104S (1965).
(10) L. M. Cummins and M. J. Fourier, *Anal. Lett.*, **2**, 403 (1969).
(11) E. T. Lin, D. C. Brater, and L. Z. Benet, *J. Chromatogr.*, **140**, 275 (1977).
(12) C. Bye, H. M. Hill, D. T. D. Hughes, and A. W. Peck, *Eur. J. Clin. Pharmacol.*, **8**, 47 (1975).
(13) R. P. Roth, E. J. Cantekin, D. Bluestone, R. M. Welch, and Y. W. Cho, *Ann. Otol. Rhinol. Laryngol.*, **86**, 235 (1977).
(14) G. E. Abraham, *J. Clin. Endocrinol. Metab.*, **29**, 866 (1969).
(15) L. T. Cheng, S. Y. Kim, A. Chung, and A. Castro, *FEBS Lett.*, **36**, 339 (1973).
(16) A. Eberhard, *Arch. Pharm.*, **253**, 62 (1915).
(17) *Ibid.*, **258**, 114 (1920).
(18) L. H. Welsh, *J. Am. Chem. Soc.*, **69**, 128 (1947).
(19) *Ibid.*, **71**, 3500 (1949).
(20) J. F. Hyde, E. Browning, and R. Adams, *J. Am. Chem. Soc.*, **50**, 2287 (1928).
(21) W. M. Hunter and F. C. Greenwood, *Nature*, **194**, 495 (1962).
(22) M. J. Barrett and P. S. Cohen, *Clin. Chem.*, **18**, 1339 (1972).
(23) B. F. Erlanger, *Pharmacol. Rev.*, **25**, 271 (1973).
(24) J. Landon and A. C. Moffat, *Analyst*, **101**, 225 (1976).
(25) V. P. Butler, *Pharmacol. Rev.*, **29**, 103 (1978).
(26) A. E. Till and L. Z. Benet, *Pharmacology*, **18**, 306 (1979).
(27) C. E. Cook, N. H. Ballentine, T. B. Seltzman, and C. R. Tallent, *J. Pharmacol. Exp. Ther.*, **210**, 391 (1979).
(28) S. J. Gross and J. R. Soares, in "Immunoassays for Drugs Subject to Abuse," S. J. Mule, I. Sunshine, M. Braude, and R. E. Willette, Eds., CRC Press, Cleveland, Ohio, 1974, pp. 3–11.
(29) K. Kawashima, A. Levy, and S. Spector, *J. Pharmacol. Exp. Ther.*, **196**, 517 (1976).
(30) J. Dickerson, D. Perrier, M. Mayersohn, and R. Bressler, *Eur. J. Clin. Pharmacol.*, **14**, 253 (1978).
(31) A. Yacobi, R. G. Stoll, G. C. Chao, J. E. Carter, D. M. Baaske, B. L. Kamath, A. H. Amann, and C. M. Lai, "Abstracts," vol. 9, American Pharmaceutical Association, Academy of Pharmaceutical Sciences, Washington, D.C., 1979, p. 108.

ACKNOWLEDGMENTS

Presented in preliminary form at the FASEB meeting, Atlantic City, April 1978 [*Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **37**, 725 (1978)].

The authors thank Dr. D. Brent and his staff of the Organic Chemistry Department, Wellcome Research Laboratories, for the mass spectral data and Dr. R. Zweidinger, Research Triangle Institute, for the comparative GLC data.

Comparative Teratogenicity of Cortisone and Phenytoin in Mice

JOSEPH M. McDEVITT, RONALD F. GAUTIERI ˣ, and DAVID E. MANN, Jr.

Received September 2, 1980, from the *Department of Pharmacology, School of Pharmacy, Temple University, Philadelphia, PA 19140.* Accepted for publication November 13, 1980.

