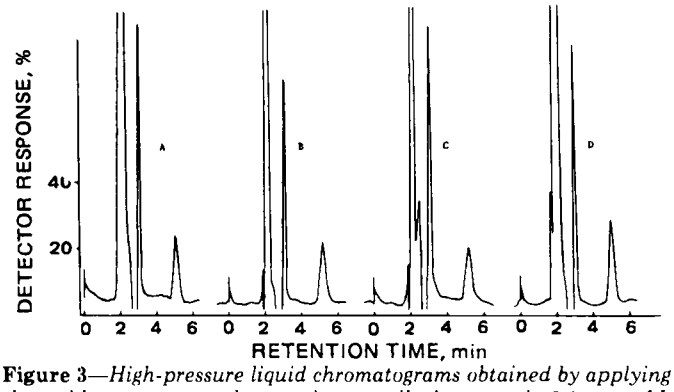

Figure 3—*High-pressure liquid chromatograms obtained by applying the residue assay procedure to tissues spiked to contain 0.1 ppm of I. The solvent system was 65% chloroform, 30.5% isooctane, 4.0% methanol, and 0.5% acetic acid. Key: A, liver; B, kidneys; C, muscle; and D, fat.*

tolerance level except liver, which had a recovery of 69% and a relative standard deviation of ~10% (Table V).

Unspiked tissues typically showed no peak other than random baseline noise in the region where I eluted (Fig. 2). The magnitude of the sulfamethazine signal obtained from tissues spiked at the tolerance limit was ~20% of full scale (Fig. 3). A favorable signal-to-blank ratio at the tolerance limit was obtained with this procedure, allowing low detection limits. The tolerance limit of 0.1 ppm of I in tissues was approximately five times the detection limit of the method.

With slight modifications, the method should be applicable to several sulfonamides currently used in veterinary medicine. Recoveries of sulfamethazine only have been demonstrated to date.

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Teratogenic Potential of Cocaine Hydrochloride in CF-1 Mice

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Abstract □ This investigation revealed that cocaine hydrochloride was teratogenic when administered in nontoxic doses to gravid CF-1 mice on Days 7–12 of gestation. The teratogenic susceptibility of the CF-1 mouse fetus to cocaine hydrochloride was evident throughout this portion of the gestation period. The early appearance of eye defects and the occurrence of skeletal defects later in gestation after cocaine hydrochloride challenge paralleled the sequence of ontogenesis.

Keyphrases □ Cocaine hydrochloride—evaluation as potential teratogen, effect on catecholamine levels in placenta, skeletal and soft tissue malformations in fetal mice □ Teratogenic drugs—cocaine hydrochloride, effect on fetal mice □ Inhibition, competitive—cocaine hydrochloride and norepinephrine, effect on fetal mice

Cocaine (benzoylmethylecgonine), the first local anesthetic discovered, not only inhibits nerve conduction but also prevents norepinephrine reuptake at nerve terminals (1). Because of the latter action, norepinephrine levels are augmented and vasoconstriction occurs (1).

BACKGROUND

Previous studies detected catecholamines in human amniotic fluid (2) and placenta (3). If the mechanisms for storage and release of norepinephrine in the placenta are comparable to those at nerve terminals, increased catecholamine levels induced by cocaine could cause placental vasoconstriction.

A relationship between placental vasoconstriction and fetal anomalies was demonstrated in studies involving mechanical obstruction of uterine and placental vascular flow by clamping, which probably causes fetal malformations through decreased oxygen availability (4–7), and by drugs such as serotonin (8) and morphine (9), which accomplish similar effects pharmacodynamically.

With respect to the autonomic neural involvement of cocaine as a potential teratogen, Furchgott *et al.* (10) detected a specific transfer site with which norepinephrine molecules combine before being transferred to storage or enzymatic inactivation loci. According to Johnson and Kahn (11), the potentiation of norepinephrine responses by cocaine results from competitive inhibition between the local anesthetic and norepinephrine molecules at these transfer sites. Thus, in pregnant mice, cocaine conceivably could induce fetal malformations by raising catecholamine levels which, in turn, cause placental vasoconstriction *via* competitive inhibition.

The purpose of this study was twofold: (*a*) to test the hypothesis that cocaine is a potential teratogen in mice, and (*b*) if the results were affirmative, to ascertain the precise days of gestation when fetal susceptibility to the teratogen is evident.

