Effects of Nematocyst Toxin of *Physalia physalis* (Portuguese Man-of-War) on the Canine Cardiovascular System.* (32008)

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Recent studies(1,2) have shown that the nematocyst toxin of *Physalia physalis* (Portuguese Man-of-War) specifically affects the conduction system in both the neurogenic heart of the land crab (*Cardisoma guanhumi*) and the myogenic heart of the rat. In earlier experiments the toxin was dissolved and injected immediately because of its extreme lability. Recently a more stable preparation of the toxin has been made, using a phosphate buffer (pH 6.3) that has prolonged activity sufficiently to allow us to observe the response to graded doses.

The present experiments were designed to study the effects of graded doses of *P. physalis* toxin upon the canine cardiovascular system, with particular emphasis on the EKG, systemic blood pressure, cardiac output, peripheral vascular resistance, and the levels of serum potassium and sodium. Effects of potassium infusion on toxin-induced arrhythmias were also evaluated.

Methods. Toxin was prepared as previously described(3), with the exception that the nematocysts were homogenized in 5 ml of isotonic Sorensen's phosphate buffer (containg 18.94 g Na_2HPO_4; 18.16 g KH_2PO_4; per liter). Following centrifugation (37,000 $\times$ *g* at 4°C for 30 min) the supernatant solution was lyophilized and stored in the cold in evacuated containers. Just prior to infusion, the lyophilized toxin was dissolved in 5% (w/v) dextrose buffered to pH 6.3 to a final concentration of 40 μg/ml.

Eight mongrel dogs, 9.6-11.0 kg, were used in this experiment. The dogs were anesthetized with sodium pentobarbital, 28.6 mg/kg, administered intravenously after a 24-hour fasting period. The left carotid artery was exposed and cannulated with PE 205 catheter. Carotid arterial pressures were recorded, using a Statham strain gauge coupled to a Grass Model 7 Polygraph. Mean pressures were derived by an electrical meaning circuit in the recorder. The jugular vein was cannulated with PE 190 catheter that was advanced centrally toward the heart and used for all infusions.

Animals were maintained on a continuous intravenous drip of 5% dextrose. Control values (Table I, Fig. 1) included carotid pressure, EKG (lead II, intradermal electrodes), serum sodium and potassium, and cardiac output (dogs #6, 7, 8).

Cardiac output was estimated by the dye-dilution method; 2.6 mg of Cardio-Green‡ in 1.0 ml of water was injected through the jugular catheter. Beginning with the injection of dye, serial samples were taken in heparinized centrifuge tubes from the carotid catheter at intervals of 0.5 to 1 second. From the centrifuged heparinized blood samples 100 λ aliquots of serum were withdrawn and diluted for optical density measurement in a Beckman DU spectrophotometer. The cardiac output was estimated by the triangle method by Nicholson and Wood(4) and Warner and Wood(5). The hematocrit was determined along with cardiac output. Serum potassium and sodium were determined with a Coleman flame photometer.

Physalia toxin was administered by intravenous drip, at a rate of 30 μg/kg/min for 0.5 to 4 minutes. After toxin administration, cardiac output was determined when carotid pressure stabilized, usually within 4 minutes.

The effect of potassium on toxin-induced cardiac arrhythmias was evaluated following injection of 10 ml of isotonic KCl during prolonged periods of ectopic ventricular beats.

Results and discussion. Cardiac arrhythmias produced by *P. physalis* toxin, like those seen

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‡ Indocyanine green; Hynson, Westcott & Dunning, Inc., Baltimore, Md.

TABLE I. Comparison of Carotid Pressure, Heart Rate, Serum Potassium, Serum Sodium, and Presence of Hemolysis in Control *vs* Experimental Periods.*

	Dog No.	Systemic blood pressure (mm Hg)	Cardiac rate/min	Serum K (mEq/l)	Serum Na (mEq/l)	Hemolysis
Control	1	160/130	160	—	—	—
	2	160/120	125	3.15	147	—
	3	135/100	108	2.90	130	—
	4	180/150	190	3.70	130	—
	5	150/120	120	3.35	142	—
	6	140/110	140	3.35	132	—
	7	140/110	160	3.15	132	—
	8	150/100	125	3.25	136	—
	Avg	152/118	141	3.26	136	—
		±15/±17	±27	±0.25	±7	
Experimental	1	240/150	150	—	—	+
	2	275/160	105	4.10	135	+
	3	335/205	200	3.90	125	+
	4	280/210	220	3.80	135	+
	5	210/160	105	4.35	132	+
	6	250/150	165	3.70	125	+
	7	270/180	190	3.90	130	+
	8	200/120	125	4.10	126	+
	Avg	258/167	158	3.98	130	+
		±43/±30	±44	±0.22	±4	

* Experimental values were obtained within 8 min of beginning of toxin administration. Dose ranged from 12 to 100 μg/kg body wt.
± = standard deviation.

in cardiac glycoside toxicity, are characterized by the establishment of ectopic pacemaker centers and the occurrence of "re-entry" extrasystoles(6).