Abstract □ Single administrations of cortisone or phenytoin to pregnant mice on Days 11–14 of gestation caused similar skeletal and dissimilar soft tissue fetal anomalies. Cortisone reduced both maternal and fetal weight, whereas phenytoin only reduced fetal weight without adversely affecting maternal weight. A correlation between fetal weight reduction and cleft palate incidence was evident for each drug. Because probit analysis of dose–response regression lines did not deviate from parallelism after drug challenge, it was concluded that cortisone and phenytoin may produce palatal anomalies in the mouse fetus by a similar mechanism.

Keyphrases □ Teratogenicity—effects of cortisone and phenytoin on mice □ Cortisone—teratogenic effects on mice □ Phenytoin—teratogenic effects on mice □ Antiepileptics—teratogenic effects of phenytoin on mice

The experimental use of various agents to elicit and inhibit cleft palate has been pursued vigorously in recent years to determine its cause and to prevent its occurrence in human newborns. Although cortisone is the prototypal agent employed for the induction of this anomaly in lower species (1), phenytoin is of particular interest because of the purported link between its use as an antiepileptic drug during pregnancy and the appearance of cleft palate in offspring (2, 3).

The purpose of this investigation was to compare the teratogenic effects of phenytoin with those of cortisone by means of dose–response relationships in mice and to ascertain whether the mechanism involved in cleft palate inception by cortisone and phenytoin is similar.

EXPERIMENTAL

Animals—CF-1 albino mice[1] (25–35 g) were used. Females were confined in groups of 10 to aggregate cages for at least 2 weeks prior to mating. Males were placed individually in metal cages (12.5 × 15 × 10 cm) with a wire mesh front and floor[2]. Adult mice were maintained on a commercial diet[3] and tap water *ad libitum*. The breeding room was equipped with an electrical system[4], which provided 12 hr of light (7:00 am–7:00 pm) and 12 hr of darkness. Room temperature was maintained between 22 and 26°.

Preparation of Solutions—Saline and cortisone acetate solutions were prepared commercially[5,6]. Fresh aliquots (10 ml) of phenytoin solution were prepared as needed on the day of injection by dissolving the drug[7] in a stock solution of 70% propylene glycol[8] (in saline). Phenytoin solutions were prepared containing 10, 14, 16, or 18 mg of phenytoin/ml of vehicle in injection volumes of 0.1–0.3 ml. All injections were made with a glass syringe[9]. The bone-staining solution was prepared by dissolving 18 mg of alizarin red-S in each liter of 1% KOH in distilled water. A 50:50 mixture of 70% ethyl alcohol and 100% glycerin was used to fix and clear the specimens. Fetuses chosen for soft tissue analysis were fixed in Bouin's solution.

Breeding Procedure and Treatment Regimen—The breeding procedure was previously described (4). Gravid females were randomly

[1] Charles River Breeding Laboratories, Wilmington, Mass.
[2] RD-T unit, Norwich Wire Works, Norwich, N.Y.
[3] Rodent Laboratory Chow 5001, Ralston-Purina Co., St. Louis, Mo.
[4] Astronomic dial time switch with "skipper," model V-45073, International Register Co., Spring Grove, Ill.
[5] Sodium chloride injection USP (0.9%), lot B7D091A, McGraw Laboratories, Division of American Hospital Supply Corp., Irvine, Calif.
[6] Cortone, lot 0613A, Merck Sharp and Dohme, West Point, Pa.
[7] Dilantin Sodium, lot PE338, Parke-Davis Co., Detroit, Mich.
[8] Lot P-810, Amend Drug and Chemical Co., Irvington, N.J.
[9] B-D 1-ml tuberculin syringe with 1.27-cm, 26-gauge needle.

