tion was observed with increasing ascorbic acid concentration. Thermodynamic parameters were calculated from Arrhenius plots at pH 3.52, 4.55, 5.45, and 6.60.

REFERENCES

(1) Z. Czuros and J. Petro, *Acta Chim. Acad. Sci. Hung.*, **7**, 199 (1955).

(2) B. S. N. Rao, *Radiat. Res.*, **17**, 683(1962).

(3) N. F. Barr and C. G. King, *J. Amer. Chem. Soc.*, **78**, 303 (1956).

(4) J. Grochmalicka, *Zesz. Probl. Postepow Nauk Roln.*, **53**, 57 (1965); through *Chem. Abstr.*, **64**, 5813f(1966).

(5) J. L. Ciminera and P. W. Wilcox, *J. Amer. Pharm. Ass., Sci. Ed.*, **35**, 363(1946).

(6) F. Giral, *ibid.*, **36**, 82(1947).

(7) F. J. Bandelin and J. V. Tuschhoff, *ibid.*, **44**, 241(1955).

(8) K. Yuriko, *Yakuzaigaku*, **25**, 131(1965).

(9) A. Bartilucci and N. E. Foss, *J. Amer. Pharm. Ass., Sci. Ed.*, **43**, 159(1954).

(10) J. R. Nixon and B. P. S. Chawla, *J. Pharm. Pharmacol.*, **17**, 558(1965).

(11) R. I. Poust and J. L. Colaizzi, *J. Pharm. Sci.*, **57**, 2119(1968).

(12) J. Tillman, *Z. Unters. Lebensm.*, **54**, 33(1927).

(13) T. Higuchi and E. Brochmann-Hanssen, "Pharmaceutical Analysis," Interscience, New York, N. Y., 1961, p. 691.

(14) "The United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965, p. 47.

(15) R. W. Herbert, E. L. Hirst, E. G. V. Percival, R. J. W. Reynolds, and F. Smith, *J. Chem. Soc.*, **299**, 1270(1933).

(16) S. S. Wilks, "Elementary Statistical Analysis," Princeton University Press, Princeton, N. J., 1964, p. 236.

(17) I. Guttman and S. S. Wilks, "Introductory Engineering Statistics," Wiley, New York, N. Y., 1965, p. 241.

(18) M. M. Taqui Khan and A. E. Martell, *J. Amer. Chem. Soc.*, **90**, 6011(1968).

(19) Y. Kato and M. Sugiura, *Gifu Yakka Daigaku Kiyo*, **6**, 59 (1956); through *Chem. Abstr.*, **51**, 5365(1957).

(20) M. M. Taqui Khan and A. E. Martell, *J. Amer. Chem. Soc.*, **90**, 3386(1968).

(21) *Ibid.*, **89**, 4176(1967).

(22) H. S. Harned and B. B. Owen, "Physical Chemistry of Electrolytic Solutions," Reinhold, New York, N. Y., 1958, pp. 665, 667, 758.

(23) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," vol. 1, W. A. Benjamin, New York, N. Y., 1966, p. 14.

(24) J. D. Ross and T. J. Morrison, *J. Chem. Soc.*, **1933**, 1016.

(25) C. Hoitsema, *Z. Phys. Chem.*, **27**, 312(1898).

(26) H. Marshall and A. T. Cameron, *J. Chem. Soc.*, **91**, 1519 (1907).

(27) I. M. Kolthoff and W. Bosch, *J. Phys. Chem.*, **36**, 1685 (1932).

(28) T. Higuchi, M. Gupta, and L. W. Busse, *J. Amer. Pharm. Ass., Sci. Ed.*, **41**, 122(1952).

(29) M. R. Valinoti and S. Bolton, *J. Pharm. Sci.*, **51**, 201(1962).

(30) P. Finholt, R. B. Paulssen, and T. Higuchi, *ibid.*, **52**, 948 (1963).

ACKNOWLEDGMENTS AND ADDRESSES

Received August 23, 1971, from the *College of Pharmacy, University of Iowa, Iowa City, IA 52240*

Accepted for publication January 5, 1972.