EXPERIMENTAL

Animals—The test animals were CF-1 mice[1], 25–30 g. Females were placed in aggregate cages, each holding 10 animals. Two weeks after their arrival, the mice were bred if they weighed at least 25 g. Males were placed individually in metal cages (12.5 × 15 × 10 cm) with a wire-mesh front and floor[2]. All animals were maintained on laboratory food[3] and tap water *ad libitum*.

The room housing the animals was protected from exposure to natural sunlight and was equipped with an electrical lighting system[4], which al-

[1] Charles River Breeding Laboratories, Wilmington, Mass.
[2] RD-T unit, Norwich Wire Works, Norwich, N.Y.
[3] Purina Laboratory Chow, Ralston Purina Co., St. Louis, Mo.
[4] Model V-45073 astronomic dial time switch with "skipper," International Register Co., Spring Grove, Ill.

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Table I—Mean Values of the Test Groups

Treatment[a]	Day	Maternal Weight Ratio, S/T[b]	Fetal Ratio, Right Horn/ Left Horn	Resorption Ratio, Right Horn/ Left Horn	Mean Fetal Weight, g	Sex Ratio, M/F	Soft Tissue Abnormalities	Skeletal Abnormalities
Untreated	—	28.2/50.9	5.6/5.3	0.3/0.2	1.13	4.7/6.2	0.0	1.3
Saline	7	30.2/56.3	6.1/5.6	0.0/0.2	1.16	8.0/3.7	0.1	3.5
	8	28.1/54.2	6.3/5.3	0.2/0.2	1.14	7.0/4.7	0.2	1.7
	9	28.4/53.3	6.3/5.1	0.3/0.0	1.17	8.3/3.1	0.1	1.6
	10	30.7/55.6	5.7/5.7	0.0/0.6	1.15	6.8/4.6	0.0	4.0
	11	30.6/53.0	5.8/5.5	0.0/0.5	1.11	6.1/5.2	0.0	2.6
	12	27.4/52.1	6.5/4.6	0.5/0.0	1.15	7.1/4.0	0.0	3.8
Cocaine	7	29.5/54.8	5.5/6.2	0.2/0.3	1.14	5.6/6.1	6.0[c]	7.7[c]
	8	28.6/50.9	5.7/5.0	0.5/0.2	1.10	6.2/4.5	2.1[c]	7.3[c]
	9	29.8/54.4	6.7/4.7	0.7/0.2	1.12	6.3[c]/5.1[c]	2.2[c]	8.6[c]
	10	30.1/52.0	5.7/4.3	1.1[c]/1.0	1.13	6.2/3.8	2.1[c]	5.2
	11	30.4/53.6	6.5/4.3	0.6/0.7	1.13	6.6/4.2	2.6[c]	6.0
	12	28.6/53.3	5.8/5.2	0.1/0.2	1.19	6.0/5.0	1.0[c]	8.1

[a] The dose of saline was 0.3 ml sc, and the dose of cocaine was 60 mg/kg sc. [b] S = starting weight, and T = terminal weight. [c] Statistically significant in comparison with respective saline controls, $p < 0.05$.

Table II—Occurrence of Exencephaly, Cryptorchidism, and Hydronephrosis on Day of Treatment (Compared with Saline)

		Exencephaly				Cryptorchidism				Hydronephrosis			
		Litters		Fetuses		Litters		Fetuses		Litters		Fetuses	
Treatment[a]	Day	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Untreated	—	8	0	43	0	8	0	43	0	8	0	43	0
Saline	7	8	0	45	0	7	1	44	1	8	0	45	0
	8	8	0	46	0	7	1	45	1	8	0	46	0
	9	8	0	44	0	7	1	43	1	8	0	44	0
	10	8	0	44	0	8	0	44	0	8	0	44	0
	11	8	0	44	0	8	0	44	0	8	0	44	0
	12	8	0	43	0	8	0	43	0	8	0	43	0
Cocaine	7	7	1	44	1	6	2	42	3	4	4[b]	37	8[b]
	8	7	1	39	2	4	4	35	6[b]	7	1	40	1
	9	4	4[b]	41	5[b]	5	3	42	4	4	4[b]	41	5[b]
	10	7	1	39	1	4	4[b]	33	7[b]	6	2	37	3
	11	8	0	44	0	5	3	39	5[b]	6	2	41	3
	12	8	0	44	0	6	2	42	2	7	1	43	1

[a] The dose of saline was 0.3 ml sc, and the dose of cocaine was 60 mg/kg sc. [b] Statistically significant in comparison with respective saline controls, $p < 0.05$.

lowed 12 hr of light (7:00 am–7:00 pm) and 12 hr of darkness. The temperature was maintained at 22–26°.

