Vincristine inhibits cell mitosis at the metaphase and dissolves the microtubule structures (18). However, it has little effect on ornithine decarboxylase activity, and this fact could be why it is ineffective in the inhibition of neuroblastoma growth.

Although bromoacetylcholine binds to the nicotinic receptor at the neuromuscular junction irreversibly (19), as does α-bungarotoxin (20), the latter compound produced little effect on neuroblastoma growth, indicating that bromoacetylcholine inhibits neuroblastoma growth through an action mechanism that differs from α-bungarotoxin, similar to action at cholinergic receptors. Neuroblastoma cells possess adrenergic, cholinergic, and nonspecific receptors but very few serotonergic receptors. It thus is understandable that neuroblastoma cell growth was not inhibited by a serotonergic neuron degenerator, 5,6-dihydroxytryptamine.

Among the routes of administration studied, intratumor administration for bromoacetylcholine, bromoacetate, and 1,3-diaminopropane and intraperitoneal administration for cyclophosphamide were the best.

In summary, it seems that drugs capable of inhibiting ornithine decarboxylase can suppress the cell growth of neuroblastoma. A more potent ornithine decarboxylase inhibitor that produces few side effects may be developed as an effective weapon to treat neuroblastoma.

REFERENCES

(1) C. Pochedly, "Neuroblastoma," Publ. Sci. Group, Inc., Acton, Mass., 1976.

(2) J. Z. Finklestein, E. Arima, P. E. Byfield, J. E. Byfield, and E. W. Fonkalsrud, Cancer Chemother. Rep., 57, 405 (1973).

(3) C. Y. Chiou, J. Pharm. Sci., 64, 469 (1975).

(4) Ibid., 66, 837 (1977).

(5) Ibid., 67, 331 (1978).

(6) S. K. Chapman, M. K. Martin, M. S. Hoover, and C. Y. Chiou, Biochem. Pharmacol., 27, 717 (1978).

(7) C. Y. Chiou and B. V. R. Sastry, ibid., 17, 805 (1968).

(8) C. Y. Chiou, N. E. Liddell, M. K. Martin, and C. J. Chu, Arch. Int. Pharmacodyn. Ther., 233, 235 (1978).

(9) A. Bjorklund, H. G. Baumgarten, and A. Nobin, Adv. Biochem. Psychopharmacol., 10, 13 (1974).

(10) K. Fuxe and G. Jonsson, ibid., 10, 1 (1974).

(11) C. Y. Chiou, C. J. Chu, and N. E. Liddell, Arch. Int. Pharmacodyn. Ther., 235, 35 (1978).

(12) U. Bachrach, Proc. Natl. Acad. Sci. USA, 72, 3087 (1975).

(13) J. L. Clark and P. Duffy, Arch. Biochem. Biophys., 172, 551 (1976).

(14) Z. N. Canellakis and T. C. Theoharides, J. Biol. Chem., 251, 4436 (1976).

(15) T. C. Theoharides and Z. N. Canellakis, Nature (London), 255, 733 (1975).

(16) P. P. McCann, C. Tardiff, P. S. Mamont, and F. Schuber, Biochem. Biophys. Res. Commun., 64, 336 (1975).

(17) K. J. Lembeck, Biochim. Biophys. Acta, 354, 88 (1974).

(18) P. Calabres and R. E. Parks, in "The Pharmacological Basis of Therapeutics," L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1975, p. 1284.

(19) C. Y. Chiou, Eur. J. Pharmacol., 26, 268 (1974).

(20) C. C. Chang, J. Formosan Med. Assoc., 59, 315 (1960).

Effect of pH, Chlorobutanol, Cysteine Hydrochloride, Ethylenediaminetetraacetic Acid, Propylene Glycol, Sodium Metabisulfite, and Sodium Sulfite on Furosemide Stability in Aqueous Solutions

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Abstract ☐ A study was conducted to determine the effects of pH, two antioxidants, a chelating agent, a preservative, and propylene glycol on furosemide stability. Aqueous solutions of furosemide containing 10% alcohol (v/v) were prepared in phosphate buffers with various pH values (5, 6, and 9) whose ionic strength was adjusted to 0.1 M with potassium chloride. Some solutions contained chlorobutanol, ethylenediaminetetraacetic acid, or sodium metabisulfite. Another set of aqueous solutions contained phosphate buffer (0.1 M), alcohol (10% v/v), and propylene glycol (40% v/v) with or without cysteine hydrochloride, ethylenediaminetetraacetic acid, and sodium sulfite. The solutions were divided into two parts, stored at 24 and 50°, and assayed frequently using a previously developed high-pressure liquid chromatographic procedure. At the lowest pH value (pH 5), furosemide appeared to be very unstable. Cysteine

hydrochloride, ethylenediaminetetraacetic acid, and sodium sulfite failed to improve the stability of furosemide. Chlorobutanol and sodium metabisulfite had an adverse effect on the stability, probably due to the fact that they decreased the pH of the solution. The pH value appears to be the only critical factor for the stability of furosemide. Buffered solutions containing propylene glycol were very stable at both temperatures for 170 days, and they tasted good.