* Present address: Invenex Pharmaceuticals, San Francisco, CA 94124

▲ To whom inquiries should be directed.

Pharmacological Aspects of Concurrent Administration of Furosemide and Skeletal Muscle Relaxants

EDWARD REYES and GLENN D. APPELT▲

Abstract □ The pharmacological aspects of concurrent administration of furosemide and the skeletal muscle relaxants, *d*-tubocurarine, succinylcholine, and chlordiazepoxide, were investigated with respect to the effects on diuresis and skeletal muscle relaxation in female albino rats. Administration of *d*-tubocurarine and chlordiazepoxide concurrently with furosemide produced no significant effects on furosemide-induced diuresis. Succinylcholine, when administered concurrently with furosemide, caused a decrease in the volume of urine excreted; other parameters of renal function were not affected. The skeletal muscle relaxant properties of *d*-tubocurarine, succinylcholine, and chlordiazepoxide were investigated upon concurrent administration of the muscle relaxants with furosemide by an *in vivo* study and an *in situ* study. The skeletal muscle relaxant action of succinylcholine was significantly potentiated by pretreatment of the animals with furosemide, both in the exercise wheel study and in the diaphragm–phrenic nerve preparation. A tendency toward an antagonism between *d*-tubocurarine and furosemide was observed on skeletal muscle relaxation both *in vivo* and *in situ*. The effect of *d*-tubocurarine on blood pressure was also antagonized by treatment of the animal with furosemide. Preliminary spectrophotometric evidence suggests that a complex between furosemide and *d*-tubocurarine exists, which may possibly explain an antagonism between the two drugs.

Keyphrases □ Furosemide and concurrent administration of *d*-tubocurarine, succinylcholine, or chlordiazepoxide—effects on diuresis and skeletal muscle relaxation, rats □ *d*-Tubocurarine administered concurrently with furosemide—effect on diuresis and skeletal muscle relaxation, rats □ Succinylcholine administered concurrently with furosemide—effect on diuresis and skeletal muscle relaxation, rats □ Chlordiazepoxide administered concurrently with furosemide—effect on diuresis and skeletal muscle relaxation, rats □ Skeletal muscle relaxants—pharmacology of concurrent administration of furosemide, rats

It has been observed that the effects of *d*-tubocurarine are sensitive to electrolyte disturbances. Ferrari *et al.* (1) reported that the paralytic activity of *d*-tubocurarine was potentiated in rabbits by pretreatment with chlorothiazide, an electrolyte-depleting diuretic (2). A pharmacological interaction between *d*-tubocurarine and

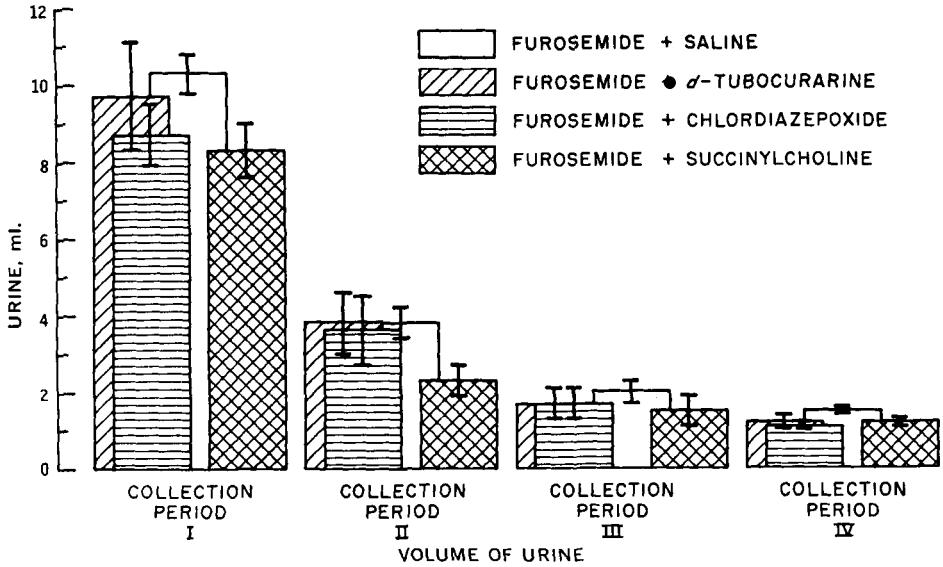

Figure 1—*Volume of urine collected for each group of six animals is shown at four different collection periods. Collection Period I is from time zero (administration of furosemide) to 90 min., Collection Period II is from 90 to 180 min., Collection Period III is from 180 to 270 min., and Collection Period IV is from 270 to 360 min.*

