Selected Pharmacological Studies of Succinic Semialdehyde

J. W. KOSH▲ and G. D. APPELT

Abstract □ Succinic semialdehyde was found to exhibit, in the rat, significant blood-pressure-lowering properties, a negative inotropic and chronotropic effect, and smooth muscle relaxant properties. No anticonvulsant activity was observed in either chemoshock or minimal and maximal electroshock procedures. Succinic semialdehyde was found to be devoid of any CNS activity in the doses employed.

Keyphrases □ Succinic semialdehyde—pharmacology □ CNS activity—succinic semialdehyde □ Anticonvulsant activity—evaluation of succinic semialdehyde □ Pharmacology—succinic semialdehyde

γ-Aminobutyric acid (I) has been implicated as one factor in the maintenance and stabilization of the convulsive threshold. Supportive experimental data demonstrate a binding of I to various neural tissues (1, 2), a correlation of lowered levels of I with the onset of convulsive seizures after convulsant hydrazide administration (3, 4), a relation between increased levels of I and a lowered incidence of convulsive episodes (5), and inhibition of epileptiform convulsions produced in cryoepilepsy (6).

The γ-aminobutyric acid pathway is of interest due to the possible feedback controls that might influence levels of I when the pathway intermediates vary in concentration. Some intermediates of the pathway, such as γ-hydroxybutyric acid and its lactone, have been implicated in the maintenance of various sleep states (7). γ-Aminobutyrylhistidine (8) and γ-guanidinobutyric acid (9), however, have an unknown pharmacological significance. One intermediate, succinic semialdehyde (II), has been shown to possess both CNS and peripheral actions (10–13), but generally only in very high doses.

A significant question that has not been defined is the relationship of succinic semialdehyde to the convulsive threshold of mammalian systems. In addition, because a paucity of information presently exists concerning the effects of succinic semialdehyde upon the cardiovascular (blood pressure and cardiac rhythm), muscle (smooth and skeletal), and central nervous (motor) systems, the purpose of the present study was to correct these pharmacological deficiencies.

EXPERIMENTAL

Chemicals, Reagents, and Animals—The succinic semialdehyde used in this study was prepared by a modification of the method of Langheld (14). Sodium hypochlorite and sodium glutamate were added together in equimolar quantities, and the reaction mixture was adjusted to pH 6.85. After heating, the pH was adjusted to 3.0, the solution was extracted with a continuous ether extractor, and the ether was evaporated under air. Succinic semialdehyde was examined for purity by determining the melting point of the 2,4-dinitrophenylhydrazone derivative and by paper chromatography. The parent compound was further examined by IR spectroscopy, refractive index, and TLC. The concentration of succinic semialdehyde was assayed enzymatically using bovine succinic semialdehyde dehydrogenase and NAD in 0.1 *M* sodium pyrophosphate buffer, pH 8.75. The total increase in absorbance at 340 nm. was utilized for the molarity calculation.

Table I—Characteristics of Succinic Semialdehyde

	Actual	Literature Value
Maximum yield	35%	—
Refractive index, 29°	1.4473	1.4485(23°)
IR spectrum:		
Carboxylic acid peak	Positive	—
Aldehydic carbonyl peak	Positive	—
TLC, silica gel, 95% ethanol	R_f 0.80 (Homogeneous)	—
Melting point, 2,4-dinitrophenylhydrazone derivative	202–204°	202.5–203.5°
Descending paper chromatography, 2,4-dinitrophenylhydrazone of succinic semialdehyde		
Solvent I [*n*-butanol–ethanol–0.5 *M* phosphate (pH 6.0), 27:3:10]	R_f 0.68	0.68
Solvent II (*n*-butanol–ethanol–1 *N* acetic acid, 27:3:10)	R_f 0.85	0.85

All animals used were male adult Sprague-Dawley rats, weighing 300–400 g. They were fed with rat chow[1] and watered *ad libitum*.