At a toxin dose of 12 μg/kg body weight EKG alterations occurred within 0.5-1.0 minutes. These included modification of the T wave, progressive reduction in the P-R interval, and finally, suppression of the P wave (Fig. 1) when an ectopic pacemaker site was activated. Since the QRS complex retained its normal shape, the ectopic center of depolarization presumably was located in the A-V nodal area. This pacemaker became dominant when its rate exceeded that of the S-A node (in Fig. 1 it was 105/min while the rate of the S-A node was only 95/min). This reaction suggests that in the dog, as in the rat

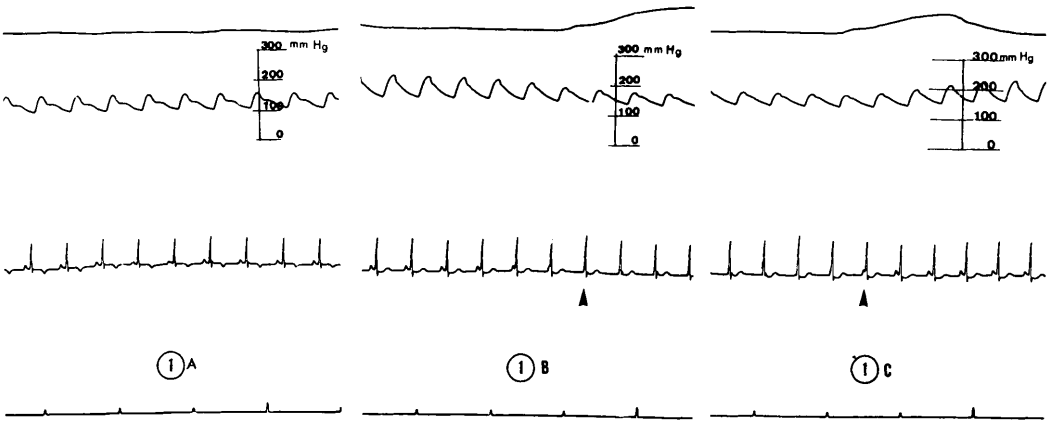

FIG. 1. *A*: Control recordings; top trace is respiration, next carotid pressure, next EKG, and the bottom trace marks time in seconds. *B*: one minute after infusion of 12 μg toxin/kg body weight. Arrow indicates where P wave merged with the QRS complex. *C*: approximately 1.5 min later. Arrow marks first obvious P wave followed by restored S-A rhythm.