Determination of Teratogenic Dose—The teratogenic dose of a suspected teratogen is based on its relationship to its toxic dose (LD_{50}). To ascertain the LD_{50} of cocaine hydrochloride, 40 female nongravid mice were selected randomly and assigned to one of five groups of eight animals each. Dosage levels for the five groups were 75, 100, 125, 150, and 175 mg/kg, respectively. Each mouse was weighed to the nearest 0.1 g, caged individually, and injected subcutaneously with a freshly prepared cocaine hydrochloride solution into the right side of the abdomen. After each injection, the mice were observed for behavioral changes and fatalities.

The estimated LD_{50} of cocaine hydrochloride in nongravid mice was 113 mg/kg (12). Nongravid animals are acceptable substitutes for gravid animals because the LD_{50} of a drug in gravid mice generally is equivalent to that in nongravid mice (13). Because a dose that produces severe toxicity and behavioral changes in female mice also will exert devastating effects on developing fetuses, the teratogenic dose should be nonlethal and elicit only minimal pharmacological responses.

Stimulation followed by depression and eventual recovery occurred in most animals receiving doses of 100 mg/kg or higher, but deaths also were noted at these levels. At the lowest dose (75 mg/kg), four animals showed no apparent effects; four became excited, developed a Straub tail, and bit the cage, and one convulsed. Although this dosage produced no deaths, it was not regarded as optimal because of excessive stimulation of the central nervous system. Therefore, another randomly selected group of animals was given a 70-mg/kg dose by the same route and procedure. At this level (below the LD_1), no deaths were noted, although convulsions still persisted. A 60-mg/kg dose was given to another group, which showed mild excitatory responses and a Straub tail in the absence of convulsions. Therefore, this dose (60 mg/kg) was selected as the teratogenic dose for this study.

Breeding Procedure and Treatment Regimen—Timed pregnancies were accomplished by placing two females in a cage with a male from 3:00 pm until 7:00 am, whereupon they were removed and examined for vaginal plugs. Animals with plugs were termed gravid, and the day of gestation coincident with their appearance was designated as Day 0. The pregnant mice then were weighed to the nearest 0.1 g[5], and each weight was recorded as the maternal starting weight.

Gravid animals were placed in individual cages identical to those of the males, where they remained undisturbed until the morning of Day 7 of gestation when they were reweighed. A weight gain of 2 g or more confirmed pregnancy. These animals then were assigned randomly to one of 13 groups of eight mice each. Six groups received single injections of cocaine hydrochloride (60 mg/kg) on Day 7, 8, 9, 10, 11, or 12, respectively; six groups received 0.3 ml of normal saline as a single injection on Days 7–12 of gestation (trauma controls). The final group represented untreated controls.

Preparation of Cocaine Hydrochloride Solution—Cocaine hydrochloride solution[6] (2%) was prepared by dissolving the drug in double-distilled water. The sodium chloride solution[7] was prepared commercially. All injections were made subcutaneously with a glass syringe[8].

Examination of Fetuses—Following treatment, each mouse was permitted to proceed to Day 18, the day prior to the termination of gestation, whereupon the terminal maternal weight was recorded. The animal then was sacrificed by cervical dislocation, and its abdomen was opened to expose the peritoneal cavity. The uterine horns were examined grossly for fetal swellings and resorption sites, appearing as small dark nodules, which were counted and recorded.

Following incision of the uterine horns, the exposed fetuses were removed, blotted dry, and weighed to the nearest 0.01 g on a torsion balance[9]. Each fetus was examined grossly for external soft tissue defects and sexed. Viability was determined by reflex movement of the limbs in response to mechanical stimulation with a blunt probe. Every other fetus

[5] Model 700 triple-beam balance, Ohaus.
[6] Merck 2209, lot L3506, Merck and Co., Rahway, N.J.
[7] Sodium chloride USP (code 2A1302), Travenol Laboratories, Deerfield, Ill.
[8] B-D 1-ml tuberculin syringe with a 1.27-cm, 26-gauge needle.
[9] Model PL-800, Torbal.