0022-3549/81/0600-0631$01.00/0

Table I—Treatment Regimen of Experimental Drugs

Group[a]	Treatment[b] on Days 11–14 (mg/kg) and Route
1	Untreated controls
2	Saline, intramuscular
3	Propylene glycol, subcutaneous
4	I (25), intramuscular
5	I (50), intramuscular
6	I (75), intramuscular
7	I (100), intramuscular
8	I (125), intramuscular
9	II (50), subcutaneous
10	II (70), subcutaneous
11	II (80), subcutaneous
12	II (90), subcutaneous

[a] Eight mice per group. [b] I is cortisone acetate; II is phenytoin.

assigned to one of three control groups or one of two treatment sections: I, cortisone; and II, phenytoin, the latter being divided into five groups each (Table I). Each control group and each treatment group consisted of eight females. Of the three control groups, one received no further treatment while the other two served as vehicle controls. One group received saline solution in a fixed volume of 0.3 ml, which was administered in a manner identical to that of cortisone; the remaining control group received 0.3 ml of 70% propylene glycol solution in a manner identical to that of phenytoin.

The treatment groups received cortisone or phenytoin on Days 11–14 of gestation. The steroid was injected intramuscularly into the thigh muscles of the hindlegs, alternating between the right and left legs on successive days, in 25-, 50-, 75-, 100-, and 125-mg/kg doses per group. Phenytoin was administered subcutaneously into the ventroposterior or dorsoanterior quadrant close to the axilla, in alternate quadrants on successive days, in 10-, 30-, 50-, 70-, and 90-mg/kg doses per group. It soon became apparent that a threshold dose for phenytoin teratogenicity (cleft palate production) would be >50 mg/kg. Therefore, the two lower dosage schedules were deleted, and a group of eight mice receiving 80 mg of phenytoin/kg was substituted.

Examination of Fetuses—On Day 18, 1 day before term, the terminal weight was recorded for each mouse[10], which then was sacrificed by cervical dislocation. The number and position of fetuses and resorption sites (metrial glands) were ascertained and recorded. The fetuses were removed, tested for viability with a blunt probe, blotted dry, and weighed to the nearest hundredth of a gram[10]. They then were examined for gross external defects and sexed.

The specimens were prepared for macroscopic examination as follows. For skeletal examination, every second fetus was prepared according to the method of Staples and Schnell (5); the remaining fetuses were fixed and decalcified in Bouin's solution in preparation for freehand razor blade sectioning (6). The palatal shelves were observed prior to sectioning, and the urogenital organs were left intact. All specimens were examined for bone and soft tissue anomalies with a binocular dissecting microscope[11].

Statistical Methods and Analysis—The degree of significance of observed variations among the experimental groups was determined by the Student t test (7). All t values were calculated on a computer[12]. The dose–response relationships for cortisone- and phenytoin-induced cleft palate were compared by probit analysis (8).

RESULTS AND DISCUSSION

Maternal Effects of Drugs—The female mice were unresponsive to intramuscular injections of saline or to initial cortisone challenges, but second and third administrations of the hormone caused lethargy that persisted for over 24 hr at the higher dosage levels. Mice injected subcutaneously with either 70% propylene glycol or phenytoin experienced excitement and discomfort. As the number of injections and dosages of the antiepileptic agent was increased, Straub tail reactions, excitement (cage circling), ataxia, and depression occurred. Ataxia and depression were most pronounced in the group receiving 90 mg of phenytoin/kg. At laparotomy, most mice that had received solutions containing the propylene glycol vehicle had inflamed injection sites, particularly in the scapular region. No deaths in gravid animals resulted from drug treatment.