furosemide, a diuretic acting at the loop of Henle (3–5), might be expected due to electrolyte imbalance induced by diuresis. Therefore, the use of furosemide concurrently with skeletal muscle relaxants of the curarine type has been contraindicated. There appears to be no experimental or clinical evidence to substantiate this speculation, although it is well founded.

In this study, experiments were conducted to determine if the activity of either furosemide or *d*-tubocurarine might be altered by concurrent administration. In an attempt to understand better the nature of any interaction that may occur, additional skeletal muscle relaxants with different modes of action when compared to *d*-tubocurarine were employed in the investigation.

EXPERIMENTAL

Diuresis and Urine Electrolyte Patterns—Female Sprague-Dawley albino rats (230–380 g.) were divided into five groups of six animals each and placed on a diet of 12 g. of laboratory chow daily. The animals were housed in pairs, and after 1 week the collection of urine was initiated. At the onset of the collection period, the animals were injected intraperitoneally with 5 mg./kg. of furosemide (6). This injection was repeated at 45-min. intervals for a 6-hr. collection period. In addition, the animals were injected with a solution of one of three different skeletal muscle relaxants or saline. The muscle relaxants used were *d*-tubocurarine chloride (0.03 mg./kg.), succinylcholine chloride (0.5 mg./kg.), and chlordiazepoxide hydrochloride (10 mg./kg.). The muscle relaxants, *d*-tubocurarine and succinylcholine, were administered at the same intervals as furosemide due to their short duration of action (7, 8). Chlordiazepoxide, possessing a more sustained duration of activity (9, 10), was administered only at the onset of the collection period. A control group received neither diuretic nor muscle relaxant. The doses of the muscle relaxants were those that caused a rat to slide off a 30° inclined plane after intraperitoneal drug administration.

Urine samples were collected every 90 min. for 6 hr. and the volumes were measured. The samples were then frozen for future analysis.

The urine samples collected were analyzed for pH, specific gravity, osmolarity, total sodium, and total potassium. The pH was determined with a pH meter[1]. The specific gravity was determined by weighing 100 μl. of the urine specimen. The osmolarity of the urine specimens was found by a melting-point determination. The melting-point method was an adaptation of that used by Jones (11). The urine samples were analyzed for sodium and potassium using standard emission flame photometry (12, 13) on a spectrophotometer[2], employing air as the support gas and acetylene as the fuel. The wavelengths used for the determination of potassium and sodium were 766.5 and 589.0 nm., respectively.

Skeletal Muscle Relaxant Properties—*In vivo* and *in situ* experiments were conducted to monitor skeletal muscle relaxation when furosemide and skeletal muscle relaxants were administered concurrently. The *in vivo* experiment, employing an exercise wheel, was intended to ascertain the effects on muscular relaxation in the absence of anesthesia.

Exercise Wheel—Female Sprague-Dawley albino rats (60–100 g.) were divided into three groups of 14. Representatives of each group were matched for weight and then housed together in the same wire-bottomed cage. Seven days before the animals were to be tested, a regimen of 12 g. of laboratory chow and deionized water was instituted. The rats then received a daily intraperitoneal injection of furosemide (5 mg./kg.) or saline. On Day 7, each rat was injected with a skeletal muscle relaxant 15 min. following the diuretic and then immediately placed on an exercise wheel. The animal was exercised until a sharp end-point of paralysis occurred or until 10 min. elapsed. Paralysis within 10 min. could most likely be ascribed to the action of the muscle relaxants (7, 8, 14); if paralysis occurred at some time greater than 10 min., fatigue might also contribute to the temporary paralysis (14). The doses of the muscle relaxants were determined from ED_{50} studies using the criteria already described and were as follows: *d*-tubocurarine chloride, 0.15 mg./kg.; succinylcholine chloride, 0.75 mg./kg.; and chlordiazepoxide hydrochloride, 15 mg./kg. The fall time for each animal was recorded, and the effectiveness of the muscle relaxants was determined for each group.