Table III—Occurrence of Anophthalmia, Malformed Lenses, and Missing Lenses on Day of Treatment (Compared with Saline)

		Anophthalmia				Malformed Lenses				Missing Lenses			
		Litters		Fetuses		Litters		Fetuses		Litters		Fetuses	
Treatment[a]	Day	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Untreated	—	8	0	43	0	8	0	43	0	8	0	43	0
Saline	7	8	0	45	0	8	0	45	0	8	0	45	0
	8	8	0	46	0	8	0	46	0	8	0	46	0
	9	8	0	44	0	8	0	44	0	8	0	44	0
	10	8	0	44	0	8	0	44	0	8	0	44	0
	11	8	0	44	0	8	0	44	0	8	0	44	0
	12	8	0	43	0	8	0	43	0	8	0	43	0
Cocaine	7	5	3	41	4[b]	3	5[b]	31	14[b]	4	4[b]	39	6[b]
	8	6	2	39	2	5	3	38	3	6	2	39	2
	9	6	2	44	2	6	2	44	2	8	0	46	0
	10	5	3	37	3	7	1	39	1	7	1	39	1
	11	5	3	40	4[b]	6	2	42	2	5	3	41	3
	12	6	2	42	2	8	0	44	0	5	3	41	3

[a] The dose of saline was 0.3 ml sc, and the dose of cocaine was 60 mg/kg sc. [b] Statistically significant in comparison with respective saline controls, $p < 0.05$.

Table IV—Occurrence of Delayed Ossification of the Skull, Paws, and Centrum on Day of Treatment (Compared with Saline)

		Delayed Ossification Skull				Delayed Ossification Paws				Delayed Ossification Centrum			
		Litters		Fetuses		Litters		Fetuses		Litters		Fetuses	
Treatment[a]	Day	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Untreated	—	8	0	45	0	3	5	36	9	8	0	45	0
Saline	7	8	0	50	0	3	5	34	16	8	0	50	0
	8	8	0	48	0	4	4	41	7	8	0	48	0
	9	8	0	48	0	7	1	43	5	8	0	48	0
	10	8	0	48	0	2	6	32	16	8	0	48	0
	11	8	0	47	0	4	4	33	14	8	0	47	0
	12	7	1	45	1	2	6	32	14	8	0	46	0
Cocaine	7	8	0	49	0	2	6	26	23	8	0	49	0
	8	7	1	42	3	2	6	22	23[b]	8	0	45	0
	9	7	1	45	1	1	7[b]	16	30[b]	7	1	45	1
	10	7	1	40	1	1	7	20	21	8	0	41	0
	11	6	2	38	5[b]	2	6	24	19	8	0	43	0
	12	2	5[b]	34	10[b]	0	7	19	25[b]	6	1	40	4[b]

[a] The dose of saline was 0.3 ml sc, and the dose of cocaine was 60 mg/kg sc. [b] Statistically significant in comparison with respective saline controls, $p < 0.05$.

Table V—Occurrence of Split Sternebrae, Malformed Sternebrae, and Crankshaft Sternebrae on Day of Treatment (Compared with Saline)

		Split Sternebrae				Malformed Sternebrae				Crankshaft Sternebrae			
		Litters		Fetuses		Litters		Fetuses		Litters		Fetuses	
Treatment[a]	Day	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Untreated	—	7	1	44	1	8	0	45	0	7	1	44	1
Saline	7	3	5	40	10	8	0	50	0	7	1	49	1
	8	5	3	43	5	8	0	48	0	7	1	47	1
	9	5	3	44	4	6	2	46	2	6	2	46	2
	10	3	5	41	7	4	4	43	5	7	1	47	1
	11	6	2	45	2	8	0	47	0	6	2	45	2
	12	4	4	41	5	4	4	41	5	6	2	43	3
Cocaine	7	2	6	39	10	2	6[b]	35	14[b]	6	2	46	3
	8	4	4	38	7	4	4[b]	37	8[b]	4	4	39	6[b]
	9	1	7[b]	33	13[b]	5	3	39	7	3	5	37	9[b]
	10	5	3	37	4	1	7	29	12[b]	7	1	40	1
	11	5	3	38	5	3	5[b]	35	8[b]	6	2	41	2
	12	3	4	34	10	2	5	38	6	6	1	43	1

[a] The dose of saline was 0.3 ml sc, and the dose of cocaine was 60 mg/kg sc. [b] Statistically significant in comparison with respective saline controls, $p < 0.05$.

was immersed in either a bone-staining solution or Bouin's fixative and processed for skeletal or soft tissue examination, respectively.

Fetuses destined for soft tissue examination were fixed and decalcified in Bouin's fixative for at least 2 weeks, whereupon they were reexamined for external anomalies with a low power binocular dissecting microscope[10] and sectioned according to a literature method (14). Fetuses designated for skeletal examination were processed according to the technique of Staples and Schnell (15). The significance of observed variations among the experimental groups was determined by the Student t test and the uncorrected χ^2 test for binomial populations (16).