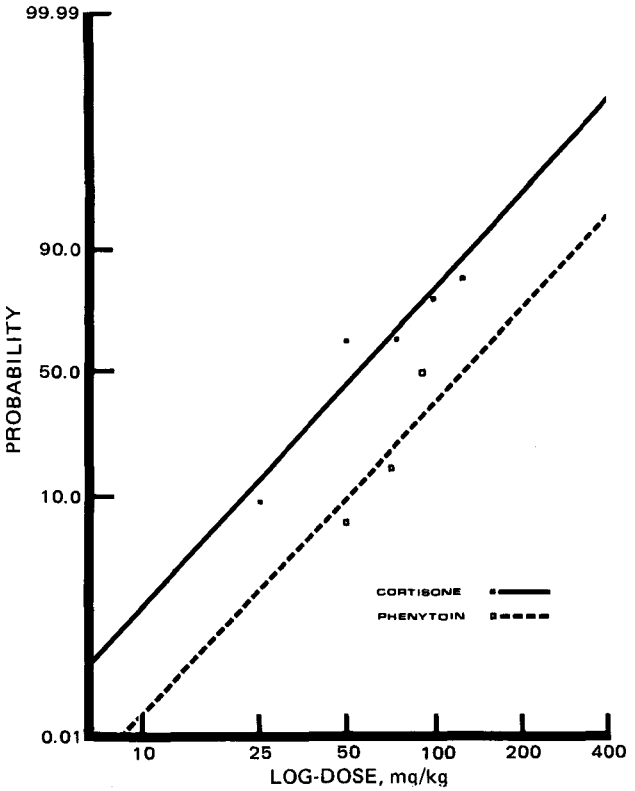

Figure 1—*Probit regression of the percent of cleft palate per litter on log-dose.*

Table II presents the mean values of maternal and fetal deviations of the test groups in relation to their respective saline or propylene glycol controls. Each group contained eight litters and ~100 fetuses.

Maternal Weight—The weight gain reflects the difference in maternal weight on Days 0 and 18 of gestation. The mean maternal weight gains among the cortisone-treated groups decreased as the dosage of the hormone was increased. Significantly lower weight gains, when compared with those in the saline group, occurred in all cortisone groups, except in the one receiving the 25-mg/kg dose. No significant differences were found when the mean maternal weight gains within the phenytoin-treated groups were compared with those of the group receiving the propylene glycol vehicle.

Fetal Resorption—Percent resorption is defined as the ratio of the number of resorption sites to the sum of the resorption sites and viable fetuses times 100. No significant differences were found among the control or cortisone-treated groups with respect to percent resorption. Within the phenytoin-treated groups, only mice that received 70- and 80-mg/kg doses had a significantly higher percent resorption ($p < 0.05$) than occurred in the group that received a dose of 50 mg/kg[13].

Fetal and Sex Ratios—No significant variations in the mean number of fetuses in either the right or left uterine horn were noted among the control or treated groups. There were no differences in the sex ratios of the phenytoin-treated and propylene glycol control groups. However, the group that received cortisone (75 mg/kg) had a significantly lower mean percentage of males when compared with the saline control.

Mean Fetal Weight—There were no significant differences in the mean fetal weight among the control groups. Within the cortisone-treated groups, an apparent dose-dependent reduction of this parameter occurred, with significant differences being evident with cortisone at 50, 100, and 125 mg/kg when compared with the saline control. In the phenytoin-treated group, a significant reduction in mean fetal weight was noted between the antiepileptic agent (90 mg/kg) and its propylene glycol control.

Production of Soft Tissue and Skeletal Anomalies—The occurrence and nature of the various soft tissue and skeletal defects representing significant deviations of the cortisone- and phenytoin-treated

[10] Torbal torsion balance model PL-800.
[11] Bausch & Lomb model ASZ30L2.
[12] Wang 2200.
[13] This intragroup comparison is not shown in Table II.