Urine and serum samples were collected from rats on an identical regimen of food and diuretic. These samples were then assayed for sodium and potassium content.

Diaphragm–Phrenic Nerve Preparation—The *in situ* experiment employed a diaphragm–phrenic nerve preparation isolated according to the procedure reported by D'Amour (15). Female Sprague-Dawley albino rats (250–300 g.) were fasted for 24 hr. before the experiment. Animals were anesthetized with sodium pentobarbital solution (25 mg./kg.) administered intraperitoneally, additional anesthetic being administered as required. The left phrenic nerve was isolated and retained by means of a ligature. The neck incision was closed to prevent excessive drying of the phrenic nerve. A thread was fastened to the diaphragm, attached to the myograph transducer unit of a Physiograph Six[3], and the tension was adjusted to allow

[1] Beckman Zeromatic II.

[2] Beckman DB-G.

[3] Narco Bio-Systems, Inc., Houston, Tex.

Table I—Effects of Skeletal Muscle Relaxants on Specific Gravity of Rat Urine in Furosemide-Induced Diuresis

Skeletal Muscle Relaxant	Urine Collection Period[a] I	II	III	IV
Saline	1.018 ± 0.002[b]	1.023 ± 0.002	1.027 ± 0.001	1.025 ± 0.002
d-Tubocurarine	1.023 ± 0.004	1.030 ± 0.002[c]	1.028 ± 0.003	1.040 ± 0.003[c]
Chlordiazepoxide	1.022 ± 0.004	1.026 ± 0.003	1.025 ± 0.003	1.030 ± 0.003
Succinylcholine	1.025 ± 0.003[c]	1.024 ± 0.002	1.032 ± 0.004	1.031 ± 0.004

[a] See text for explanation of collection period. Each value represents the arithmetic mean from six experimental animals. [b] Standard error of the mean. [c] Value significant at $p < 0.05$.

Table II—Effects of Skeletal Muscle Relaxants on pH of Rat Urine in Furosemide-Induced Diuresis

Skeletal Muscle Relaxant	Urine Collection Period[a] I	II	III	IV
Saline	6.49 ± 0.04[b]	6.51 ± 0.11	6.32 ± 0.08	6.36 ± 0.07
d-Tubocurarine	6.25 ± 0.02	5.79 ± 0.24[c]	5.97 ± 0.24	6.06 ± 0.22
Chlordiazepoxide	5.91 ± 0.09[c]	5.82 ± 0.17[c]	6.02 ± 0.16	6.05 ± 0.21
Succinylcholine	5.98 ± 0.15[c]	6.37 ± 0.35	6.03 ± 0.24	6.04 ± 0.23

[a] See text for explanation of collection period. Each value represents the arithmetic mean from six experimental animals. [b] Standard error of the mean. [c] Value significant at $p < 0.05$.

Table III—Effects of Skeletal Muscle Relaxants on Urinary Sodium Excretion in Furosemide-Induced Diuresis in Rats

Skeletal Muscle Relaxant	Urine Collection Period[a] I	II	III	IV
Saline	1.91 ± 0.25[b]	0.89 ± 0.13	0.46 ± 0.08	0.32 ± 0.02
d-Tubocurarine	2.48 ± 0.37	1.01 ± 0.22	0.39 ± 0.10	0.29 ± 0.04
Chlordiazepoxide	1.81 ± 0.39	0.83 ± 0.16	0.35 ± 0.12	0.23 ± 0.03[c]
Succinylcholine	2.24 ± 0.28	0.60 ± 0.14	0.29 ± 0.10	0.25 ± 0.04

[a] See text for explanation of collection period. Each value represents the arithmetic mean of meq. Na excreted from six experimental animals × 10^{-3}. [b] Standard error of the mean. [c] Value significant at $p < 0.05$.

recording of diaphragm contractions. The neck incision was reopened and the phrenic nerve was cut headward. The isolated phrenic nerve was then available for stimulation by means of an electric stimulator.