Methods—*Pentylenetetrazol Seizure Test*—The convulsive threshold for pentylenetetrazol was considered to be one episode of clonic spasms that persisted for at least 5 sec. (15). Pentylenetetrazol[2] was administered intraperitoneally (30–70 mg./kg.), and the animals were observed for 15 min. Sodium phenobarbital[3] (20–40 mg./kg.), trimethadione[4] (200–300 mg./kg.), and succinic semialdehyde (up to 400 mg./kg.) were screened for their ability to alter the pentylenetetrazol seizure threshold. In each instance, pentylenetetrazol was injected at the time of known peak anticonvulsant effect for the particular drug being tested (16).

Maximal Electroshock and Minimal Electroshock Threshold—In both procedures the electrical a.c. stimulus was delivered by an electroshock apparatus[5] in which ma. output and duration of stimulus could be controlled. The stimulus was applied *via* corneal electrodes. For maximal electroshock testing, a stimulus of 150 ma. for 200 msec. was used and the drugs screened were phenobarbital (2–10 mg./kg.), diphenylhydantoin[6] (10–40 mg./kg.), and succinic semialdehyde (up to 600 mg./kg.). For minimal electroshock threshold, a stimulus of 37 ma. for 200 msec. was used and the drugs screened were trimethadione (100–300 mg./kg.), phenobarbital (30–40 mg./kg.), and succinic semialdehyde (up to 600 mg./kg.). In the maximal electroshock assay, the drugs were assayed for their ability to reduce or block hind-limb extension. In the minimal electroshock threshold assay, the ability to block or reduce the duration of clonus was observed.

Cardiovascular—A physiograph (DMP-4A) was used for monitoring both cardiovascular studies. Carotid blood pressure de-

[1] Purina.
[2] Knoll Pharmaceutical Co.
[3] Merck Chemical.
[4] Abbott Laboratories.
[5] Wahlquist.
[6] Parke-Davis.

Table II—Influence of Trimethadione, Sodium Phenobarbital, and Succinic Semialdehyde upon the Pentylenetetrazol Seizure Threshold in the Rat

Pentylenetetrazol Dose, mg./kg. i.p.	Pretreatment, Intraperitoneal	Percent Convulsing[a]
30	None	16.7
40	None	50.0
50	None	71.5
60	None	83.5
	Trimethadione, mg./kg.[b]	
40	200	0.0
50	200	33.3
60	200	100.0
50	250	16.7
60	250	50.0
70	250	66.7
60	300	37.5
70	300	62.5
80	300	83.0
	Sodium Phenobarbital, mg./kg.[c]	
40	20	16.5
45	20	33.3
50	20	66.6
40	30	0.0
50	30	33.3
60	30	100.0
50	40	0.0
60	40	33.3
70	40	50.0
	Succinic Semialdehyde	
No anticonvulsant effect observed with doses up to 400 mg./kg.		

[a] At least six animals per test group. [b] Pretreatment 1 hr. [c] Pretreatment 2 hr.

termination was performed by standard methods after anesthetization with sodium pentobarbital (35 mg./kg. i.p.). Administration of succinic semialdehyde was made *via* a jugular vein cannula.

Isolated perfused heart studies were performed according to standard techniques. Administration of succinic semialdehyde was accomplished by addition to the perfusing fluid.

Skeletal Muscle—The procedure used involved the sciatic nerve and gastrocnemius muscle *in situ* (17). The sciatic nerve was isolated, and the gastrocnemius muscle was freed at the Achilles tendon and tied securely with string. The sciatic nerve was stimulated at a frequency of 2/sec., 0.2-msec. duration, and 20 v. Succinic semialdehyde was administered *via* intraperitoneal injection. A physiograph monitor was utilized for the muscle contraction record.

Smooth Muscle—Ileum strips were suspended in an isolated smooth muscle chamber at 37°, containing Locke–Ringer solution, and aerated with compressed air. Smooth muscle contractions were monitored using a smooth muscle myograph and recorded with the aid of a physiograph. Solutions of succinic semialdehyde were introduced directly into the bathing medium.

