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Pharmacological Properties of Hypobranchial Gland of *Thais haemastoma* (Clench)

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Abstract □ The aqueous extract of the hypobranchial glands of *Thais haemastoma*, administered intravenously to anesthetized cats at doses equivalent to 60–90 mg. of fresh gland/kg. body weight, produced an increase in the blood pressure accompanied by tachycardia. These effects were partially blocked by hexamethonium bromide but were not modified by atropine or adrenergic blockade. In the isolated rabbit heart preparation, the extract caused an increase in the heart rate, coronary outflow, and amplitude of cardiac contractions. The extract produced marked contractions in the guinea pig ileum, rabbit duodenum, and rat uterus, which were blocked by atropine and hexamethonium bromide, and induced contractions in the frog *rectus abdominis* muscle, which were abolished by *d*-tubocurarine. It also blocked the conductions in the frog nerve–muscle preparations. It appeared that two active components were present in the hypobranchial gland extract; one produced a direct stimulatory effect on the blood pressure and heart actions and the other acted as a neuromuscular blocking agent of depolarizing type. The LD_{50} in mice was found to be 215 mg./kg.

Keyphrases □ *Thais haemastoma*—pharmacological properties of hypobranchial gland, toxicity □ Hypobranchial glands, *Thais haemastoma*—pharmacology, toxicity □ Toxicity—hypobranchial gland of *Thais haemastoma*

Thais haemastoma is a sea snail which belongs to a family of *Muricidae*. *Thais* usually feeds on oysters, and with its sharp rasplike device called the radula, it can drill through an oystershell in a few hours. Because of its carnivorous eating habit, *Thais* often presents a considerable economic threat to oyster farming. The drilling seems to be aided by acids or enzymes secreted from its salivary gland or tongue, but these secretions are not necessary for drilling. Apparently, *Thais* injects a poison into a molluscan prey at the moment it opens the shell. The poison is considered to be located in the hypobranchial gland or purple gland, which is part of the skin and appears as a conspicuous, folded glandular structure on the roof of the mantle cavity. The gland appears as three narrow, elongated parallel bands or zones oriented in an anteroposterior direction (1). Fisher (2) claimed that it functions as a genital organ, a viewpoint also held by Jullien (3, 4). Erspamer and Glassar (5) suggested that a neurotoxin, murexine, is used by the gastropods for food procurement and as a defense mechanism; however, no further information supports this speculation.

A review of the literature revealed no pharmacological study on this marine animal. Therefore, this study was undertaken to investigate the toxicity and pharmacological properties of the hypobranchial gland of *Thais haemastoma* (Clench), obtained from the Gulf of Mexico.

EXPERIMENTAL

Materials—One hundred and twenty specimens were collected near the Biloxi bridge, Biloxi, Miss., and the surrounding areas where the oysterbeds exist. Most *Thais* were taken by dredges and some in trawls. The salinity and temperature varied from 24.9 to 30.0 p.p.t. and from 15.0 to 24.9°, respectively.

The hypobranchial glands from 30 animals were weighed (10 g.) and homogenized in a blender (Waring) for 5 min., using distilled water as a solvent. The homogenate was centrifuged at 9000 r.p.m. for 5 min., and the yellowish supernatant liquid was used for pharmacological investigations. The doses used were expressed in terms of the fresh gland weight.

Toxicity Studies—General acute toxicity and various toxic manifestations were studied in mice. Male albino mice, weighing 15–22 g., were randomly grouped with five animals in each group. They were administered with the aqueous extract of hypobranchial

Table I—Effect of Hypobranchial Gland Extract of *Thais haemastoma* on Locomotor Activity of Mice (Activity Counts/15 Min.)[a]

