solutions up to f_{PG} of 0.50 were of equivalent thermodynamic activity (directly related to chemical potential) with respect to the permeant and, since diffusivity adjusted fluxes were invariant within experimental error, the controlling influence on mass transport was the activity in the donor phase. This also is in accord with the studies of Garrett and Chemburkar (12) utilizing ethanol–water mixtures as a solvent system.

These data sharply contrast with those presented by Poulsen *et al.* (23) which were obtained using a seemingly similar system. Fluocinolone acetonide release from propylene glycol–water gels into isopropyl myristate was measured as a function of f_{PG}. These investigators found that maximum release for a fixed concentration of the steroid was obtained from "vehicles containing the minimum amount of propylene glycol necessary to dissolve the steroid completely." When a significant amount of undissolved drug was present, drug release rates plunged sharply, which was attributed to dissolution-rate control. Diffusion layers formed as the drug was leached from the gel were likely also contributive. These data have received wide acceptance, and it is generally believed that good topical drug availability depends on formulating to have full solution and also saturation. It is difficult to choose between thermodynamic control and dissolution-diffusion layer control as the best model for topical drug availability at this time. Poulsen (24) published some *in vivo* data consistent with the latter. Factors that point to this mechanism are the high viscosities of topical preparations and the lack of mixing at the surface of the skin (after initial application). However, the applied layer is extremely thin and many vehicles tend to dissolve in or fluidize on the skin surface. For a thin application of a fluid vehicle containing a drug with appreciable solubility, thermodynamic control is a distinct possibility. Further studies directed at unraveling this riddle are contemplated.

REFERENCES

(1) G. L. Flynn and T. J. Roseman, *J. Pharm. Sci.*, **60**, 1788 (1971).

(2) J. T. Doluisio and J. V. Swintosky, *ibid.*, **53**, 597(1964).

(3) A. M. Agostini and J. H. Schulman, "Surface Activity and the Microbial Cell," 3rd ed., Gordon and Breach, New York, N. Y., 1965, p. 37.

(4) M. A. Augustine and J. Swarbrick, *J. Pharm. Sci.*, **59**, 314 (1970).

(5) J. Perrin, *ibid.*, **56**, 411(1967).

(6) M. H. Yunker and S. Borodkin, *ibid.*, **60**, 52(1971).

(7) G. L. Flynn and E. W. Smith, *ibid.*, **60**, 1713(1971).

(8) R. G. Stehle, doctoral thesis, University of Michigan, Ann Arbor, Mich., 1970.

(9) C. F. Most, *J. Appl. Polym. Sci.*, **11**, 1019(1970).

(10) T. J. Roseman and W. I. Higuchi, *J. Pharm. Sci.*, **59**, 353 (1970).

(11) J. Haleblian, R. Runkel, N. Mueller, J. Christopherson, and K. Ng, *ibid.*, **60**, 541(1971).

(12) E. R. Garrett and P. B. Chemburkar, *ibid.*, **57**, 944, 949 (1968).

(13) *Ibid.*, **57**, 1401(1968).

(14) K. Sundaram and F. A. Kincl, *Steroids*, **12**, 517(1968).

(15) H. A. Daynes, *Proc. Roy. Soc., Ser. A*, **97**, 286(1920).

(16) R. M. Barrer, *Trans. Faraday Soc.*, **35**, 628(1939).

(17) B. J. Zwolinski, H. Eyring, and C. E Reese, *J. Phys. Chem.*, **53**, 1426(1949).

(18) R. G. Stehle and W. I. Higuchi, *J. Pharm. Sci.*, **56**, 1367 (1967); see also *Reference 8*.

(19) T. Higuchi, *J. Soc. Cosmet. Chem.*, **11**, 85(1960).

(20) C. A. M. Hogben, D. J. Tocco, B. B. Brodie, and L. S. Schanker, *J. Pharmacol. Exp. Ther.*, **125**, 275(1959).

(21) A. Beckett and A. C. Moffatt, *J. Pharm. Pharmacol.*, **21**, 1445 (1969).