Table III—Minimal Electroshock Threshold: Effect of Trimethadione, Sodium Phenobarbital, and Succinic Semialdehyde on the Minimal Electroshock Threshold in the Rat[a]

Treatment	Dose, mg./kg.	Number in Group	Duration of Clonus, sec.	Percent Cloning
Saline	—	7	23.2	100
Trimethadione	100	6	18.7	66.6
	200	12	23.2	50.0
	300	12	18.8	41.4
Sodium phenobarbital	30	6	15.3	50.0
	35	6	16.5	33.3
	40	6	—	0
Succinic semialdehyde	No anticonvulsant effect observed with doses of succinic semialdehyde up to 400 mg./kg.			

[a] Stimulus was applied corneally at 37 ma. for 200 msec.

Table IV—Maximal Electroshock: Effect of Diphenylhydantoin, Sodium Phenobarbital, and Succinic Semialdehyde on Maximal Electroshock in the Rat[a]

Treatment	Dose, mg./kg.	Number in Group	Hind-Limb Duration, sec.	Extension Percent
Saline	—	12	6.0	100
Diphenylhydantoin	10	6	5.3	100
	20	6	3.5	66.6
	30	6	4.3	33.3
	40	6	—	0
Sodium phenobarbital	2	6	4.8	83.3
	3	6	4.4	50.0
	6	6	5.7	16.6
	10	6	—	0
Succinic semialdehyde	No anticonvulsant effect was observed with doses of succinic semialdehyde up to 600 mg./kg.			

[a] Stimulus was applied corneally at 150 ma. for 200 msec.

CNS—The open-field method of determining motor activity was employed. The animal was placed on a 90 × 120-cm. grid, divided into 48 squares measuring 15 × 15 cm. The animal was given a positive count each time the rat crossed from one square to another, monitoring only the position of one limb.

RESULTS

Purity of Succinic Semialdehyde—Succinic semialdehyde, as prepared by a modification of the method of Langheld (14), gave a maximum yield of 35% (Table I). The refractive index after lyophilizing was 1.4473 at 29°. The published (18) value is 1.4485 at 23°. A positive peak for a carboxylic acid group and an aldehydic group was observed in the IR spectrum of the aldehyde in chloroform. Reference scan 13467 in Sadtler's index (19) of the ethyl ester of succinic semialdehyde contains a similar aldehyde peak. TLC of succinic semialdehyde developed in 95% ethanol produced a homogeneous spot with an R_f of 0.80.

A melting point of 202–204° was obtained for the 2,4-dinitrophenylhydrazone of succinic semialdehyde. This value is in agreement with published values of 202.5–203.5° (20) and 201° (8). Descending paper chromatograms of the same hydrazone produced an R_f of 0.68 in Solvent I and an R_f of 0.85 in Solvent II (Table I). Both R_f values are in agreement with the data of Erwin (21).

In the chemoshock assay, pretreatment with trimethadione and sodium phenobarbital produced the expected elevation of the seizure threshold. The ED_{50} convulsive dose for pentylenetetrazol was elevated from 40 mg./kg. in the control group to approximately 65 mg./kg. at a dosage of 300 mg./kg. trimethadione. For sodium

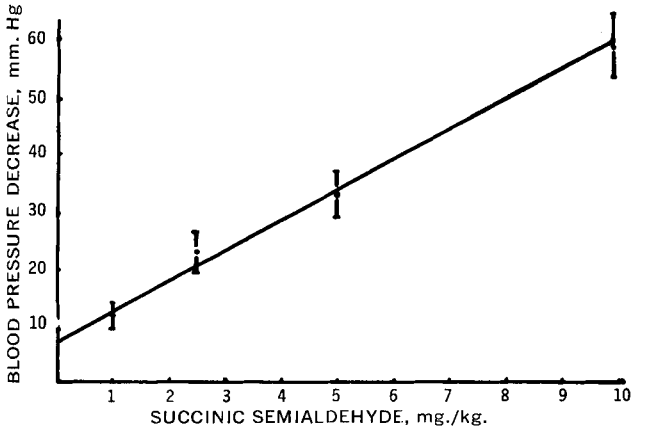

Figure 1—*Effect of succinic semialdehyde on rat carotid blood pressure. Blood pressure was determined as discussed in the text under* Methods. *Brackets around plotted points represent standard errors of blood pressure changes.*