Minutes	75 mg./kg. Control	75 mg./kg. Hypobranchial Gland	150 mg./kg. Control	150 mg./kg. Hypobranchial Gland
15	451.3 ± 95.3	482.6 ± 178.0	482.8 ± 119.1	123.8 ± 60.5[b]
30	376.3 ± 56.3	318.8 ± 145.3	457.3 ± 153.3	77.3 ± 25.7[b]
45	305.0 ± 81.7	191.6 ± 89.5	370.1 ± 77.0	46.1 ± 5.2[c]
60	229.6 ± 50.1	116.0 ± 39.4	328.1 ± 54.9	34.6 ± 9.9[c]
75	220.0 ± 37.3	65.0 ± 29.6[b]	312.0 ± 86.1	20.0 ± 5.6[b]
90	104.5 ± 20.3	23.8 ± 5.3[c]	291.0 ± 89.9	43.3 ± 9.9[b]
105	125.1 ± 16.3	42.8 ± 13.9[c]	211.1 ± 33.3	19.3 ± 5.3[c]
120	176.0 ± 23.8	45.0 ± 11.8[c]	158.6 ± 11.6	20.8 ± 5.1[c]
Total	2003.1 ± 278.1	1285.0 ± 368.4	2608.3 ± 590.8	385.5 ± 60.6[c]

[a] Each value represents the mean ± standard error for a group of six mice. [b] $p < 0.05$ when compared with the control. [c] $p < 0.01$ when compared with the control.

glands and housed in individual cages provided with food and water. The toxic symptoms and behavioral changes were observed for 48 hr. The LD_{50} was estimated according to the method of Horn (6).

Pharmacological Studies—*Locomotor Activity*—Albino mice[1] were used for motor activity studies. An automatic timer provided the 8 a.m.–8 p.m. light schedule, and the air conditioning maintained the temperature between 22 and 24° in the room housing the animals. All mice were allowed a minimum of 7 days to adapt to these environmental conditions.

Measurement of the spontaneous locomotor activity was recorded by means of three photocell activity cages (actometers)[2], consisting of a circular runway 9 cm. wide and 20 cm. deep. Six photocells were located in the outside wall 3 cm. above the floor, and the entire 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 (7).

Blood Pressure and Respiration—The effects of the toxin on blood pressure, respiration, and heart were studied in anesthetized cats. Overnight fasted cats (2.5–3.5 kg.) of both sexes were anesthetized with 35–40 mg./kg. i.p. of sodium pentobarbital. The right common carotid was exposed at the neck region and cannulated with a polyethylene cannula, which was connected to a previously calibrated (linear core) pressure transducer[3] for a blood pressure recording. Respiration was recorded from chest electrodes, which were connected to an impedance pneumograph[3]. 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[4] with standard transducer cables for recording. The ECG recordings were interpreted according to the description of Burch and Winsor (8) and Friedman (9).

All 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; *i.e.*, both vagi were cut at the cervical level and the animals were injected with atropine (3 mg./kg. i.v.), and complete parasympathetic blockade was ensured when no response was obtained with a test dose of acetylcholine (5 mcg./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 a complete blockade of the 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.). To determine 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 the toxin on the isolated rabbit heart was studied by Langedorff's technique, as modified by Anderson and Craver (10). The chest was opened and the heart was removed. It was placed into an aerated 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 a cannula was tied to it. A hook with thread was fastened on the tip of the ventricle, and the heart was perfused. The thread was passed through a pulley and attached to a myograph[5], which was connected to the physiograph[4] by standard transducer cables for recording. A constant pressure was maintained throughout the perfusion.

Isolated Hind Quarter of the Rat—The action of the hypobranchial gland extract on the blood vessels was studied with the hind-quarter preparation of albino rats (11). 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 placed on a circular muslin attached to a wire resting on a glass funnel. A marriotte bottle containing Ringer's solution was connected to the cannula by a rubber tubing, and the preparation was perfused until the perfusage was free from blood. The outflow from the vessels passed through the muslin into the funnel and was measured dropwise. A constant pressure was maintained throughout the perfusion, and all injections were made directly into the connecting rubber tubing.

Isolated Smooth Muscle—Rabbits, 1–2 kg., and guinea pigs, 300–500 g., of both sexes were used. The animals were sacrificed and the intestines exposed. An actively contracting loop of ileum (guinea pig) or duodenum (rabbit) of 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 on a holder and the other end attached to the myograph. The preparation was suspended in an organ bath with Tyrode's solution, which was maintained at 37° and aerated with 95% O_2–5% CO_2.