Table II—Mean Values of Test Groups

Treatment Group[a]	Maternal Weight, start/gain, g	Percent Resorption	Fetal Ratio, right/left	Percent Male/Female	$\bar{X}$ Fetal Weight, g	Soft Tissue Defects[b]	Skeletal Defects[b]	Percent Soft Tissue Defects[c]	Percent Skeletal Defects[c]
1	30.5/26.2	3.7	6/5	48.4/51.6	1.17	0.8	1.3	14.5	19.2
2	29.4/26.2	8.4	6/6	50.0/50.0	1.15	0.8	2.0	14.0	29.5
3	30.9/23.1	9.0	5/6	57.9/42.1	1.09	0.7	1.7	10.1	24.2
4	27.9/23.0	9.4	5/5	43.6/56.4	1.16	0.6	3.2	12.9	44.1
5	30.0/20.7[d]	18.9	6/4	39.6/61.4	1.01[d]	4.1[d]	5.8[d]	70.7[d]	76.7[d]
6	29.5/18.7[d]	13.1	4/5	35.2/64.8[d]	1.06	4.0[d]	9.5[d]	68.3[d]	87.0[d]
7	29.4/17.5[d]	16.3	4/5	56.0/44.0	0.88[d]	4.0[d]	8.8[d]	77.5[d]	95.9[d]
8	29.1/16.4[d]	21.8	5/4	53.5/46.5	0.87[d]	4.5[d]	8.7[d]	85.8[d]	100.0[d]
9	29.3/22.2	2.8	6/4	50.8/49.2	1.18	1.2	1.7	26.3	22.4
10	31.7/22.7	12.1	5/6	52.1/47.9	1.01	1.7	3.5	24.9	53.7[e]
11	33.7/22.8	14.5	5/4	60.0/40.0	1.04	1.7	6.2	26.7	57.2[e]
12	30.3/20.7	7.9	6/5	44.6/55.4	0.89[e]	6.2[e]	10.1[e]	60.0[e]	75.8[e]

[a] Eight mice per group. [b] Mean number of defects per litter. [c] Mean percent of fetuses displaying at least one defect per litter. [d] Significance ($p < 0.05$) when compared to the saline group. [e] Significance ($p < 0.05$) when compared to the propylene glycol group.

groups from their saline and propylene glycol controls are presented in Tables III and IV, respectively.

With the exception of cleft palate, which appeared in the fetuses of both treatment groups, most soft tissue anomalies caused by single intramuscular injections of cortisone on Days 11–14 of gestation were unlike those observed after single subcutaneous administrations of phenytoin on the same days of gestation.

An inverse relationship between fetal weight and cleft palate incidence has been well documented for cortisone teratogenicity (9, 10). A similar association between reduced fetal weight and phenytoin-induced cleft palate also was reported (11). However, these are dose-dependent responses, as shown in the present study and others (11–14), which probably explain why fetal weight loss was not reported when low doses of phenytoin were used (15, 16).

An inverse relationship appeared to exist between the occurrence of a small protuberance on the forepaw and cortisone dosage, whereas a direct relationship was evident between the dosage level of the steroid and the emergence of other soft tissue anomalies, *i.e.*, cleft palate. The former situation demonstrates that the presence of one defect may be dependent on the presence or absence of another. This type of variable dependency may account for the nonlinear response observed for sternebral defects in the cortisone-treated group. The frequently appearing anomaly, crankshaft sternebrae, could positively influence the occurrence of fused sternebrae because sternebral ossification sites would be positioned closer to each other. Therefore, anomaly frequency in response to a given dose should be considered individually before conclusions concerning the teratogenic potential of a suspected agent are made.

Cryptorchidism seemed to be directly dependent on phenytoin dosage but was not related to cortisone dosage. Ovarian displacement after phenytoin treatment also appeared to be dose related. The reason why ectopic ovarian development occurred only on the left side of the fetus remains enigmatic.

In addition to the soft tissue defects listed in Table III, other soft tissue anomalies observed in the phenytoin-treated groups were curled tail, connective tissue infiltration of the cerebral hemispheres, cerebral hemorrhage, fused digit, ectrodactyly, malformed hindpaw, malformed kidney, and cleft lip. These soft tissue defects and those observed in the cortisone-treated groups, *i.e.*, curled tail, exencephaly, and fused digit, were not statistically significant when compared with the respective treatment or control groups.