(22) R. J. Scheuplein, *J. Invest. Dermatol.*, **45**, 334(1965).

(23) B. J. Poulsen, E. Young, V. Coquilla, and M. Katz, *J. Pharm. Sci.*, **57**, 928(1968).

(24) B. J. Poulsen, *Brit. J. Dermatol., Suppl. 6*, **82**, 49(1970).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 26, 1971, from *The Upjohn Co., Kalamazoo, MI 49001*

Accepted for publication September 3, 1971.

▲ To whom inquiries should be directed.

Toxicological and Pharmacological Studies on Sea Anemone, *Calliactis polypus* (Hormathiidae)

C. L. HUANG*▲ and G. N. MIR†

Abstract □ Tentacles of *Calliactis polypus* were homogenized and centrifuged, and the supernatant liquid was lyophilized. Mice receiving a phosphate buffer solution of the lyophilized material, ranging between 7.5 and 15.0 mg./kg. i.p., demonstrated a stuporous condition and respiratory distress; some died within 24 hr. The LD_{50} in mice was 32 mg./kg. Doses of 5–15 mg./kg. produced a decrease in the blood pressure and bradycardia. These effects were abolished by bilateral vagotomy and atropinization. With higher doses, various arrhythmias and ECG abnormalities were observed. When tested on isolated rabbit heart, the extract caused a decrease in heart rate, force of contraction, and rate of coronary outflow. The extract produced contraction in the isolated rabbit duodenum, guinea pig ileum, and rat uterus by parasympathetic stimulation. These effects were abolished by atropine.

Keyphrases □ *Calliactis polypus* (sea anemone)—toxicology, pharmacology □ Sea anemone—toxicology, pharmacology □ Toxicity—*C. polypus* (sea anemone)

The sea anemone is one of the most abundant of the seashore animals. Approximately 1000 species are found in the ocean. They vary in size from a few millimeters to 0.5 m. or more in diameter. Most species are sessile and are attached to objects of various kinds but are, nevertheless, able to creep about to some extent. When they are covered by water and undisturbed, the body and tentacles have a flowerlike appearance (1).

Table I—Effects of *Calliactis polypus* Toxin on Locomotor Activity (Activity Counts/15 Min.)[a]

Minutes	I (5 mg./kg.) Control	I (5 mg./kg.) *Calliactis*	II (10 mg./kg.) Control	II (10 mg./kg.) *Calliactis*
15	592.1 ± 83.8	251.6 ± 44.3[b]	451.3 ± 94.4	107.5 ± 29.5[b,c]
30	343.0 ± 41.0	141.1 ± 60.0[d]	376.3 ± 56.3	59.3 ± 13.3[b]
45	256.6 ± 24.4	72.6 + 15.0[b]	305.0 ± 81.7	43.1 ± 6.4[d]
60	202.3 ± 17.7	103.6 ± 18.1[b]	229.6 ± 50.1	40.6 ± 10.1[b,c]
75	166.8 ± 18.1	57.8 ± 24.5[b]	220.0 ± 37.3	36.3 ± 11.8[b]
90	169.6 ± 21.5	54.1 ± 28.8[d]	119.6 ± 10.9	16.5 ± 6.6[b]
105	158.6 ± 25.8	45.8 ± 25.1[d]	125.1 ± 16.3	19.1 ± 6.8[b]
120	197.3 ± 14.0	25.8 ± 7.9[b]	176.0 ± 23.8	16.8 ± 7.7[b]
Total	2080.0 ± 153.8	769.5 ± 152.0[b]	2003.1 ± 278.1	339.5 ± 58.1[b,c]

[a] Each value represents the mean ± the standard error for a group of six mice. [b] $p < 0.01$ when compared with the control group. [c] $p < 0.05$ when 5-mg./kg. dose was compared with the 10-mg./kg. dose of *C. polypus*. [d] $p < 0.05$ when compared with the control group.