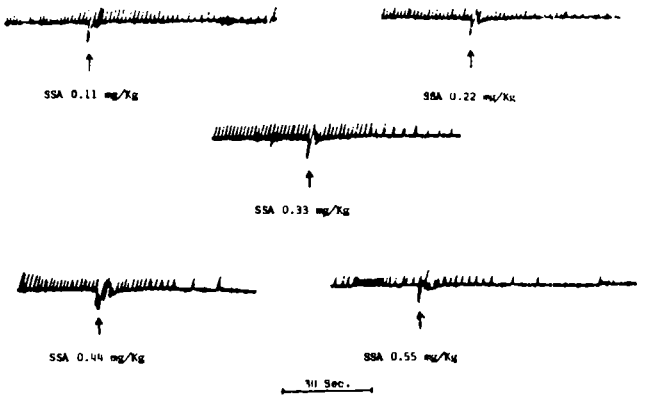

Figure 2—*Effect of succinic semialdehyde (SSA) on the isolated heart of the rat. Succinic semialdehyde was introduced into the perfusion fluid in doses ranging from 0.11 to 0.55 mg./kg. (original whole body weight).*

phenobarbital, the ED_{50} (40 mg./kg.) was elevated to 70 mg./kg. pentylenetetrazol. Pretreatment with succinic semialdehyde at doses up to 400 mg./kg. i.p. did not alter the threshold (Table II).

In the minimal electroshock assay, trimethadione reduced the percent of animals cloning from 100 to 41% at a dosage of 300 mg./kg. Sodium phenobarbital at 40 mg./kg. lowered the percent of animals cloning to 0%. Succinic semialdehyde at doses up to 400 mg./kg. did not affect the minimal electroshock threshold (Table III). Phenobarbital and trimethadione appeared to reduce clonus duration when compared to the control, but the amount of reduction did not appear related to dosage.

The percent of animals exhibiting hind-leg extension in the maximal electroshock studies was abolished with diphenylhydantoin at 40 mg./kg. Phenobarbital produced the same effect at a dosage of 10 mg./kg. However, no anticonvulsant effect was seen with succinic semialdehyde at doses up to 600 mg./kg. (Table IV). No trend on hind-limb extension duration was observed by pretreatment with any of the three compounds. Duration of tonic extension was reduced from the control value by phenobarbital and diphenylhydantoin.

Succinic semialdehyde injected at a dosage of 1–10 mg./kg. i.v. produced significant lowering of carotid blood pressure. At a dose of 10 mg./kg., an average drop in blood pressure of 55 mm. Hg was observed (Fig. 1).

A negative inotropic and chronotropic effect was observed on the isolated heart at dosages ranging from 0.11 to 0.55 mg./kg. (based on whole body weight) (Fig. 2). At a dose of 0.55 mg./kg. of succinic semialdehyde, cardiac contractility appeared to be almost nonexistent.

Smooth Muscle—Succinic semialdehyde was shown to possess definite smooth muscle (ileum) relaxant properties. At a concentration of 7×10^{-4} *M* in the tissue chamber, succinic semialdehyde appeared to be 1/3500 as potent as epinephrine (Table V).