Isolated Uterus Preparation—Young female rats, weighing 150–200 g., were used. Estrus was induced by injecting 0.1 mg./kg. s.c. of stilbestrol 24 hr. prior to the experiment. The animal was sacrificed, and both horns of the uterus were isolated. The horns were transferred to a dish containing warm DeJalon's solution (NaCl, 9.0; KCl, 0.42; glucose, 0.5; $NaHCO_3$, 0.5; and $CaCl_2$, 0.03 g./l.), and each horn was cut longitudinally into two strips. The threads were tied to the end of the strip (about 2 cm. in length) and mounted in an organ bath (25 ml.) containing DeJalon's solution, which was maintained at 30–37°, pH 7.4, and aerated with 95% O_2–5% CO_2. The preparation was left in the bath for 30 min. to equilibrate.

Frog Rectus Abdominis *Muscle Preparation*—The frog *rectus abdominis* was obtained from *Rana pipiens* according to the method of Burn (11). The frog was decapitated and pithed. The muscle was dissected from the pelvic girdle to its insertion in the cartilage of the pectoral girdle. Threads were attached to both ends of the muscle, and an initial weight of 0.5–1.0 g. was applied on it. The preparation was kept at room temperature (25°) in frog Ringer's solution, which was aerated with 95% O_2–5% CO_2. An interval of about 30 min. was allowed for the preparation to stabilize.

[1] Southern Animal Farms, Prattville, Ala.
[2] Model R064, Woodard Research Corp., Herndon, Va.
[3] E & M Instrument Co., Inc., Houston, Tex.
[4] NARCO, model DMP-4A.
[5] Model B-655, E & M Instrument Co., Inc., Houston, Tex.

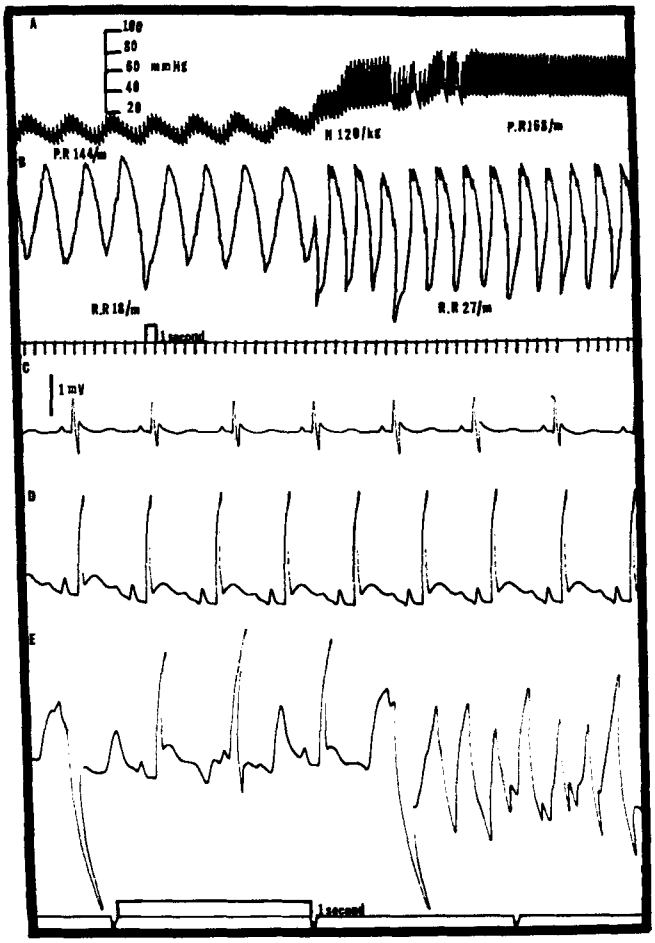

Figure 1—*Effect of hypobranchial gland extract of* Thais haemastoma *on: A, blood pressure*; B, respiration; C, ECG (control); D, ECG at dose level of 100 mg./kg.; and E, ECG at dose level of 150 mg./kg. H = hypobranchial gland extract, PR = pulse rate, RR = respiratory rate, m = minute, and mv = millivolt. (* 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, which is shown on the scale.)*

Nerve–Muscle Preparation—The frog was decapitated, pithed, and pinned prone on a frog board. The *gastrocnemius* was isolated at the tendon, and a thread was fastened around it. The tendon was cut free from its attachment to the heel and the muscle connected to the myograph with a thread. The skin on the back of the animal above the urostyle was dissected. The sciatic nerve was exposed, and a sleeve electrode connected to the stimulator cable was placed around it. Both single and continuous stimuli of 2-msec. duration at 25 v. were applied.