Following the isolation of the phrenic nerve, furosemide (5 mg./kg.) was administered intraperitoneally. After 15 min. the phrenic nerve was stimulated and a muscle relaxant was administered at the same dosage level used in the *in vivo* experiment. The phrenic nerve was then stimulated every minute until blockade of the stimulus was observed, or for 20 min. The procedure followed for chlordiazepoxide differed from that of the other two muscle relaxants in that the muscle relaxant was administered at the same time as the anesthetic. Furosemide was then administered immediately after the first diaphragm response to stimulation, followed 15 min. later by stimulation at 1-min. intervals. A control animal for each muscle relaxant received saline in place of furosemide. Upon termination of the experiment, the isolation of the phrenic nerve was confirmed by autopsy, tracing the nerve from the diaphragm to the neck.

Blood Pressure—Male Sprague-Dawley albino rats (250–350 g.) were anesthetized by an intraperitoneal injection of sodium pentobarbital (35 mg./kg.). The trachea, the jugular vein, and the carotid artery were cannulated. The blood pressure was measured from the carotid artery[4]. For each of six animals, the blood pressure was measured after the administration of either saline, *d*-tubocurarine (0.09 mg./kg.), or furosemide (5 mg./kg.) *via* the jugular vein. Another group of six animals was pretreated with furosemide (5 mg./kg.); 15 min. later, *d*-tubocurarine (0.09 mg./kg.) was administered and the blood pressure was measured. The percentage change in blood pressure after the administration of each drug was then determined.

In addition, the blood pressure was monitored after intravenous injection of succinylcholine or chlordiazepoxide.

Absorption Spectrum—The absorption spectrum for furosemide was determined from 190 to 400 nm. using 10^{-6} mole in pH 7.4 phosphate buffer on a double-beam spectrophotometer, the total volume of which was 3.0 ml. The furosemide absorption spectrum was then examined upon the addition of 1×10^{-5} and 1.5×10^{-4} mmole of *d*-tubocurarine, succinylcholine, or chlordiazepoxide.

[4] Using the E&M Linear-Core, a fluid-pressure transducer, connected to a Physiograph Six.

RESULTS

Diuresis and Electrolyte Patterns—The effects produced by *d*-tubocurarine, chlordiazepoxide, and succinylcholine on furosemide-induced diuresis are recorded in Fig. 1 and in Tables I–V. The mean urine volumes collected for each of four consecutive 90-min. periods are shown in Fig. 1, with the standard error of the means plotted as vertical lines through the points. The volumes of urine collected for animals administered furosemide in conjunction with a skeletal muscle relaxant are compared with the urine volumes of rats receiving furosemide and saline. Significant changes in the volume of urine voided were obtained with the combination of furosemide and succinylcholine at all four collection periods. Chlordiazepoxide in combination with furosemide also produced significant volume differences in the last collection period (270–360 min.), which was manifested as a volume decrease similar to that produced by succinylcholine.

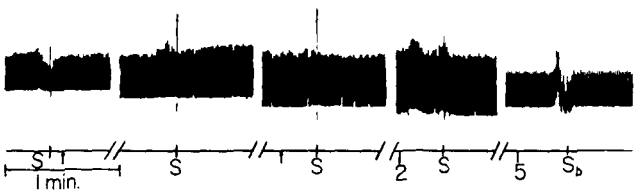

Figure 2—*Response of the diaphragm to phrenic nerve stimulation after the intraperitoneal administration of furosemide and* d-*tubocurarine (0.17 mg./kg.). The S denotes stimulation of phrenic nerve with a corresponding diaphragm contraction, S_b denotes stimulation of nerve without a corresponding diaphragm contraction, and the arrows denote injection of furosemide and* d-*tubocurarine in respective order of administration. The numerals refer to the time in minutes after the administration of* d-*tubocurarine.*

Table IV—Effects of Skeletal Muscle Relaxants on Urinary Potassium Excretion in Furosemide-Induced Diuresis in Rats