Keyphrases ☐ Furosemide—stability in aqueous solutions, effect of formulation factors ☐ Diuretics—furosemide, stability in aqueous solutions, effect of formulation factors ☐ Stability—furosemide in aqueous solutions, effect of formulation factors

Furosemide (I) is a widely used diuretic, but little information is available concerning the stability of this drug in dosage forms. Rowbotham et al. (1) reported that aqueous furosemide solutions undergo hydrolysis and photochemical degradation. Quantification of photochemical degradation products of furosemide by the USP XIX (2) UV assay procedures was not successful. A stability-indicating assay for furosemide using high-pressure

liquid chromatography (HPLC) was developed by Ghanekar et al. (3). It also was reported that an aqueous furosemide solution containing sorbitol and 10% alcohol (v/v) had limited stability. The pH of the solution was adjusted to ~8.5. However, it was difficult to maintain the pH value of the solution, which caused rapid decomposition.

The objectives of the present investigation were to study

Table I—Prepared Solutions [a] (0.5 mg of Furosemide/ml)

Solution	Buffer Solution (±0.1) pH	Other Ingredients
1	5	None
2	5	0.2% Chlorobutanol
3	5	0.05% III
4-6	5	0.1, 0.2, and 0.4% V, respectively
7-9	6	0.1, 0.2, and 0.4% V, respectively
10-12	9	0.1, 0.2, and 0.4% V, respectively
13	8.4	None
14	8.2	0.02% II
15	8.4	0.05% IV
16	8.3	0.05% II and 0.05% IV
17	8.0	0.05% II, 0.05% IV, and 0.05% III

[a] All solutions contained 10% alcohol (v/v) since the stock solution was made in alcohol; Solutions 13-17 contained propylene glycol, and the pH values of the final solutions were those obtained after diluting 1:10 with water.

Table II—Assay Results at Room Temperature

Solution	Percent of Label Claim 35 Days	90 Days	152 Days	170 Days	240 Days	pH$_f$ (±0.1)
1	—	100.0	—	95.9	96.2	5.2
2	97.7	—	—	96.9	96.5	5.1
3	96.4	93.3	—	—	94.7	5.2
4	5.2[a]	—	—	—	—	3.3
5	15.1[a]	—	—	—	—	2.9
6	44.8[a]	—	—	—	—	2.8
7	91.8	—	81.1[b]	—	—	5.0
8	94.7	—	15.9[c]	—	—	4.5
9	98.3	—	93.0[b]	—	—	5.4
10	98.1	—	98.0	82.1	—	7.5
11	95.2	—	78.6[b]	—	—	6.9
12	91.7	—	60.5[b]	—	—	5.7
13	—	100.6	—	99.7	—	8.4
14	—	100.7[b]	—	100.8	—	8.2
15	—	99.5	—	99.5	—	8.4
16	—	100.8	—	100.4	—	8.3
17	—	100.9	—	100.2	—	8.0

[a] Crystals were found in the solution, so it was not reassayed. [b] Color had changed to light yellow (from colorless). [c] Fungus.

the effect of pH, chlorobutanol, cysteine hydrochloride monohydrate (II), ethylenediaminetetraacetic acid (III), sodium sulfite (IV), and sodium metabisulfite (V) on the stability of furosemide in aqueous solutions and to develop an appropriate buffering system to maintain the adjusted pH value.

EXPERIMENTAL

Chemicals and Reagents—All chemicals and reagents were USP, NF, or ACS grade and were used without further purification. Furosemide[1] powder was used as received.

Apparatus—The apparatus was the same as that reported previously (3), except that a variable-wavelength detector[2] set at 254 nm was used. The column also was the same as that reported previously (3).

Preparation of Solutions—A stock solution of furosemide was prepared by dissolving 1.25 g of furosemide in enough alcohol to make 250 ml. The solutions for stability studies were prepared by diluting an appropriate quantity of the stock solution with a buffer of an appropriate pH value (0.05 M phosphate buffers of pH 5, 6, and 9 whose ionic strength was adjusted to 0.1 M with potassium chloride). The phosphate buffers were prepared according to the USP procedure (4). Before the solutions were diluted to volume, additional ingredients, if any, were added.