RESULTS

Toxicity Studies—With doses of 25–100 mg./kg., there was an initial increase in the muscular tension, followed by a relaxation of varied duration depending upon the dose. Doses higher than 100 mg./kg. produced an immediate muscular relaxation. With lethal doses, mild convulsions were noted in some animals before death. The LD_{50} in mice was found to be 215 mg./kg.

Pharmacological Properties—*Locomotor Activity*—As shown in Table I, 75 mg./kg. of the toxin did not produce a significant change in the motor activity for the first 75 min. However, there was a significant reduction in the motor activity after this period. With 150 mg./kg., significant changes in the motor activity were observed within the first 15 min. and continued for the next 2 hr.

Effect on Blood Pressure and Respiration of the Cat—Intravenous injection of the toxin in graded doses from 30 to 50 mg./kg. produced little effect on the blood pressure, pulse rate, and respiration in anesthetized cats. Doses from 60 to 90 mg./kg. showed a transient rise in the blood pressure (20–40 mm. Hg), with a slight increase in the heart rate and respiratory rate. Larger doses of the substance, from 100 to 150 mg./kg., produced an immediate and persistent rise (30–50 mm. Hg) in the blood pressure (Fig. 1), accompanied by an increase in the pulse rate and respiratory rate which lasted for approximately 30 min. The pressor effect was not blocked either by atropinization or bilateral vagotomy, except by hexamethonium bromide which was able to antagonize partially the effect on the blood pressure.

Effect on ECG of the Cat—With doses from 100 to 150 mg./kg., there was an increase in the amplitude of the P wave and R wave and an increase in the P-T interval (Fig. 1D) followed by an irregular ventricular beat. Before the animal died, the characteristic pattern of the ventricular beat was lost, and most of the beats presented a rapid sequence of broad oscillation, on top of which a rudimentary Q-R-S complex is indicated by notching (Fig. 1E). Dichloroisoproterenol, a β-adrenergic blocker, reduced the effect of the toxin on the heart, particularly when irregular ventricular rhythms appeared.

Effect on Isolated Rabbit Heart– The control values (10 observations) for coronary flow rate (2.3 ± 0.4 ml./min.), heart rate (65 ± 8 beats/min.), and amplitude of cardiac contractions (25 ± 5 mm. deflection) were consistent throughout the experiment. The injection of various doses of toxin into the coronary perfusion circuit brought about changes in all three parameters. Four milligrams injected into the perfusion fluid (Fig. 2A) produced an immediate increase in the coronary flow rate (3.5 ± 0.3 ml./min.), heart rate (135 ± 5 beats/min.), and amplitude of cardiac contractions (30 ± 3 mm. deflection). The effect lasted for 5 min. or more.

When 8 mg. of the toxin was injected into the perfusion fluid, stimulatory effects were noted in all three parameters. Initially, several irregular beats were observed (Fig. 2B) for the first 10–15 sec. but, thereafter, the heart became regular and continued beating