RESULTS AND DISCUSSION

The results of this study support the hypothesis that cocaine possesses teratogenic potential. Significant occurrences of soft tissue abnormalities, as compared with those of the controls, were observed on each day of cocaine treatment. Significant skeletal anomalies were observed on Days 7–9 of cocaine treatment as compared with the controls (Table I). Following the subcutaneous administration of cocaine hydrochloride (2%) at 60 mg/kg, a significant incidence of fetuses with exencephaly, cryptorchidism, hydronephrosis, anophthalmia, malformed or missing lenses, delayed ossification of the skull, paws (17), or centrum, extra ribs, malformed or crankshaft sternebrae (18), and split or butterfly xiphoid processes occurred when compared with those observed in the respective control groups (Tables II–VI).

Significant differences also were noted in the resorption (Day 10) and sex ratio (Day 9) when compared with those of the controls (Table I). Data in Table I indicate that cocaine did not alter the maternal mean terminal weights when compared to those of the controls, nor did the maternal weight gain during gestation appear to indicate fetal size or development. Furthermore, the initial starting weight of gravid mice was not responsible for any changes in the mean fetal weight (*i.e.*, there was no apparent

[10] Model ASZ30L2, Bausch & Lomb.

Table VI—Occurrence of Split Xiphoid, Butterfly Xiphoid, and Extra Ribs on Day of Treatment (Compared with Saline)

		Split Xiphoid				Butterfly Xiphoid				Extra Ribs			
		Litters		Fetuses		Litters		Fetuses		Litters		Fetuses	
Treatment[a]	Day	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Untreated	—	8	0	45	0	8	0	45	0	8	0	45	0
Saline	7	8	0	50	0	8	0	50	0	8	0	50	0
	8	8	0	48	0	8	0	48	0	7	1	47	1
	9	8	0	48	0	8	0	48	0	8	0	48	0
	10	8	0	48	0	8	0	48	0	7	1	45	3
	11	8	0	47	0	8	0	47	0	6	2	44	3
	12	7	1	45	1	8	0	46	0	8	0	46	0
Cocaine	7	6	2	47	2	6	2	47	2	6	2	45	4[b]
	8	6	2	40	5[b]	6	2	42	3	6	2	42	3
	9	5	3	43	3	6	2	44	2	7	1	45	1
	10	8	0	41	0	8	0	41	0	6	2	39	2
	11	7	1	42	1	6	2	40	3	5	3	40	3
	12	7	0	44	0	4	3[b]	40	4[b]	3	4[b]	40	4[b]

[a] The dose of saline was 0.3 ml sc, and the dose of cocaine was 60 mg/kg sc. [b] Statistically significant in comparison with respective saline controls, $p < 0.05$.

correlation between fetal growth retardation and increased susceptibility to teratogenesis).

The central stimulatory effects of cocaine hydrochloride observed in the dams were minimal, and no fatalities occurred in the treated groups. These results agree with those of previous studies in which other teratogenic drugs also were nonlethal (19). Because of the mild excitability and nonlethality observed with the dose of cocaine hydrochloride employed in this study, there appears to be no correlation between the production of fetal malformations and increased maternal toxicity.

A critical period exists when a maximum frequency of teratogenic insults occurs. Although these periods overlap for different organs and structures, there is a definite time of susceptibility to teratogens, as was confirmed by this investigation. Interference with developmental or biochemical processes at a specific time during ontogenesis by either drug treatment or hypoxia causes defects that parallel the development period of the affected organ or structure. For example, the days of gestation during which the most prominent occurrence of defects was noted in this study corresponded to those reported previously (6, 8, 9, 19).

In addition, there was a correlation between cocaine hydrochloride and other specific treatments and conditions with respect to the production of comparable anomalies on similar days of gestation. For example, the most prominent day for the occurrence of exencephaly and sternebrae defects in this study was Day 9. Previous studies with different agents (8, 19) and hypoxic conditions (6, 20) also found maximum responses for exencephaly and vertebral defects on Day 9 of gestation. It appears that cocaine may decrease placental transfer of oxygen as a contributing factor to its teratogenicity, because hypoxic conditions elicit similar anomalies (*i.e.*, exencephaly) at similar times of the gestation period (6, 8, 19, 20).

A significant incidence of skeletal abnormalities appeared late in gestation whereas eye defects occurred early, paralleling closely the period of ontogenesis for these structures (21, 22) (Tables III and IV). The period of susceptibility of the CF-1 mouse fetus to the teratogenic effects of cocaine hydrochloride was evident throughout the range of drug administration in this study (Days 7–12) and was not confined to any particular day.

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