Skeletal Muscle Relaxant	Urinary Collection Period[a] I	II	III	IV
Saline	2.32 ± 0.18[b]	0.38 ± 0.05	0.35 ± 0.05	0.31 ± 0.02
d-Tubocurarine	2.46 ± 0.34	0.38 ± 0.07	0.27 ± 0.07	0.26 ± 0.05
Chlordiazepoxide	2.18 ± 0.24	0.37 ± 0.07	0.36 ± 0.06	0.23 ± 0.03[c]
Succinylcholine	1.99 ± 0.26	0.38 ± 0.07	0.20 ± 0.07	0.24 ± 0.03

[a] See text for explanation of collection period. Each value represents the arithmetic mean of meq. K excreted from six experimental animals × 10^{-3}. [b] Standard error of the mean. [c] Value significant at $p < 0.05$.

Table V—Effects of Skeletal Muscle Relaxants on Osmolarity of Rat Urine in Furosemide-Induced Diuresis

Skeletal Muscle Relaxant	Urine Collection Period[a] I	II	III	IV
Saline	244 ± 51[b]	380 ± 75	543 ± 92	712 ± 8
d-Tubocurarine	354 ± 52[c]	454 ± 91	391 ± 93	550 ± 28[c]
Chlordiazepoxide	243 ± 54	485 ± 124	400 ± 98	510 ± 100
Succinylcholine	262 ± 83	389 ± 91	445 ± 104	461 ± 28[c]

[a] See text for explanation of collection period. Each value represents the arithmetic mean in mosm. from six experimental animals. [b] Standard error of the mean. [c] Value significant at $p < 0.05$.

The effects of concurrent administration of furosemide and skeletal muscle relaxants on pH, specific gravity, osmolarity, total sodium, and total potassium are shown in Tables I–V. No consistent significant changes or trends were noted in these diuretic patterns when data from furosemide-treated animals were compared to values obtained from animals treated with furosemide and *d*-tubocurarine, succinylcholine, or chlordiazepoxide.

Skeletal Muscle Relaxant Properties—The effects of furosemide on the skeletal muscle relaxant properties of *d*-tubocurarine, succinylcholine, and chlordiazepoxide in the exercise wheel experiment are shown in Table VI. The fall times of the animals that received a skeletal muscle relaxant along with furosemide were compared to fall times of animals that were administered the muscle relaxants and saline. The percent effectiveness of the drug combinations was also determined. The group receiving succinylcholine concurrently with furosemide had a fall time approximately half that of the saline control. The fall time for animals receiving *d*-tubocurarine and furosemide was increased significantly from that of animals receiving *d*-tubocurarine and saline. The percent effectiveness of the muscle relaxant, succinylcholine, was increased to 100% by concurrent administration of furosemide, while the effectiveness of *d*-tubocurarine was decreased to 13%. There was no significant change in the muscle relaxant properties of chlordiazepoxide.

Table VI—Effects of Furosemide on Fall Time of Rats Treated with Skeletal Muscle Relaxants

Skeletal Muscle Relaxant	Percent Affected within 10 min.	Percentage (σ)
d-Tubocurarine	Saline	47 ± 13.3[b]
	Furosemide[a]	13 ± 8.9
Succinylcholine	Saline	50 ± 13.4
	Furosemide[a]	100 ± 2.7
Chlordiazepoxide	Saline	57 ± 13.2
	Furosemide	78 ± 8.2

[a] Value significant at $Z < 1.96$. [b] Standard error of a percent (σ%).

Table VII—Electrolyte Levels following 7 Days of Diuretic Administration

	Saline	Furosemide
Electrolytes excreted in urine, meq./6 hr.		
Na[a]	0.019 ± 0.005[b]	0.085 ± 0.003
K	0.017 ± 0.005	0.028 ± 0.003
Serum electrolytes, meq./l.		
Na	155.25 ± 2.5	162.58 ± 3.8
K	5.78 ± 0.32	5.73 ± 0.45

[a] Value significant at $p < 0.05$. [b] Represents standard error of the mean.

Total sodium and total potassium in the urine and the serum levels of the two electrolytes after 7 days of diuretic administration are shown in Table VII.