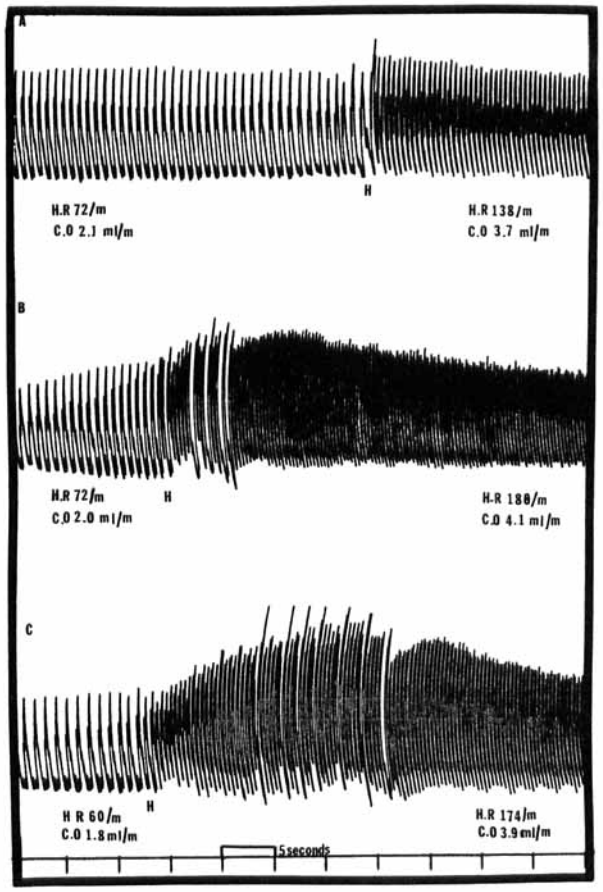

Figure 2—*Effect of hypobranchial gland extract of* Thais haemastoma *on isolated rabbit heart. Key: A, 4 mg.; B, 8 mg.; and C, 12 mg. H = hypobranchial gland extract, HR = heart rate, CO = coronary outflow, and m = minute.*

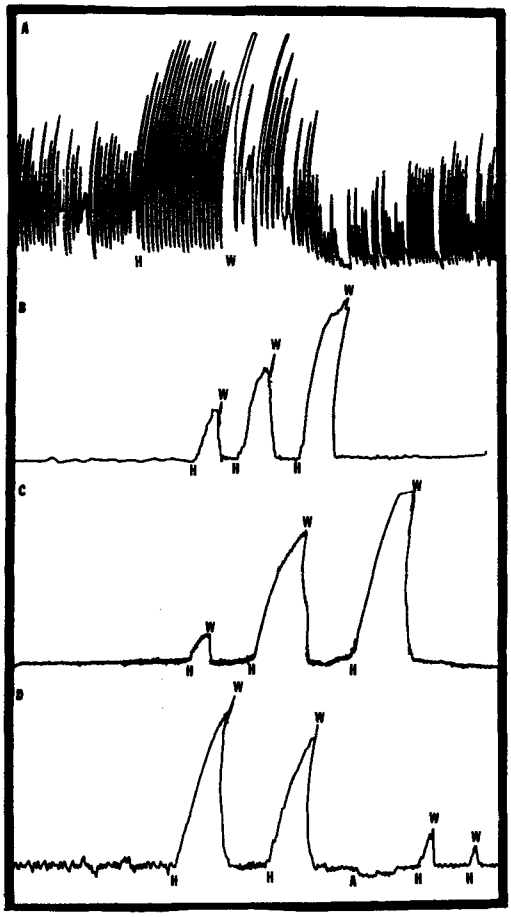

Figure 3—*Effect of hypobranchial gland extract of* Thais haemastoma *on: A, isolated rabbit duodenum (300 mcg./ml.); B, guinea pig ileum (200, 400, and 600 mcg./ml.); C, rat uterus (200, 400, and 600 mcg./ml.); and D, guinea pig ileum (showing blockade of the effect of the toxin, 300 mcg./ml., by atropine sulfate, 0.1 mcg./ml.). H = hypobranchial gland extract, W = wash, and A = atropine sulfate.*

normally for about 10 min. The values for the three parameters were: coronary outflow, 4.0 ± 0.2 ml./min.; heart rate, 185 ± 5 beats/min.; and amplitude of cardiac contractions, 32 ± 2 mm. deflection.

Immediately following the injection of a 12-mg. dose, the heart started beating irregularly (Fig. 2C), which lasted for more than 40 sec. The values for the three parameters were: coronary outflow, 4 ± 0.2 ml./min.; heart rate, 180 ± 5 beats/min.; and amplitude of cardiac contractions, 32 ± 2 mm. deflection. The effect of this dose lasted for 15 min. or longer; then all three parameters declined slowly and ultimately returned to the control levels.