Cortisone and phenytoin treatment produced fetuses with similar skeletal malformations that primarily involved the sternebral and cranial (supraoccipital) bones; this finding is in agreement with previous results (15). The following skeletal defects were observed at insignificant frequencies in their respective groups: missing sternebrae, malformed and split xiphoid processes (cortisone and phenytoin), ectopic sternebrae (cortisone, phenytoin, and untreated), and displaced sternebrae, *i.e.*, due to rotation of the sternebral ossification sites 30° off center (cortisone).

Phenytoin also interfered specifically with skeletal digit development. Delayed ossification of the digits was dose dependent (Table IV). Malformed and hypoplastic digits have been reported in human infants whose epileptic mothers were maintained on phenytoin therapy during pregnancy (17, 18).

Rugh (19) accumulated data that related organogenesis to gestational age in the mouse. Thus, from the results obtained in this and other studies that employed similar dosage schedules (12, 13, 15) of cortisone and phenytoin, it is evident that the majority of the skeletal abnormalities induced by these agents occur at sites where chondrification centers develop on Days 15–16 (supraoccipital bone, sternum, and distal phalanges). These findings indicate that the critical time for phenytoin- and cortisone-induced skeletal anomalies is several days prior to genesis of the affected tissue.

The critical periods reported for phenytoin- and cortisone-induced cleft palate are remarkably similar to those required for the production of sternebral, supraoccipital, and digital malformations. Single injections of cortisone (20) and phenytoin (12, 21) were most effective in producing cleft palate when given to mice on Day 12 of gestation, although maximum responses were obtained from multiple injections over Days 12–14. It has been determined (22) that murine palatogenesis begins on Day 14

Table III—Significant Occurrence[a] and Nature of Soft Tissue Anomalies

Group[b]	Cleft Palate	Fifth-Digit Protuberance	Crypt-orchidism	Ectopic Ovary
1	—	14.5	—	—
2	—	14.1	—	—
3	—	10.1	—	—
4	8.8	2.1[c]	4.1	—
5	62.1[c,e]	12.7	7.7	—
6	64.2[c,e]	0.0[c,f]	2.1	—
7	77.5[c,e]	0.0[c,f]	2.1	—
8	83.8[c,e]	2.1[c]	10.8[c]	—
9	6.3	7.1	2.1	3.1
10	15.5	0.0	4.9	0.0
11	21.1[d]	0.0	4.3	0.0
12	49.5[d,g,h]	0.0	11.3[d,g]	12.8[d,h,i]

[a] Mean percent of defects per litter. [b] Eight mice per group. Significance ($p < 0.05$) is indicated by superscripts as compared with [c] saline and [d] propylene glycol controls and treated groups: cortisone, [e] 25 and [f] 50 mg/kg, and phenytoin, [g] 50, [h] 70, and [i] 80 mg/kg.

Table IV—Significant Occurrence[a] and Nature of Skeletal Defects

Group[b]	D.O.P.	Split Supraocc.	Stern Dft.	Crank. St.	Split St.	Fused St.
1	2.1	—	17.1	8.8	4.2	2.1
2	10.0	—	21.6	21.6[c]	0.0	5.0
3	9.2	—	16.8	16.8	1.8	1.8
4	7.1	27.5[d]	21.6	16.0	0.0	10.0
5	1.8	58.3[d,f]	41.4	22.8	6.4[d,f]	24.2[d,f]
6	2.5	72.7[d,f]	71.5[d,f]	31.3	9.6	54.8[d,f]
7	0.0	93.8[d,f,g]	56.0[d,f]	15.8	17.9[d,f]	31.9[d,f]
8	16.7	95.8[d,f,g]	51.7[d,f]	20.4	19.2[d,f]	25.8[d,f]
9	2.5	0.0	20.0	10.7	1.8	10.0
10	21.2	0.0	39.2[e]	8.5	30.3[e,h]	0.0
11	43.7[e,h]	6.0	41.1	30.1	24.3[e,h]	8.5
12	54.0[e,h]	20.0[e]	60.9[e,h,i]	36.4[e,h,i]	13.8[e,h,i]	8.7