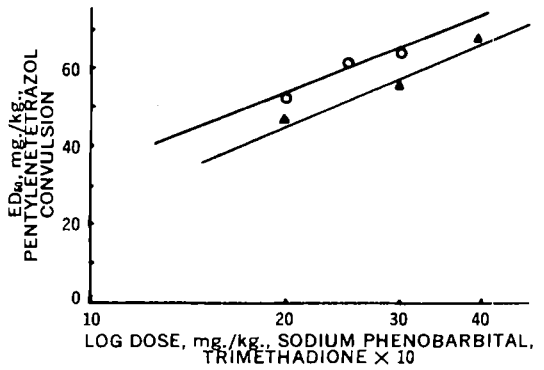

Figure 3—*Comparison of modification of convulsive dose response to pentylenetetrazol by sodium phenobarbital and trimethadione. Plotted is the dose, in milligrams per kilogram, of pentylenetetrazol (ordinate) that produced convulsions in 50% of the animals tested* versus *the logarithmic dose of phenobarbital or trimethadione (abscissa). Key: --○—, trimethadione; and —▲—, sodium phenobarbital.*

Table V—Effect of Succinic Semialdehyde on Smooth Muscle (Ileum) Tissue

Succinic Semialdehyde Final Concentration, *M*	Average Percent Relaxation[a,b]	Standard Error	Range
1.8×10^{-4}	43	±6.7	25–63
3.6×10^{-4}	79	±10.4	50–122
7.3×10^{-4}	105	±12.9	73–132

[a] Percent relaxation values are based on the relaxation produced by epinephrine at 2×10^{-7} *M* final concentration. [b] At least six animals per group.

No CNS effects were seen in the gross appearance screen for succinic semialdehyde. With doses of succinic semialdehyde up to 400 mg./kg., no modification of motor activity was observed as determined by the open-field grid test. In addition, no activity was observed with the skeletal muscle preparation at doses up to 400 mg./kg. i.p.

DISCUSSION

Succinic semialdehyde was shown previously to have only slight pharmacological activity, and only in very large doses (11, 12, 22). In contrast to other data, succinic semialdehyde in the present studies had pharmacological activity in very small dose ranges, acting peripherally. In addition, succinic semialdehyde exhibited dramatic activity upon cardiac contractility. A direct depressant effect was observed with very small doses in the perfusing fluid (Fig. 2). Effects of succinic semialdehyde can be seen also upon smooth muscle tissue (ileum) in doses that approach physiological quantities (Table V). In addition, significant reductions in blood pressure are seen with doses of succinic semialdehyde as low as 1–10 mg./kg. (Fig. 1).

Although part of the blood pressure decrease can be explained in cardiac slowing (bradycardia), relaxation of vascular smooth muscle is also a possible component since definite relaxant activity was observed on ileum tissue. A possible brief period of tolerance to the effect of high doses of succinic semialdehyde was noted, which lasted approximately 5 min. After 5 min., the full effect of succinic semialdehyde could again be seen.

It was demonstrated in the present study that succinic semialdehyde injected intraperitoneally does not modify convulsive seizure thresholds nor their durations caused by pentylenetetrazol or minimal and maximal electroshock stimuli (Tables II–IV). Succinic semialdehyde applied intracisternally might produce different results under these experimental conditions since the barriers of biochemical oxidation of succinic semialdehyde and crossing brain membranes would be removed.

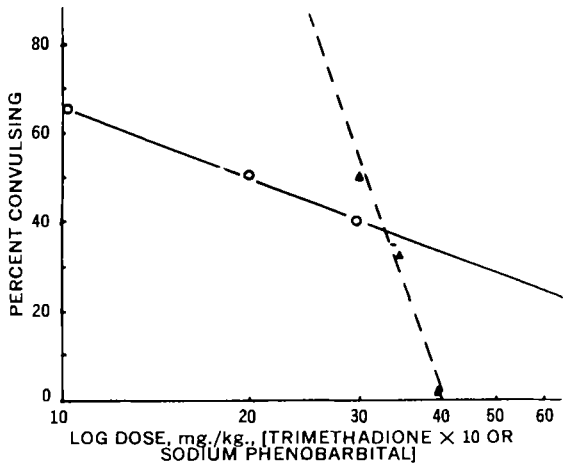

Figure 4—*Modification of minimal electroshock threshold by trimethadione or sodium phenobarbital. The corneal stimulus applied was 37 ma. for 200 msec. Key: —○—, trimethadione; and --▲--, sodium phenobarbital.*