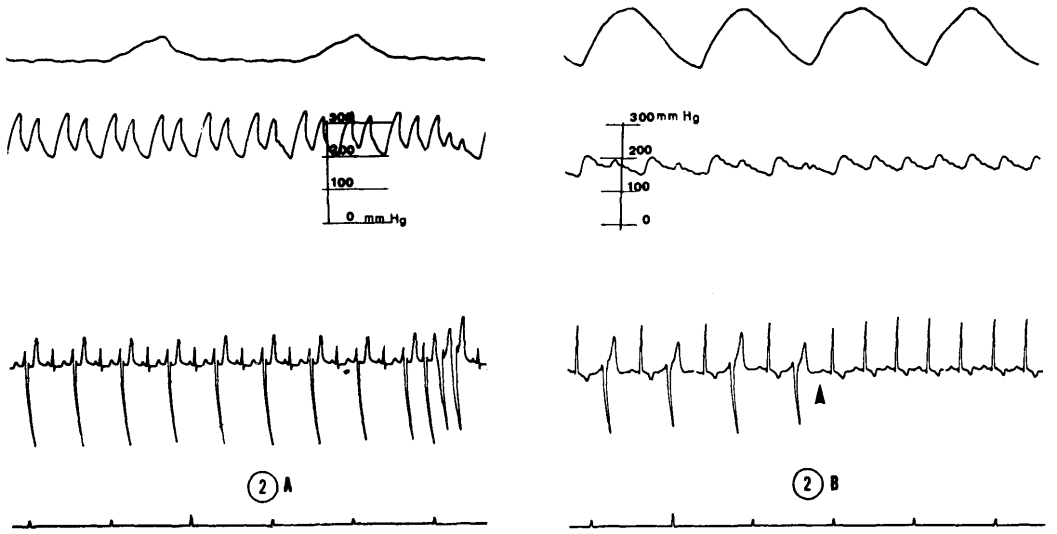

FIG. 2. *A*: recordings taken 1.5 min after infusion of 50 μg toxin/body weight. Bigeminous rhythm was followed by bursts of extrasystoles. Note absence of P wave in ectopic beats. *B*: following 25 min of continuous bigeminal rhythm 10 ml of isotonic KCl was injected. This record was made 1.5 min later. Arrow marks the restoration of normal S-A rhythm.

(2), the tissues in the vicinity of the A-V node are especially susceptible to *P. physalis* toxin. This ectopic center dominated the heart for variable periods up to 1 minute, after which normal S-A nodal rhythm was restored (Fig. 1). While the P wave was suppressed ventricular filling was incomplete, causing carotid pressure to fall. There was no retrograde conduction from the ectopic locus, so the P wave maintained its normal shape until it was absorbed by the ventricular complex. Summation of the P and R waves occurred in the QRS complex marked by the arrow in Fig. 1 *B*. In the next 2 patterns, the P wave appeared after the QRS complex, and thereafter was entirely masked by ventricular repolarization. This sequence of changes was reversed when the rate of the ectopic pacemaker slowed (Fig. 1 *C*). This wandering pacemaker cycle was repeated several times before the ectopic A-V pacemaker locus was established. The sequence of changes could be reversed readily and normal sinus rhythm reestablished by injection of 10 cc isotonic KCl.

At toxin doses of 50-100 μg/kg body weight, the initial period of A-V nodal rhythm was soon followed by "re-entry" or coupled extrasystoles similar to those described by Vasalle *et al* in ouabain-induced arrhythmias(6). Bigeminous rhythms were most typical, often changing to a series of coupled beats (Fig. 2 *A*). At times 20 extrasystoles followed a single normal cardiac cycle. These bursts of extrasystoles contained no P wave after the initiating beat. Administration of 10 cc isotonic KCl at this time restored a normal rhythm as shown in Fig. 2 *B*. In this experiment, KCl was injected after 25 minutes of bigeminous rhythm, restoring a normal EKG within 1.5 minutes.

Preliminary studies in this laboratory suggest that *P. physalis* toxin, like ouabain, inhibits the Na-K ATPase system of crustacean gill tissue, thus reducing active ion transport. The cardiac arrhythmias reported here could result, therefore, from depletion of intracellular potassium. Reversal of the arrhythmias by KCl infusion could result from restoration of normal intracellular K concentration.

After toxin administration (Table I) serum K was significantly elevated ($P = <0.001$) while the level of serum Na was depressed ($P = 0.1\text{-}0.05$). This could result from passive diffusion of ions down concentration gradients after active transport of Na and K had been inhibited by the toxin. Cardiac glycosides also elevate serum K levels (7,8,9). It was calculated that the intracel-

TABLE II. Comparison of Cardiac Output in Control and Experimental Periods.*

	Dog No.	Cardiac output (l/min)	Mean systemic blood pressure (mm Hg)	Peripheral resistance†	Heart rate/min	Stroke vol (ml/min)
Control	6	1.14	120	105	150	7.60
	7	1.02	90	88	160	6.38
	8	1.26	100	79	125	10.08
	Avg	1.14	103	91	145	8.02
Experimental	6	1.95	200	103	165	11.81
	7	2.00	225	112	170	11.77
	8	2.02	150	74	130	15.53
	Avg	1.99	192	96	155	13.03

* Experimental values were obtained when pressure increases stabilized, usually within 4 min following toxin administration.

† Peripheral resistance is mean systemic blood pressure/cardiac output.

lular K (10 mEq K/liter cellular water) released by the hemolyzed erythrocytes would be insufficient to account for all the increased serum K. Approximately 1.6% of the cells were hemolyzed. This would release sufficient K to raise serum levels only about 0.11 mEq/liter.

The observed increase in serum K, however, may have protected the heart and prevented arrhythmias, since additional toxin, infused slowly into a dog already treated with toxin, had no further effect on the EKG. Protection, in this instance, may indicate that active transport was restored. This might be similar to the mechanism by which increased K levels counteract the inhibitory effects of ouabain on erythrocyte membrane transport(10). Rapid injections of 200 μg toxin/kg body weight, however, in a previously injected dog caused additional episodes of ventricular extrasystoles terminating in ineffectual ventricular beats and cardiovascular collapse (Fig. 6).