Toxicities have been observed with sea anemones, particularly with *Rhodactis howesii* (2–6) in which the LD_{99} of the aqueous homogenate was demonstrated to be 6.2 mg. for 20-g. mice. The active principle was considered to be neurotoxic, and it also acted as an anticoagulant. The toxin from *Condylactis gigantea* (7) was extremely potent. The partially purified toxin had an LD_{50} of less than 1 mg./kg. Recently, aminoethylphosphonic acid and methylaminoethylphosphonic acid were isolated from *Anthopleura xanthogrammica* (8, 9).

Calliactis polypus (hormathiidae) is a common seashore animal found in the northern Gulf of Mexico. Its sting is known to cause a mild itching at the sting site; however, this species has received little scientific attention. Therefore, this study was undertaken to investigate the toxicity and pharmacological properties of the extract of this species of sea anemone obtained from the Mississippi Sound.

MATERIALS AND METHOD

Thirty *C. polypus*[1] were collected by trawls around Ship Island. The salinity was 30.0–33.0 p.p.t. and the temperature ranged from 10.0 to 14.9°. Tentacles of the animals were removed, weighed, and homogenized in chilled distilled water for 5 min. The homogenate[2] was centrifuged at 9000 r.p.m. for 5 min., and the yellowish-red supernatant liquid was separated and lyophilized. The lyophilized material was dissolved either in distilled water or 0.1 *M* phosphate buffer (pH 6.5) and used in the studies reported here.

Toxicity Studies—General acute toxicity and various toxic manifestations were studied on mice. Male albino mice, weighing 16–23 g., were randomly grouped, with five animals in each group. They were treated intraperitoneally with aqueous solutions of the lyophilized extract of sea anemone and housed in individual cages provided with food and water. The toxic symptoms and behavioral changes of the animals were observed for 48 hr. The LD_{50} was estimated according to the method of Horn (10).

Pharmacological Studies—*Locomotor Activity*—Albino mice[3] were used in the locomotor activity studies. An automatic timer provided an 8 a.m.-to-8 p.m. light schedule in the room housing the animals, and air conditioning maintained the room temperature between 22 and 24°. All mice were allowed to adapt to these conditions for a minimum of 7 days before use in the experiment.

Measurement of spontaneous locomotor activity was recorded by means of three photocell activity cages (photoactometers)[4]. Each actometer consisted of a circular runway 9 cm. wide and 20 cm. deep. Six photocells were located in the outside wall, 2.54 cm. (1 in.) above the floor. The actometer was covered with a sound-attenuating box. Electromechanical counters registered an activity count each time the animal traversed one-sixth the circumference of the runway. By means of a stopwatch and the counter, counts were taken every 15 min. for 2 hr. The statistical evaluation of the data in this study involved use of one-factor analysis of variance (11).

Blood Pressure and Respiration—The effect of the toxin on blood pressure, respiration, and heart was studied in anesthesized cats. Overnight fasted cats (2.5–3.5 kg.) of both sexes were anesthetized with 35–40 mg./kg. i.p. sodium pentobarbital. The right common carotid artery was exposed at the neck region and cannulated with a polyethylene cannula, which was then connected to a previously calibrated linear core pressure transducer[5] for measurement of blood pressure. Respiration was recorded from chest electrodes, which were connected to an impedance pneumograph[5]. While recording respiration, the same electrodes were connected to an ECG preamplifier for obtaining standard Lead II electrocardiograms. The ECG preamplifier was calibrated in such a manner that each deflection represented 1 mv. in amplitude and 10 mm. in height. The pressure transducer, pneumograph, and ECG preamplifier were connected to a physiograph[6]. The ECG recordings were interpreted according to the description of Burch and Winsor (12) and Friedman (13).