[a] Mean percent of defects per litter. [b] Eight mice per group. Significance ($p < 0.05$) is indicated by superscripts as compared with controls: [c] untreated, [d] saline, and [e] propylene glycol, and with treated groups: cortisone, [f] 25 and [g] 50 mg/kg, and phenytoin, [h] 50 and [i] 70 mg/kg. D.O.P. = delayed ossification of the paws; Split Supraocc. = split supraoccipital bone, Stern. Dft. = occurrence of at least one sternebral defect, Crank St. = crankshaft sternebrae, Split St. = split sternebrae, and Fused St. = fused sternebrae.

and terminates on Day 15.

Several studies linked the alteration of events common to palatogenesis and bone formation, including glycosaminoglycan (23) and collagen formation (24, 25), and the activities of enzymes (alkaline phosphatase, acid phosphatase, and β-glucuronidase) (26) and hormones (parathyroid hormone and calcitonin) (27) to the production of cortisone-induced cleft palate. Because of these relationships and the similarities that exist between cortisone- and phenytoin-induced skeletal malformations, it can be concluded that, qualitatively, the teratogenic mechanism of action of these agents in the formation of skeletal anomalies is similar.

A graphical approximation of the regression lines of the mean percent of cleft palate per litter on log-dose for cortisone- and phenytoin-treated groups appears in Fig. 1. These lines are the result of the second iteration of the method of probit analysis described by Finney (8). The original iteration was performed on lines fitted independently from the results obtained from the test groups without constraint of parallelism. The results from the second stage were employed to calculate the common slope, which was used to construct the curves in Fig. 1. Heterogenicity and parallelism were both examined by χ^2 tests. Because the dose–response regression lines for cortisone- and phenytoin-induced cleft palate did not deviate from parallelism, the teratogenic mechanism of action of these agents in causing this anomaly may be similar. The relative potency of phenytoin as a cleft palate-inducing teratogen was calculated, according to the method of Finney (8), to be from 14.54 to 69.18% that of cortisone.

REFERENCES

(1) F. C. Fraser and T. D. Fainstat, *Pediatrics*, **8**, 527 (1951).

(2) E. V. Kuenssberg and J. D. E. Knox, *Lancet*, **1**, 198 (1973).

(3) J. W. Hanson and D. W. Smith, *J. Pediatr.*, **87**, 285 (1975).

(4) R. S. Thompson and R. F. Gautieri, *J. Pharm. Sci.*, **58**, 406 (1969).

(5) R. E. Staples and V. L. Schnell, *Stain Technol.*, **39**, 61 (1964).

(6) J. G. Wilson, in "Teratology Principles and Techniques," J. G. Wilson and J. Warkany, Eds., University of Chicago Press, Chicago, Ill., 1965, p. 267.

(7) G. G. Simpson, A. Roe, and R. C. Lewontin, "Quantitative Zoology," Harcourt, Brace, New York, N.Y., 1960, pp. 176, 186.

(8) D. J. Finney, "Probit Analysis," 3rd ed., Cambridge University Press, Cambridge, England, 1971, pp. 50–80, 100–122.

(9) F. C. Fraser, in "Teratology Principles and Techniques," J. G. Wilson and J. Warkany, Eds., University of Chicago Press, Chicago, Ill., 1965, pp. 21–56.

(10) H. Loevy, *Anat. Rec.*, **145**, 117 (1963).

(11) R. B. Paulson, G. W. Paulson, and S. Jreissaty, *Arch. Neurol.*, **36**, 832 (1979).

(12) R. D. Harbison and B. A. Becker, *Teratology*, **2**, 305 (1969).

(13) F. M. Sullivan and P. R. McElhatton, *Toxicol. Appl. Pharmacol.*, **40**, 365 (1977).

(14) H. Fritz, D. Muller, and R. Hess, *Toxicology*, **6**, 323 (1976).

(15) J. J. Mitala, D. E. Mann, Jr., and R. F. Gautieri, *J. Pharm. Sci.*, **67**, 377 (1978).