Another set of solutions was prepared by dissolving an appropriate quantity of furosemide in 50.0 ml of 0.2 M aqueous dibasic potassium phosphate solution and then adding 10 ml of ethanol and bringing the solution to volume (100.0 ml) with propylene glycol. Additional ingredients, if any, were dissolved before bringing the solution to volume. The pH values of these solutions were determined after diluting 5.0 ml to 50.0

Table III—Assay Results at 50°

Solution	Percent of Label Claim 35 Days	90 Days	170 Days	240 Days	pH$_f$ (±0.1)
1	94.7	—	93.5	94.5	5.2
2	80.9	—	51.8	3.5[c]	3.3
3	94.8	—	88.7	79.3	5.2
4	15.3[a]	—	—	—	3.0
5-12[b]					
13	—	100.1	99.5	—	8.4
14	—	100.1[c]	99.2	—	8.2
15	—	99.4	99.4	—	8.4
16	—	100.7	100.0	—	8.3
17	—	101.1	100.5	—	8.0

[a] Crystals were found in the solution, so it was not reassayed. [b] Not studied at 50° since they were not stable even at room temperature. [c] Color had changed to light yellow.

ml with water and are reported in Table I. All of the solutions prepared are listed in Table I.

The solutions were divided into two portions, stored at 24 and 50°, and assayed frequently using the HPLC method reported previously (3), except that the absorbance unit for full-scale deflection was 0.1 instead of 0.16 and the injection volume was 20.0 instead of 40.0 μl. The results, which were calculated as previously reported (3), are presented in Tables II and III. Sample chromatograms are presented in Figs. 1 and 2.

RESULTS AND DISCUSSION

For convenience, the results from Solutions 1-12 (Table I), which did not contain any propylene glycol, will be discussed first, followed by the results from Solutions 13-17 (Table I), which contained 40% (v/v) propylene glycol.
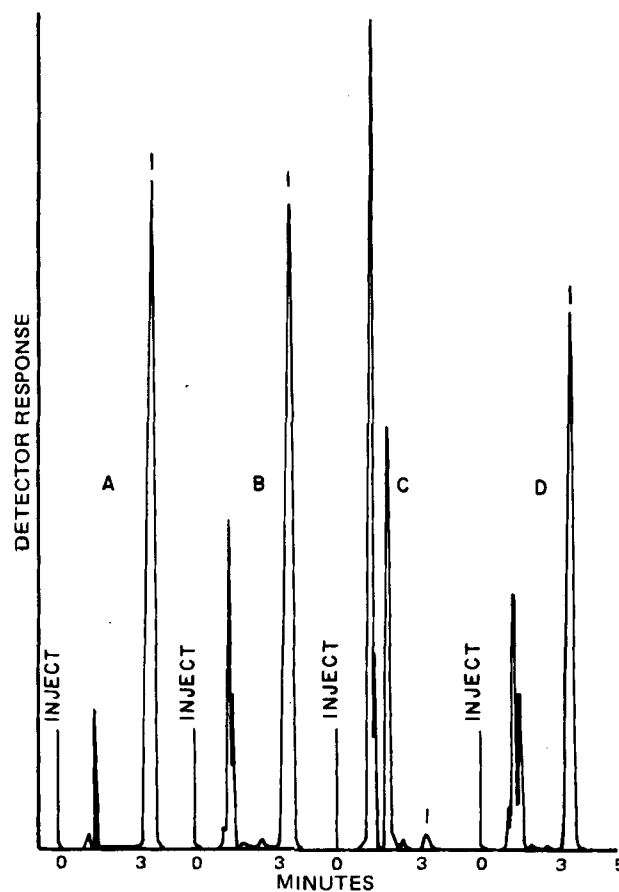

Figure 1—Sample chromatograms where peak 1 is from furosemide. Key: A, standard solution of I; B, solution of I (0.05%) in pH 5 buffer after 240 days of storage at 50°; C, same as B except that it also contained 0.2% chlorobutanol; and D, same as B except that it also contained 0.05% III.
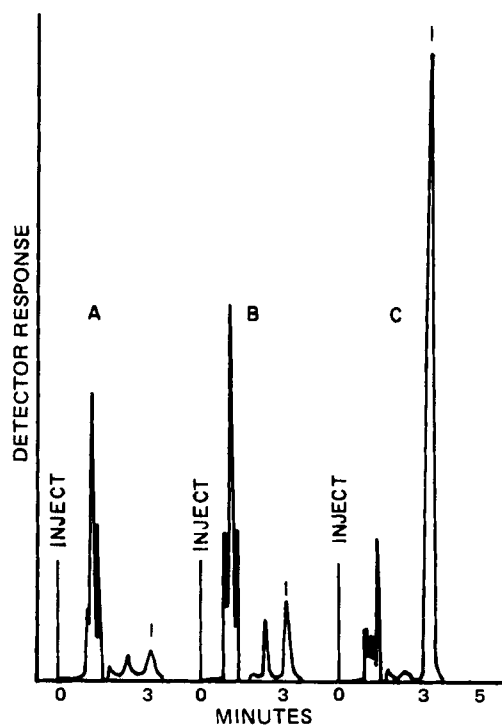
Figure 2—*Sample chromatograms where peak 1 is from furosemide. Key: A, solution of I (0.05%) in pH 5 buffer with 0.1% sodium metabisulfite after 170 days of storage at room temperature; B, same as A except that pH 6 buffer was used; and C, same as A except that pH 9 buffer was used.*