All the injections were made through the right femoral vein, which was exposed and cannulated with a venous polyethylene cannula. The cholinergic activity of the toxin was studied in bilaterally vagotomized and completely atropinized animals; for example, both vagi were cut at the cervical level and the animals were injected with atropine (3 mg./kg. i.v.). A complete parasympathetic blockade was ensured when no response was obtained with a test dose of acetylcholine (5 mg./kg. i.v.). Autonomic ganglia were blocked by the administration of hexamethonium bromide (5 mg./kg. i.v.) in three successive doses. The animals were injected with phenoxybenzamine (3 mg./kg.) for complete blockade of peripheral sympathetic α-receptors, which was ensured by observing reversal of the action of epinephrine (5 mcg./kg. i.v.) on the blood pressure. β-Receptors were blocked by dichloroisoproterenol (4 mg./kg. i.v.). For determining the action on the baroreceptors of the carotid sinus, carotid occlusion was performed three times for 30 sec., both before and after the administration of the toxin.

Isolated Heart Preparation—The effect of toxin on isolated rabbit heart was studied to investigate the effects on the cardiac muscle by Langendorff's technique as modified by Anderson and Craver (14). The chest was opened and the heart was removed. It was dipped into oxygenated Locke's solution and gently squeezed to remove the blood from the aorta. The aorta was freed from its attachment to the pulmonary artery, and the cannula was tied to it. When the cannula was fixed in place, a hook with a thread was fixed on the tip of the ventricle and the preparation was perfused. The thread was passed through a pulley and attached to a myograph (model B0655)[6], which was connected to a physiograph[6] for recording.

The pressure of the perfusate was maintained constant so that the volume of the coronary outflow would be proportional to the changes in the diameter of the coronary vessels.

[1] Identified by Dr. G. Gunter, Gulf Coast Research Laboratory, Ocean Springs, Miss.
[2] Microscopic examination of the homogenate indicated that practically all the nematocysts had discharged.
[3] Southern Animal Farm, Prattville, Ala.
[4] Model RO64, Woodard Research Corp., Herndon, Va.
[5] E & M Instrument Co., Inc., Houston, Tex.
[6] NARCO model DMP-4A.

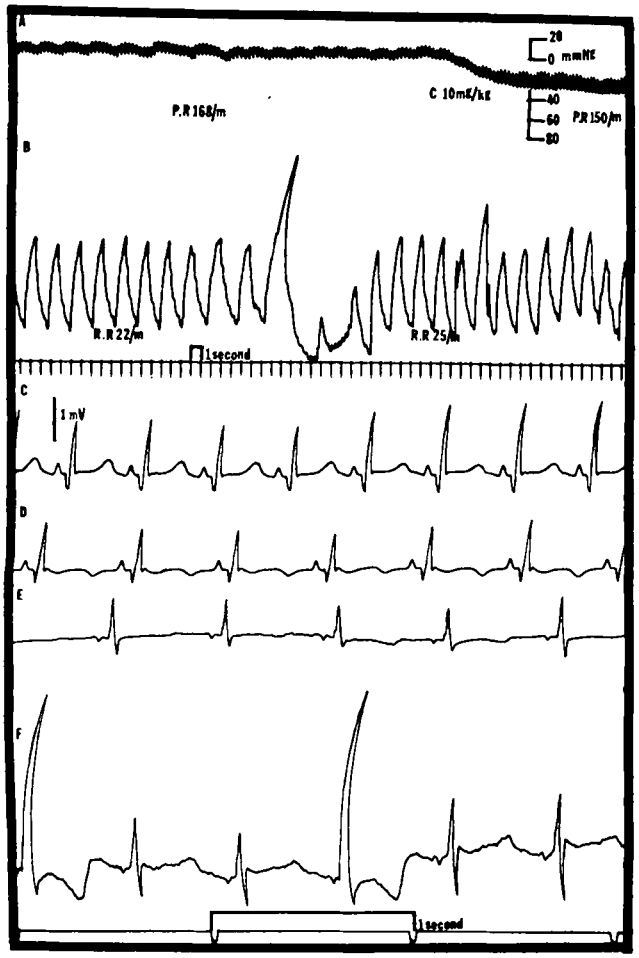

Figure 1—*Effect of* C. polypus *toxin on: A, blood pressure*; B, respiration; C, ECG control; and D, E, F, various changes in ECG at the dose level of 20, 25, and 30 mg./kg. of the toxin, respectively. Key: C,* C. polypus *toxin; PR, pulse rate; RR, respiration rate; and mV, millivolt. *No initial blood pressure is shown on the scale; instead the scale is divided in such a manner that initial blood pressure reads as 0.00 mm. Hg. The pressure transducer was calibrated for a 100-mm. Hg rise or fall in the blood pressure, as shown in the scale.*