Effect on Isolated Rabbit Duodenum—The isolated loop of the rabbit duodenum responded to 300 mcg./ml. of the toxin (Fig. 3A) with contractions. The high doses of the toxin produced an intense spasm of the intestinal loop, followed by rhythmic movements of increasing amplitude. The stimulatory effect of the duodenum loop lasted for more than 15 min. and ceased abruptly after washing with physiological saline solution.

Effect on Guinea Pig Ileum—Like the rabbit duodenum, guinea pig ileum responded to the toxin with contractions. The threshold concentration was 200–600 mcg./ml., with a good dose–response relationship (Fig. 3B). Atropine sulfate in doses (0.1 mcg./ml.) that partially abolished the effect of acetylcholine (0.1–0.2 mcg./ml.) also abolished the effect of the toxin (Fig. 3D). Hexamethonium bromide completely abolished the effect of the toxin on the guinea pig ileum.

Effect on Isolated Uterine Muscle—The toxin caused contractions of the uterine strip. The threshold concentration was 200–600 mcg./ml., and a good dose–response relationship was obtained (Fig. 3C). Atropine sulfate partially blocked the effect of the toxin on the uterine muscle.

Effect on Isolated Frog Rectus Abdominis *Muscle*—When added to the fluid bath containing the *rectus abdominis*, the toxin produced contractions with a slower onset of time than that of acetylcholine but with a comparable amplitude. The threshold dose was 300 mcg./ml. This effect was blocked by 16 mcg./ml. of *d*-tubocurarine (Fig. 4A).

Effect on Frog Nerve–Muscle Preparation—The toxin (20 mg.) injected into the dorsal lymph sac of the frog produced a complete neuromuscular blockage (Fig. 4B) characterized by the loss of contractions and the action potentials when the motor nerve was stimulated.

DISCUSSION

Hypobranchial gland toxin of *Thais haemastoma* was found to be toxic to mice, having an LD_{50} of 215 mg./kg. In gross observations and motor activity studies, the toxin produced muscular relaxation of varied duration depending upon the dose.

The toxin at various dose levels produced a hypertensive effect. The conclusion that the hypertensive effect produced by the toxin was due partially to autonomic ganglionic stimulation was indicated by the partial blockage of the rise in the arterial blood pressure by hexamethonium bromide.

The ECG recordings from the cats injected with the toxin showed an increase in the P wave amplitude, P-T interval, and R wave amplitude. With higher doses, ventricular beats of various types were observed, which seemed to emanate from different ventricular centers. However, no distinct evidence of auricular activity was noted and, finally, the heart went into ventricular fibrillation. Evidence indicated that the cardiac arrhythmia was partially blocked by β-adrenergic blocking agents, suggesting that part of the effects of the toxin on the heart may be mediated through β-receptors.

The effects on the isolated heart preparation indicated that the toxin was a powerful cardiac stimulant. All three parameters (heart rate, coronary outflow, and amplitude of cardiac contractions) were increased.

The effects on the ECG and isolated heart preparation suggested that the toxin acted directly on β-receptors of the myocardium, cells of the pacemaker, and the conduction system. Cardiac contractions were more powerful, cardiac output was enhanced, and the work of the heart was markedly increased by the toxin. The action of the toxin in inducing arrhythmias and, to some extent, in inducing cardiac automaticity was antagonized by β-blocking agents.

The rise in the blood pressure may be due to two factors: (*a*) autonomic ganglion stimulation, and (*b*) increased cardiac output observed in the isolated heart preparation.

The toxin produced contractions in the rabbit duodenum, guinea pig ileum, and rat uterus. A constant dose–response relationship