(16) K. M. Massey, *J. Oral Ther. Pharmacol.*, **2**, 380 (1966).

(17) M. Barr, A. K. Poznanski, and R. D. Schmickel, *J. Pediatr.*, **84**, 254 (1974).

(18) R. M. Hill, W. M. Verniaud, M. G. Horning, L. B. McCulley, and N. F. Morgan, *Am. J. Dis. Child.*, **127**, 645 (1974).

(19) R. Rugh, "The Mouse: Its Reproduction and Development," Burgess Publishing, Minneapolis, Minn., 1970, pp. 208–295.

(20) F. G. Biddle and F. C. Fraser, *Genetics*, **84**, 743 (1976).

(21) J. E. Gibson and B. A. Becker, *Proc. Soc. Exp. Biol. Med.*, **128**, 905 (1968).

(22) G. Callas and B. E. Walker, *Anat. Rec.*, **145**, 61 (1963).

(23) R. M. Jacobs, *ibid.*, **150**, 271 (1964).

(24) R. M. Pratt and C. T. G. King, *Arch. Oral Biol.*, **16**, 1181 (1971).

(25) Y. Shapira and S. Shoshan, *ibid.*, **17**, 1699 (1972).

(26) M. E. Teaford and A. A. White, *Proc. Soc. Exp. Biol. Med.*, **117**, 541 (1964).

(27) R. E. Waterman, G. C. Palmer, S. J. Palmer, and S. M. Palmer, *Anat. Rec.*, **188**, 431 (1977).

ACKNOWLEDGMENTS

Abstracted in part from a thesis submitted by J. M. McDevitt to Temple University in partial fulfillment of the Master of Science degree requirements.

Oxidative Degradation of 6-Selenoguanosine in Aqueous Solutions

A. J. REPTA ˣ and Y. A. BELTAGY *

Received August 8, 1980, from the *Pharmaceutical Chemistry Department, School of Pharmacy, University of Kansas, Lawrence, KS 66044.* Accepted for publication November 17, 1980. *Present address: Pharmaceutical Chemistry Department, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.

Abstract □ The degradation of 6-selenoguanosine (NSC 137679)(I) in water and in various buffer systems was investigated. Drug degradation in aqueous media was monitored by high-pressure liquid chromatography. Some kinetic aspects of the degradation of I in various buffer systems at 25° also were studied spectrophotometrically. The degradation, which requires oxygen, involves autoxidation of I to the corresponding diselenide, which produces a selenide and metallic selenium in the presence of oxygen. This degradation pathway differs from that reported for the oxidation of related thio compounds.

Keyphrases □ 6-Selenoguanosine—oxidative degradation in aqueous solutions, kinetics □ Autoxidation, aqueous—6-selenoguanosine, selenium compounds, pharmacokinetics □ Kinetics—6-selenoguanosine, oxidative degradation in aqueous solutions, selenium compounds □ Antineoplastics—6-selenoguanosine, selenium compounds, oxidative degradation in aqueous solutions, kinetics

During a study of selenium analogs of physiologically active sulfur compounds, numerous selenium analogs were synthesized for biochemical investigation. 6-Selenopurine was synthesized (1) and found to be active as an antileukemic agent (2), but it was unstable and toxic to the host (3). Subsequently, 6-selenopurine-9-β-D-ribonucleoside was prepared, but it was even less stable than 6-selenopurine under the same conditions (3).

In a search for a more stable analog, 6-selenoguanosine (NSC 137679) (I) was synthesized (4); it was found to be an active inhibitor of several experimental tumors *in vitro* (5, 6) and *in vivo* (7, 8). Although I was reported to be more stable than preceding analogs (4, 9), it still undergoes sufficiently rapid degradation in aqueous solutions such that its formulation in a dosage form suitable for evaluation of anticancer activity presented serious problems (10).

While considerable information has been published

0022-3549/81/0600-0634$01.00/0