The effects of furosemide on the isolated phrenic nerve preparations are shown in Table VIII and illustrated in Fig. 2. The animal was pretreated with furosemide, and Fig. 2 shows that blockade to nerve stimulation occurred at 5 min. The animal receiving *d*-tubocurarine with no furosemide showed blockade to nerve stimulation at 2 min. Furosemide treatment decreased the time for blockade to stimulation by 6 min., from 8 to 2 min., for saline–succinylcholine-treated animals. Furosemide was shown to produce no change in the effect of chlordiazepoxide on the muscle–nerve preparation.

Blood Pressure—The effects of *d*-tubocurarine, furosemide, and concurrent administration of furosemide with *d*-tubocurarine on carotid blood pressure are shown in Table IX. The mean carotid blood pressure was decreased 63% by the administration of *d*-tubocurarine (0.09 mg./kg.). The rats pretreated wih furosemide (5 mg./kg.) showed a decrease in carotid blood pressure of 15% when the skeletal muscle relaxant, *d*-tubocurarine, was administered. The decrease in carotid blood pressure was changed significantly by pretreatment of the rats with the diuretic, furosemide.

The intravenous administration of succinylcholine (0.1 mg./kg.) failed to produce any change in carotid blood pressure. However, the intravenous injection of chlordiazepoxide (10 mg./kg.) resulted in a decrease in carotid pressure. Since furosemide–chlordiazepoxide coadministration did not produce significant changes in the muscle relaxant properties of chlordiazepoxide, further studies were not deemed necessary.

Absorption Spectrum—Figure 3 compares the spectrum, from 190 to 400 nm., of furosemide with the spectrum of furosemide in combination with two concentrations of *d*-tubocurarine. Furose-

Table VIII—Effects of Furosemide on the Rat Phrenic Nerve–Diaphragm Preparation after Treatment with Skeletal Muscle Relaxants

Skeletal Muscle Relaxant	Time for Blockade, min.	
d-Tubocurarine	Saline	2.6 ± 0.76[b]
	Furosemide[a]	5.5 ± 0.87
Succinylcholine	Saline	8.8 ± 0.86
	Furosemide[a]	3.0 ± 0.14
Chlordiazepoxide[c]	Saline	>20
	Furosemide	>20

[a] Value significant at $p < 0.05$. [b] Represents standard error of the mean. [c] No blockade achieved within 20 min.

Table IX—Effects of Furosemide on the Hypotensive Properties of *d*-Tubocurarine in Rats

Drug or Drugs Administered	Percent Change in Carotid Blood Pressure
Furosemide + saline	1.6 ± 1.8[a]
Furosemide + *d*-tubocurarine	−15.3 ± 6.6
Saline + *d*-tubocurarine	−63.2[b] ± 8.2

[a] Represents standard error of mean. [b] Value significant at $p < 0.05$.

mide produced three absorption peaks: at 330, 277, and 227 nm. Upon addition of 10^{-5} mmole of *d*-tubocurarine, the absorption peak at 277 nm. was shifted to 271 nm. with a concurrent decrease in absorption from 0.68 to 0.50. The peak at 227 nm. was completely abolished. Addition of 1.5×10^{-4} mmole *d*-tubocurarine produced similar results, a peak shift from 277 to 265 nm. and a decrease of 0.34 in absorption.

The absorption spectrum of furosemide was not changed with the addition of either succinylcholine or chlordiazepoxide.

DISCUSSION

These data suggest that an interaction exists between the diuretic, furosemide, and each of the skeletal muscle relaxants, *d*-tubocurarine and succinylcholine. No indication of interaction was noted between chlordiazepoxide and furosemide. With the exception of reduced urine output in the case of succinylcholine, no other effects on the diuretic patterns of furosemide-induced diuresis were noted with any of the muscle relaxants. However, furosemide was found to have a pronounced effect on the muscle relaxant properties of succinylcholine. The succinylcholine fall time was significantly decreased in rats that had received furosemide daily for 1 week as compared to saline-injected control animals. However, no significant difference in the total serum sodium or serum potassium was noted in the furosemide-treated animals as compared to saline-treated animals. In addition, it is unlikely that serum electrolyte changes could explain the furosemide potentiation of the succinylcholine blockage of the rat phrenic nerve preparation because of the brief experimental test period. The mechanism for the potentiation remains to be elucidated.