Solutions 1-12 (without Propylene Glycol)—The results indicate that Solutions 1-12 were not very stable for 170 days either at room temperature or at 50° (Tables II and III). Furthermore, the additional ingredients, ethylenediaminetetraacetic acid, chlorobutanol, or sodium metabisulfite, did not improve furosemide stability. In fact, chlorobutanol and sodium metabisulfite had an adverse effect on its stability, especially when added with pH 5 buffers. This effect may be due to a decrease in the pH of the solutions (Table III). Furosemide is known to be unstable at lower pH values (3). The pH values of most solutions changed after storage (Tables II and III).

The effect of sodium metabisulfite (as the concentration was raised from 0.1 to 0.4%) on furosemide stability is interesting. In Solutions 4-6 and 7-9 (Table II), the stability of furosemide improved as the concentration of sodium metabisulfite was increased, perhaps due to initial oxidation of metabisulfite to sulfate by the oxygen present. The sulfate ion increased the rate of decomposition (oxidation) of furosemide. A similar scheme using epinephrine has been reported (5).

Furthermore, the additional amount of sodium metabisulfite, after all of the oxygen was consumed, protected furosemide from the oxidation effect of the sulfate ion. This effect also has been reported (5).

The described effects were not found in Solutions 10-12, probably due to their high pH values (Table II). In these solutions, the effect of pH appears to be more prominent. Moreover, in solutions with basic pH values, furosemide does not appear to be susceptible to oxidation. The same may be true of solutions with pH values above 5.5.

Crystals of furosemide were found in several solutions after 35 days, especially in those kept at room temperature (Table II, Solutions 4-6). Furosemide is known to be poorly soluble in aqueous systems, especially acidic ones.

Several other solutions (Solutions 7, 9, 11, and 12) became discolored after 152 days (Table II). The cause of this discoloration was not determined.

It was not possible to treat the data mathematically because of very little decomposition (Solutions 1-3) or changes in pH values (Solutions 4-12).

Solutions 13-17 (with Propylene Glycol)—Solutions 13-17 proved to be very stable at both 24 and 50°. Considering experimental errors, the results of both temperatures were almost 100% for all solutions at 170 days (Tables II and III). Furthermore, the pH values of the solutions did not change (Tables II and III). However, the addition of cysteine hydrochloride monohydrate (II), ethylenediaminetetraacetic acid, sodium sulfite, or their combinations did not improve furosemide stability. Although the solution containing II became discolored, the loss in furosemide potency was negligible. Compound II apparently was oxidized in this solution. This discoloration did not occur when II was present in combination with sodium sulfite due to the protection provided by the sodium sulfite. Nevertheless, the addition of these ingredients is not desirable. In weakly basic solutions, furosemide does not appear to be susceptible to oxidation.

The only critical factor in furosemide stability is the pH of the solution, which must be slightly basic and not change on storage. A stable liquid dosage form of furosemide can be prepared by dissolving an appropriate quantity of furosemide powder in 0.2 M aqueous dibasic potassium phosphate solution (50% of the total volume), adding alcohol (10% v/v), and bringing the solution to volume with propylene glycol. Since the solution did not lose potency at 50° for ~6 months, it should be stable for at least 2 years at room temperature. A panel of three persons approved the taste of the product.

REFERENCES

(1) P. C. Rowbotham, J. B. Standrod, and J. K. Sugden, *Pharm. Acta Helv.*, **51**, 305 (1976).

(2) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 213.

(3) A. G. Ghanekar, V. D. Gupta, and C. W. Gibbs, *J. Pharm. Sci.*, **67**, 808 (1978).

(4) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, pp. 653, 654.

(5) R. Bonevski, J. M. Culjat, and L. Balint, *J. Pharm. Sci.*, **67**, 1474 (1978).