Isolated Hind Quarter of Rats—The effect of the *Calliactis* extract on the blood vessels was tested on the hind-quarter preparation of albino rats (15). The animals were sacrificed and eviscerated. A cannula was tied to the abdominal aorta, and the body wall and vertebral column were cut above the point of cannulation. The hind quarter of the rat was then laid on a piece of muslin attached to a wire resting on a glass funnel. A marriotte bottle full of Ringer's solution was connected by a rubber tubing to the cannula. The vessels of the rat were perfused until the perfusate was free from blood. The outflow from the vessels passing dropwise through the muslin and the funnel was measured by counting the drops per minute. All the injections were made directly into the connecting rubber tubing.

Isolated Smooth Muscle—Rabbits weighing 1–2 kg. and guinea pigs weighing 300–500 g. of both sexes were used. The animals were sacrificed and the intestines were exposed. An actively contracting loop of ileum (guinea pig) or duodenum (rabbit), 4–6 cm. in length, was selected and cut. This was suspended in a 25-ml. bath containing Tyrode's (guinea pig ileum) or Ringer's solution (rabbit duodenum). One end of the loop was tied to the hook of a holder and the other end attached to the myograph[6]. The whole preparation was suspended in an organ bath which was kept at 37° and aerated with 95% O_2 and 5% CO_2.

Isolated Uterus Preparation—Young female rats weighing 150–200 g. were used. Estrus was induced by injecting subcutaneously 0.1 mg./kg. of diethylstilbestrol 24 hr. before the animal was used. After the animal was sacrificed, the abdomen was opened and both the horns of the uterus were isolated. The horns were transferred to a dish containing warm DeJalon solution (NaCl, 9.0; KCl, 0.42; glucose, 0.5; $NaHCO_3$, 0.5; and $CaCl_2$, 0.03 g./l.). Each horn was cut open and divided longitudinally so that four strips could be obtained from one animal. The threads were tied to the end of each strip (about 2 cm. in length), which was then mounted in an organ bath of 25-ml. capacity. The DeJalon solution, maintained at 30–37° and pH 7.4, was oxygenated with 95% O_2 and 5% CO_2. The preparation was equilibrated in the bath for 30 min. before use.

RESULTS

Toxicity Studies—Doses of 5–10 mg./kg. i.p. in mice depressed motor activity, but no other behavioral changes or reactions were observed. Lethal doses produced the same effects for the first 15–30 min., and later the animals demonstrated a stuporous condition and died within 1 hr. The LD_{50} in mice was 32 mg./kg.

Pharmacological Studies—*Locomotor Activity*—As shown in Table I, *Calliactis* toxin reduced the motor activity in mice. The absorption of the toxin was rapid, as indicated by the significant reduction in motor activity in the first 15 min. There was a significant difference ($p < 0.05$) between the two doses (5 and 10 mg./kg.), indicating a dose-response relationship.

Effect on Blood Pressure and Respiration of the Cat—Five experiments were carried out on anesthetized cats with different doses of toxin ranging from 5 to 15 mg./kg. At these doses, a 20–40-mm. Hg depression of arterial blood pressure was observed (Fig. 1A). The fall in blood pressure did not last for more than 15 min., after which it returned to normal. As the blood pressure fell, there was a slight decrease in pulse rate which lasted for the same period of time. No significant change in respiratory rate was observed (Fig. 1B). The fall in blood pressure was completely blocked by atropine sulfate (3 mg./kg.). A similar blood pressure response to a carotid occlusion was obtained before and after the administration of the toxin.