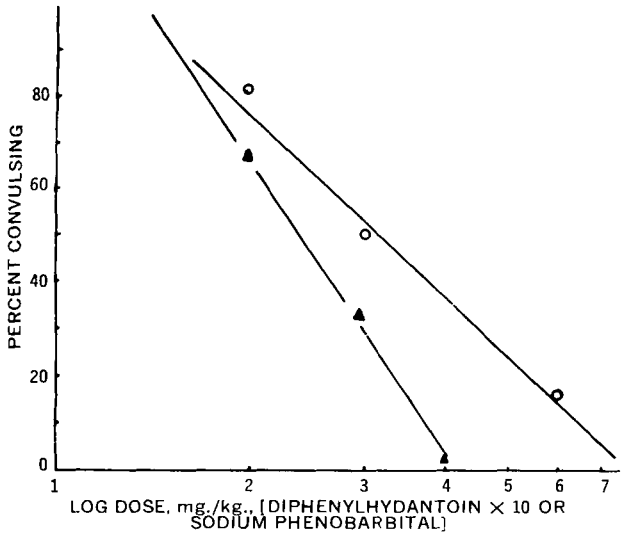

Figure 5—*Modification of maximal electroshock by sodium phenobarbital or diphenylhydantoin. The corneal stimulus applied was 150 ma. for 200 msec. Key: —○—, sodium phenobarbital; and —▲—, diphenylhydantoin.*

A plot of the ED_{50} convulsant dose of pentylenetetrazol against the corresponding respective logarithmic dose of trimethadione or phenobarbital produces similar elevations of the seizure threshold as evidenced by the parallel lines (Fig. 3). It can be stated from these data that these two agents are possibly acting similarly, but they differ in the dose required. Similar slopes indicate that the compounds may be acting at similar sites since it appears that competition by those agents with pentylenetetrazol give similar protective effects with the same percent increase in dose of either sodium phenobarbital or trimethadione.

It is not possible to draw similar conclusions about these two agents with respect to minimal electroshock threshold seizure tests. Nonparallel lines result, indicating possible activity by these two agents at different sites, possibly at multisites (Fig. 4). In view of the type of convulsions produced by pentylenetetrazol and minimal electroshock stimuli, as well as the difference in severity of the two types of convulsions, it is reasonable to expect that the anticonvulsive agents would not be acting similarly in each convulsive type.

Diphenylhydantoin and sodium phenobarbital result in dissimilar sloping lines when dose–effect curves are plotted with respect to modifying maximal electroshock (Fig. 5). Similar slopes of lines would not be expected since it was shown that the action of diphenylhydantoin is that of preventing the spreading of the local electrical discharge in the cerebral cortex (23, 24). Sodium phenobarbital acts by elevating the threshold for spontaneous electrical discharge (25, 26).

The half-life of most aldehydes in the body is of very short duration due to the extreme rapidity of oxidation to the acid form. It is possible that, in the present investigation, the dose of succinic semialdehyde necessary to elicit CNS activity was never reached. Parameters such as the effect of extremely high doses and the appearance of the aldehyde in the brain are currently being investigated.

CONCLUSIONS

1. Succinic semialdehyde administered intraperitoneally does not exhibit anticonvulsant properties against pentylenetetrazol nor minimal electroshock at doses up to 400 mg./kg. in the rat. No anticonvulsant activity was observed against maximal electroshock at doses up to 600 mg./kg.

2. Significant blood-pressure-lowering properties were observed in the rat by intravenous administration of succinic semialdehyde at doses ranging from 1 to 10 mg./kg.

3. A negative inotropic and chronotropic effect was observed on the isolated rat heart by doses of succinic semialdehyde ranging from 0.11 to 0.55 mg./kg.