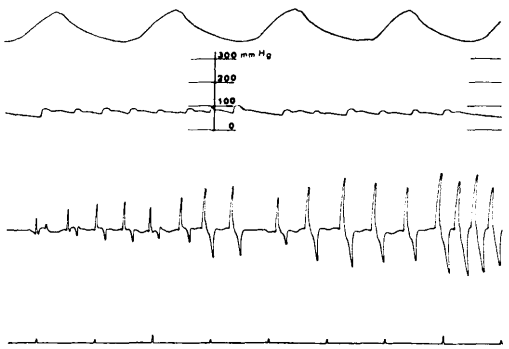

FIG. 3. Recording taken 1 min after rapid injection of 200 μg toxin/kg body weight.

Cardiac output studies showed that the elevated carotid pressure resulted primarily from increased stroke volume (Table II). This might result from the rapid change in electrolytes induced by the toxin, specifically from decreased intracellular K, shown to increase myocardial force(11). However, catecholamine release cannot be ruled out as a contributing factor.

In addition to the cardiovascular effects, the toxin had a marked influence upon the respiratory system. The breathing rate increased from 6/min to 60/min. Since blood gas and pH analyses were not done, it was impossible to determine if this was a direct effect or one mediated by chemoreceptors in response to altered pO_2, pCO_2, or pH.

Summary. Nematocyst toxin from *Physalia physalis,* when injected intravenously into dogs, activated ectopic cardiac pacemaker centers and induced "re-entry" extrasystoles; these effects could be reversed by infusion of KCl. After injection of a fatal dose, these arrhythmias progressed into ineffectual ventricular beats and cardiovascular collapse. In addition to cardiac arrhythmias, sub-lethal doses of toxin caused increased systemic blood pressure and cardiac output, elevation of serum K, decrease of serum Na, elevated breathing rate, and hemolysis.

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Bushbush, Ieri and Lukuni Viruses, Three Unrelated New Agents Isolated from Trinidadian Forest Mosquitoes.* (32009)

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During arbovirus studies carried out in the forests of eastern Trinidad from 1954 to 1964 a number of new agents were encountered, 10 of which have now been formally described(1-10). The present paper describes a further 3 new virus agents, antigenically unrelated, which are represented by 7 strains isolated exclusively from Trinidadian forest mosquitoes.

It is proposed to call these 3 viruses Bushbush, Ieri and Lukuni. The name Bushbush is derived from Bush Bush forest, a swamp island in the Nariva swamp, eastern Trinidad. Ieri, meaning "land of the humingbird," is an Amerindian name for Trinidad. Lukuni, the Amerindian word for people, refers to the inhabited forest camp site where the virus was first encountered.

Materials and methods. The methods used at this laboratory for capturing mosquitoes and processing them for virus isolation have been described previously(11). The techniques used for preparing viral antigens and carrying out hemagglutination-inhibition (HI) tests were similar to those of Clarke and Casals(12). Complement-fixation (CF) tests were done according to the method of Kerr(13). The neutralization (N) test was done by a method described previously(4).

Viruses were tested for sensitivity to sodium desoxycholate (DCA) by the method of Theiler(14). Titrations were done in 2-day-old mice inoculated intracerebrally (i.c.) with 0.02 ml of virus suspension. Titration endpoints were calculated by the method of Reed and Muench(15).

Primary hamster kidney cell cultures were grown and maintained as described by Kissling(16).

Results. The 7 isolates consist of 1 strain of Bushbush virus, 3 of Ieri virus and 3 of Lukuni virus. Table I provides pertinent data about their mosquito sources. In the following account, further details of isolation and information about identification and characterization are given separately for each virus.

1. Bushbush virus. Isolation. The single strain of this virus, TRVL 26668, was recovered from *Culex amazonensis* caught in Bush Bush forest (Table I). The mosquitoes were held overnight at ambient temperatures, sorted and stored at approximately −55°C until inoculated into mice 6 days later. Two-day-old (infant) mice inoculated i.c. with a portion of the mosquito suspension first showed signs of illness on day 8 postinoculation (p.i.). The virus was adapted relatively slowly, and not until the 4th brain passage did it kill all infant mice in the group, with deaths occurring on days 4-9 p.i. In January

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