Effect on ECG of the Cat—At doses between 5 and 15 mg./kg., the toxin did not produce a significant change in the ECG patterns. However, 20 mg./kg. and higher doses produced various ECG changes, namely, an increase in the P-R interval, a decrease in the

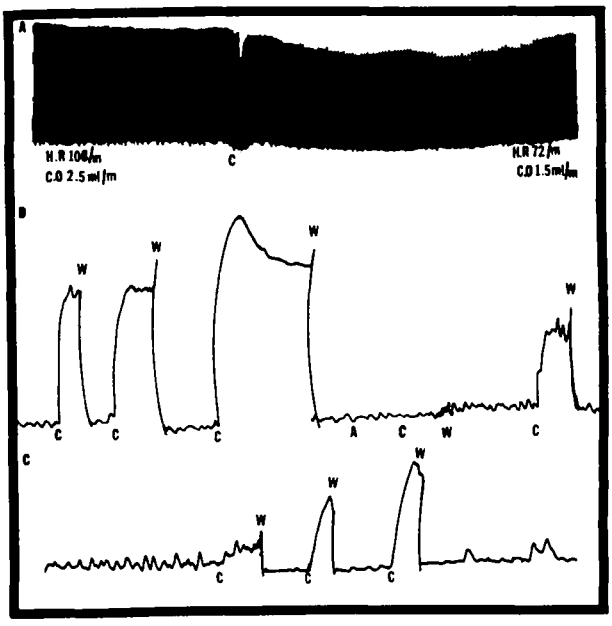

Figure 2—*Effects of sea anemone* (C. polypus) *extract on the isolated rabbit heart, isolated guinea pig ileum, and isolated rat uterus. Key: A, effect of 3-mg. dose of* Calliactis *toxin injected into the perfusion fluid of isolated rabbit heart preparation; B, effect of 25, 50, and 75 mcg./ml. of the toxin on the isolated guinea pig ileum; C, effect of 25, 50, and 100 mcg./ml. of the toxin on the isolated rat uterus; C,* C. polypus*; HR, heart rate; CO, coronary outflow; /m, per minute; W, wash; and A, atropine sulfate, 0.1 mg./ml.*

R wave amplitude, an inversion of the T wave, and an increase in the duration of the S-T segment (Fig. 1D). With 25 mg./kg., there was an inversion of the P wave, a depression of the R wave amplitude, and an inverted T wave. A 30-mg./kg. dose of the toxin produced a condition in which a premature contraction was followed by two normal beats (Fig. 1F). Atropine (3 mg./kg.) reversed the cardiac abnormalities, but it could not reverse the effect of the toxin at a dose higher than 30 mg./kg.

Effect on Isolated Rabbit Heart—The toxin at doses of 0.2–3.0 mg. injected into the perfusion fluid had little effect on the coronary outflow, heart rate, and amplitude of cardiac contractors. With a 3-mg. dose, there was a slight decrease in all three parameters (Fig. 2A), but the effect diminished within 2 min. Doses higher than 4 mg./kg. produced irregular cardiac contractions, which usually resulted in cessation of the heart action.

Effect on Isolated Guinea Pig Ileum—Guinea pig ileum responded to the toxin with contractions. The threshold concentration was 25 mg./ml., and a dose–response relationship was observed with 50 and 75 mg./ml. Atropine in doses that completely abolished the action of acetylcholine and histamine also abolished the spasmogenic action of the toxin (Fig. 2B).

Effect on Isolated Uterus of the Rat—The toxin in 25-, 50-, and 75-mg./ml. doses caused the contraction of the isolated uterine strips of rat. The effect was found to be dose dependent, and it was abolished by atropine sulfate (Fig. 2C).

DISCUSSION

The *Calliactis* toxin was found to be toxic to mice, having an LD_{50} of 32 mg./kg. In gross behavioral and motor activity studies, the toxin appeared to have CNS depressant action.