4. Succinic semialdehyde was shown to possess smooth muscle (rat ileum) relaxant properties approximately 1/3500 as potent as epinephrine.

5. No effect was observed on gross motor activity in the rat as monitored by physical appearance and open-field testing after intraperitoneally administered succinic semialdehyde at doses up to 400 mg./kg.

6. No effect was observed on skeletal muscle in the rat with intraperitoneally administered doses of succinic semialdehyde up to 400 mg./kg.

REFERENCES

(1) P. M. Orkland and E. A. Kravitz, *J. Cell. Biol.*, **49**, 75(1971).

(2) Z. Gottesfeld and K. A. C. Elliott, *J. Neurochem.*, **18**, 683 (1971).

(3) K. F. Killam and J. A. Bain, *J. Pharmacol. Exp. Ther.*, **119**, 255(1957).

(4) J. D. Wood and D. E. Abrahms, *J. Neurochem.*, **18**, 1017 (1971).

(5) C. F. Essig, *Int. J. Neuropharmacol.*, **2**, 199(1963).

(6) H. Moussatche and R. B. Cordeiro, *J. Pharm. Pharmacol.*, **23**, 382(1971).

(7) R. H. Roth and N. J. Giarman, *Biochem. Pharmacol.*, **19**, 1087(1970).

(8) S. Udenfriend, J. J. Pisano, and J. D. Wilson, "Regional Neurochemistry," Pergamon, New York, N. Y., 1961, pp. 348–350.

(9) F. Irreverre, R. L. Evans, A. R. Hayden, and R. Silber, *Nature*, **180**, 704(1957).

(10) B. Weber, C. Baron, and J. Olympie, *Agressologie*, **5**, 369 (1964).

(11) H. Laborit, C. Wermuth, and B. Weber, *ibid.*, **4**, 321(1963).

(12) H. Laborit, B. Weber, B. Delbarre, and H. Pavlovichova, *ibid.*, **6**, 303(1965).

(13) B. Weber, *ibid.*, **4**, 437(1963).

(14) K. Langheld, *Ber.*, **42**, 392(1909).

(15) E. A. Swinyard, *J. Amer. Pharm. Ass., Sci. Ed.*, **38**, 201 (1949).

(16) E. A. Swinyard, W. C. Brown, and L. S. Goodman, *J. Pharmacol. Exp. Ther.*, **106**, 319(1952).

(17) F. E. D'Amour, F. R. Blood, and D. A. Belden, Jr., "Manual for Laboratory Work in Mammalian Physiology," 3rd ed., University of Chicago Press, Chicago and London, 1966, Experiment 29.

(18) I. Heilbron, "Dictionary of Organic Compounds," vol. 1, Oxford University Press, New York, N. Y., 1953, p. 45.

(19) "Sadtler's Index," Spectra 13467, 1958.

(20) H. Waelsch, "Methods in Enzymology," vol. 3, Academic, New York, N. Y., 1957, p. 572.

(21) V. G. Erwin, thesis, University of Colorado, Denver, Colo., 1965.

(22) R. U. Ostrovskaya, *Khim. Farm. Zh.*, **3**, 21(1969); through *Chem. Abstr.*, **72**, 109609(1969).

(23) F. Morrell, W. Bradley, and M. Ptashne, *Neurology*, **9**, 492 (1959).

(24) D. M. Woodbury and D. W. Esplin, *Proc. Ass. Res. Nerv. Ment. Dis.*, **37**, 24(1959).

(25) E. W. Maynert, in "Drill's Pharmacology," 4th ed., McGraw Hill, New York, N. Y., 1971, p. 256.

(26) J. E. P. Toman, S. Loewe, and L. S. Goodman, *Arch. Neurol. Psychiat.*, **58**, 312(1947).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 12, 1972, from the *School of Pharmacy, University of South Carolina, Columbia, SC 29208*

Accepted for publication July 7, 1972.

▲ To whom inquiries should be directed.