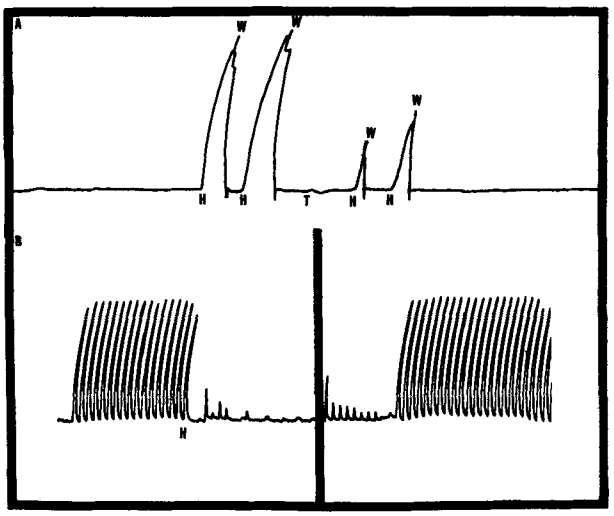

Figure 4—*Effect of hypobranchial gland extract of* Thais haemastoma *on: A, frog* rectus abdominis *muscle (30 mcg./ml.); and B, frog nerve–muscle preparation (20 mg. injected into the dorsal lymph sac). H = hypobranchial gland extract, W = wash, and T =* d*-tubocurarine.*

was noted. Both atropine sulfate and hexamethonium bromide reduced the spasmogenic effect of the toxin, suggesting that the toxin had a nicotinic effect. The muscarinic action, if any existed, was very weak.

On the isolated skeletal muscle, the toxin produced contractions which were blocked by *d*-tubocurarine. The contracture response in the skeletal muscle and the blockage of the transmission of nerve impulses in the frog nerve–muscle preparation suggested that the toxin was a depolarizing blocking agent.

The results of the experiments on the isolated tissues were in accord with the findings of Erspamer and Glasser (5) and Quilliam (12) on murexine obtained from *Murex trunculus*. The *Thais* toxin produced a blockage in the vertebrate neuromuscular transmission similar to that produced by murexine. The blockades produced by murexine and *Thais* toxin were preceded by a muscular fasciculation which was antagonized by *d*-tubocurarine.

Erspamer and Glasser (5) observed that murexine caused a biphasic response in blood pressure, namely, a transient fall followed by a rise in the blood pressure which was abolished by hexamethonium bromide. On the isolated rabbit heart preparation, murexine in smaller doses had little effect, but higher doses showed depression of the heart actions. Unlike murexine, the toxin from the hypobranchial gland of *Thais haemastoma* did not exhibit an initial fall in the blood pressure but rather produced a sustained rise which was partially antagonized by hexamethonium bromide. The observation that the toxin produced a simultaneous increase in the heart rate, coronary outflow, and force of contraction was in accord with earlier observations of Roaf and Nierenstein (13), in which they suggested that hypobranchial glands of *Purpura lapillus* had a physiological activity similar to that of adrenaline.

These observations suggested that the toxin of the hypobranchial gland of *Thais haemastoma* contained two principles: (*a*) murexine, which was shown by the effect of the toxin on isolated smooth and skeletal muscles and its neuromuscular blocking properties, and (*b*) another substance, which acted as a cardiotonic agent as indicated by the effect of the toxin on the blood pressure, intact heart, and isolated heart preparations.

SUMMARY

Hypobranchial gland of *Thais haemastoma* produced a yellowish viscid slime. The slime was found to be toxic to mice, having an LD_{50} of 215 mg./kg. The toxin produced muscular relaxation of varied duration in mice. The toxin produced a hypertensive effect in anesthetized cats. This effect seemed to be partially due to ganglionic stimulation, as indicated by a partial blockage by hexamethonium bromide. The rest of the hypertensive effect could be attributed to an increased cardiac output, observed in both intact and isolated hearts. The effects on the ECG and isolated heart suggested that the toxin acted directly on β-receptors of the myocardium, cells of the pacemaker, and conduction system. Cardiac contractions were more powerful, cardiac output was enhanced, and the work of the heart was markedly increased by the toxin. The action of the toxin in inducing arrhythmias and, to some extent, in inducing cardiac automaticity was antagonized by a β-blocking agent. The toxin produced contractions in various smooth muscles, which were blocked by hexamethonium bromide, suggesting that the toxin had a nicotinic action. The toxin produced contractions in the isolated skeletal muscles which were blocked by *d*-tubocurarine. The toxin blocked conduction in the frog nerve–muscle preparation, suggesting that the toxin is a neuromuscular blocking agent of the depolarizing type.

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