The toxin in varying dose levels produced a transient fall in arterial blood pressure. The effect was completely blocked by atropine sulfate, indicating that the fall in blood pressure was mediated through parasympathetic stimulation. This observation was further confirmed by *in vitro* studies on guinea pig ileum and rat uterus. The toxin produced contractions which were completely blocked by atropine sulfate.

The dose levels which produced an effect on blood pressure had little effect on the ECG. With 20 mg./kg., there was an increase in P-R interval, a greater duration of the Q-R-S complex, a prolonged S-T segment, and an inversion of the T wave, indicating a slight degree of A-V block, depression of ventricular depolarization, and ischemia of the heart. Thirty milligrams per kilogram produced an inversion of the P wave and a depression of the R wave amplitude, indicating local conduction blocks throughout the ventricles. This was followed by ventricular fibrillation at 30 mg./kg. or above. In this case, a premature contraction was followed by two normal beats. This is produced by a complete A-V block of every fourth impulse that reaches it from the auricles.

The effect of higher doses of toxin on the ECG seems to be independent of the nervous regulation of the heart.

SUMMARY

C. polypus (sea anemone) toxin was found to be toxic to mice, having an LD_{50} of 32 mg./kg. The toxin exhibited a CNS depressant property and also reduced the motor activity in mice. The toxin produced a transient fall in blood pressure and stimulated the isolated guinea pig ileum and rat uterus. All these effects could be abolished by atropine sulfate, indicating thereby that the effect on the blood pressure and isolated smooth muscles was due to the cholinergic stimulation. The toxin in smaller doses did not have any effect on the intact or isolated heart, but larger doses caused various irregularities of the heart action. This effect was not abolished by atropine, which indicated that at higher dose levels the toxin acted indirectly on either the cardiac muscles or the conduction system. The toxin had little effect on the isolated rat hind quarters and the skeletal muscles.

REFERENCES

(1) B. W. Halstead, "Poisonous and Venomous Marine Animals of the World," vol. I, U. S. Government Printing Office, Washington, D. C., 1965, p. 314.

(2) H. Lenhoff, *Biol. Bull.*, **126,** 115(1964).

(3) E. J. Martin, *Pac. Sci.*, **14,** 403(1960).

(4) *Ibid.*, **17,** 302(1963).

(5) E. J. Martin, *Comp. Biochem. Physiol.*, **25,** 169(1968).

(6) J. Wood and T. L. Lentz, *Nature*, **201,** 88(1964).

(7) B. I. Shapiro, *Toxicon*, **5,** 253(1967).

(8) F. A. Shelburne and L. D. Quin, *Biochem. Biophys. Acta*, **148,** 595(1967).

(9) J. S. Kittredge, A. F. Isbell, and R. R. Huges, *Biochemistry*, **6,** 289(1967).

(10) H. J. Horn, *Biometrics*, **12,** 311(1956).

(11) L. A. Edwards, "Experimental Design in Psychological Research," Holt, Reinhart and Winston, New York, N. Y., 1960, p. 117.

(12) G. E. Burch and T. Winsor, "A Primer of Electrocardiography," Lea & Febiger, Philadelphia, Pa., 1954, p. 65.

(13) H. H. Friedman, "Outline of Electrocardiography," McGraw-Hill, New York, N. Y., 1963, p. 99.

(14) F. F. Anderson and B. N. Craver, *J. Pharmacol. Exp. Ther.*, **93,** 135(1948).

(15) J. H. Burn, "Practical Pharmacology," Blackwell Scientific Publications, Oxford, England, 1952, p. 65.

ACKNOWLEDGMENTS AND ADDRESSES

Received April 26, 1971, from the *Department of Pharmacology, School of Pharmacy, University of Mississippi, University, MS 38677*

Accepted for publication September 3, 1971.

The authors express their appreciation to Dr. G. Gunter for arranging for the collection of the specimens.

* Present address: World Life Research Institute, Colton, CA 92324

† Present address: Materials Science Toxicology Laboratories, University of Tennessee Medical Center, Memphis, TN 38103

▲ To whom inquiries should be directed.