among individuals within a litter (50–100%), and litter size is large (about 10 offspring average). Except for the spontaneous hemorrhagic necrosis of apparent infectious origin mentioned above, background incidence of cephalic malformations is negligible.

Renwick (9) suggested that there might be

a possible relationship between maternal consumption of blighted potatoes and fetal anencephaly/spina bifida (ASB) in humans. Among possible teratogens suggested by him were the endogenous solanum steroidal alkaloids. Because the veratrum teratogens and the solanum alkaloids bear considerable structural similarity, we believe the golden hamster will be an assay ideally suited to study whether potato alkaloids are teratogenic, indeed much better than the rat assay that we earlier thought might be useful (4). We are presently using the assay to measure teratogenicity of a variety of naturally occurring steroidal alkaloids and synthetic analogs from both the veratrum and solanum groups.

Summary. Golden hamster fetuses were extremely sensitive to the teratogenic action of jervine and cyclopamine, the steroidal alkaloid teratogens from *Veratrum californicum.* Cebocephaly, harelip/cleft palate, exencephaly, and a cranial bleb were the common deformities produced by dosing on the seventh day of gestation. Sprague–Dawley derived albino rats were susceptible to cyclopamine but not to jervine, and at an incidence very much lower than that of hamsters. Cebocephaly and microphthalmia were the common deformities. The terata were observed as a consequence of sixth- to ninth-day dosings. Single-day dosing produced no terata. Swiss Webster mice were apparently resistant to the teratogens.

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