An earlier report indicated that chlorothiazide potentiated the muscle relaxation produced by *d*-tubocurarine (1). Under the conditions of these experiments, *d*-tubocurarine muscle relaxation was apparently antagonized by furosemide. An explanation for this observation is not available at this time, although the possibility exists that an electrostatic attraction between *d*-tubocurarine and furosemide may result in complexation, thereby reducing the muscle relaxant potency of *d*-tubocurarine. This appears possible since furosemide is widely distributed in all tissues, including skeletal muscle (16). The reversal of the hypotensive effect of *d*-tubocurarine by furosemide tends to support this speculation. In addition, preliminary spectrophotometric data suggest complexation, although further work is required to explain these observations.

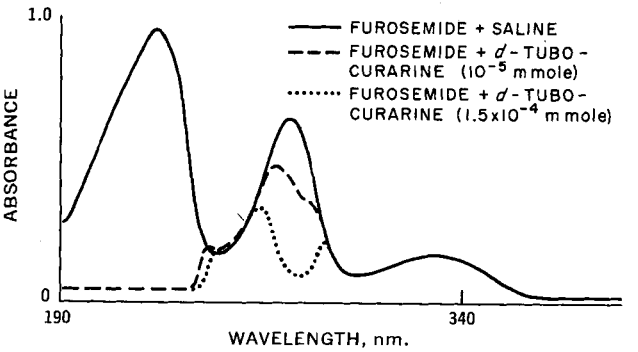

Figure 3—*Furosemide absorption spectrum. The curves compare the spectrum of furosemide prior to and after the addition of* d-*tubocurarine.*

REFERENCES

(1) W. Ferrari, G. L. Gessa, and G. Sangiorg, *Nature*, **184,** 1235 (1959).

(2) "Handbook of Experimental Pharmacology—Diuretics," vol. 24, H. Herken, Ed., Springer-Verlag, New York, N. Y., 1969, p. 398.

(3) J. B. Hook and H. E. Williamson, *Proc. Soc. Exp. Biol. Med.*, **118,** 372(1965).

(4) P. Peltola, *Acta Med. Scand.*, **177,** 777(1965).

(5) R. J. Timmerman, F. R. Springman, and H. E. Wilson, *Curr. Ther. Res.*, **6,** 88(1964).

(6) W. Suki, F. C. Rector, and D. W. Seldin, *J. Clin. Invest.*, **44,** 1458(1965).

(7) "Burroughs Wellcome and Co. Products for Use Before-During-After Anesthesia," Burroughs Wellcome and Co., 1968, p. 3.

(8) "Drill's Pharmacology in Medicine," J. R. DiPalma, Ed., McGraw-Hill, New York, N. Y., 1965, p. 681.

(9) L. O. Randall, *J. Pharmacol. Exp. Ther.*, **129,** 163(1960).

(10) L. H. Sternback and E. Reeder, *J. Org. Chem.*, **26,** 1111 (1961).

(11) L. L. Jones, *J. Cell. Comp. Physiol.*, **18,** 79(1941).

(12) C. L. Fox, Jr., *Anal. Chem.*, **23,** 137(1951).

(13) M. D. Willard, "Instrumental Methods of Analysis," D. Van Nostrand, Princeton, N. J., 1968, p. 352.

(14) S. E. Freeman, *J. Pharmacol. Exp. Ther.*, **162,** 10(1968).

(15) F. E. D'Amour, "Manual for Laboratory Work in Mammalian Physiology" 3rd ed., University of Chicago Press, Chicago, Ill., 1965.

(16) B. Calesnik, J. A. Christensen, and M. Richter, *Proc. Soc. Exp. Biol. Med.*, **123,** 17(1966).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 26, 1971, from the *School of Pharmacy, University of Colorado, Boulder, CO 80302*

Accepted for publication December 10, 1971.

▲ To whom inquiries should be directed.