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Parotoid Secretions of *Bufo blombergi* and *B. peltocephalus.* (25770)

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Bufo blombergi, a giant toad of Colombia, was recently discovered by Rolf Blomberg(1, 2). Each toad weighs one kg or more. *B. peltocephalus* (or *peltacephalus*(3)), a toad well known in Cuba, is comparable in size to *B. marinus.* In contrast with other species, the longitudinal axis of its parotoid glands is perpendicular to the body axis(4). Both *B. blombergi* and *B. peltocephalus* were in the collections of the Dept. of Amphibians and Reptiles of New York Zoological Park. Since no chemical and pharmacological information is available about their parotoid secretions, it was felt desirable to make a preliminary examination. By permission of Dr. James A. Oliver, Director of the New York Zoological Park, one of us (K.K.C.) expressed the parotoid secretions from 2 animals of each species into 2 glass vessels, according to procedure previously described(5). The light yellow secretion of the Colombian toad was milky, slimy, and quickly developed a consistency of chewing gum. It had no special odor. Secretion of the Cuban toad was light gray, creamy, and had a slight scent. Both secretions were air-dried and then desiccator-dried. The material from *B. blombergi,* total 2.156 g, remained light yellow. It was very difficult to pulverize in a mortar because of stickiness. Chunks were unavoidable. No other toad secretion has shown this property. The venom of *B. peltocephalus,* total weight 0.237 g, turned black on drying, and could easily be ground into a fine powder. Sneezing, a bitter taste, and numbness of tongue were noticed during grinding. Various extracts were prepared for detection of catecholamines, indolethylamine derivatives, cholesterol, and cardiotonic substances.

Results. 1. *Catecholamines.* Ten-mg samples were repeatedly extracted with 0.1 N HCl, filtered, and diluted to 10 ml. When ammoniacal alcohol was layered over extracts of both toad secretions, a rose-red color, typical of catecholamines, developed at the junction(6). Intravenous injection of these solutions in pithed cats was followed by a rapid rise of carotid blood pressure with prompt recovery, the pressor action of *B. peltocephalus* being greater than that of *B. blombergi.* Quantitative comparisons were attempted by paper chromatography(7,8). The acid extracts were spotted on Whatman No. 1 paper and developed with water-saturated phenol by ascending chromatography in an atmosphere equilibrated with concentrated HCl and phenol. Air-dried tapes were sprayed with potassium ferricyanide in phosphate buffer, pH 7.8, to make the spots visible. Estimations of epinephrine content were made by comparing spot intensities with those produced by known quantities of epinephrine. Epinephrine concentrations in dried secretions of *B. peltocephalus* and *B. blombergi* were 10% and 2% respectively. Best estimates of *nor*-epinephrine in *B. peltocephalus* were 0.5 to 1%. The existence of this base in the secretion of *B. blombergi* could not be detected by the

method employed. Use of a spectrofluorometer would be necessary to establish any trace of *nor*-epinephrine in this species. Presence of *nor*-epinephrine in parotoid secretions of Chinese and marine toads has been independently proved(9,10). The high content of catecholamines in *B. peltocephalus* would explain the black color after drying due to oxidation. The presence of epinephrine in *B. blombergi* corrects an earlier conclusion based on a less sensitive method, that no epinephrine was present(11). 2. *Indolethylamines.* Samples of 10 mg of dried secretions of both species were crushed in 2 ml of 95% ethanol and allowed to stand at room temperature for 96 hours. Water was added to each extract, followed by filtration and washing through sintered glass to give a volume of 15 ml. The extracts when treated with a *p*-dimethylaminobenzaldehyde test solution U.S.P.(12) gave a blue-green color that was more intense with *B. peltocephalus.* A similar color was observed with serotonin. When the color reagent was modified by substitution of sodium nitrite for ferric chloride(13), a deep purple was produced, which turned red upon standing. Serotonin again duplicated the results. The color reaction does not necessarily indicate presence of serotonin in the secretions of the 2 toads, but it may be evidence of its analogs, especially since in other toads derivatives of 5-hydroxyindolethylamine have been identified(14). 3. *Cholesterol.* A 5-mg sample of each dried toad poison was treated with potassium hydroxide in ethanol, and incubated for 30 minutes at 37°C(15). The whole was extracted with petroleum ether and the supernatant layer was evaporated to dryness. The residue was dissolved in 0.25 ml of chloroform and an aliquot of 0.05 ml (equivalent to 1 mg of the secretion) was spotted on paraffin-treated paper for chromatography, by means of a system of cellosolve, *n*-propanol, methanol and water(16). Cholesterol was found to be present in each of the 2 secretions, decidedly more in *B. blombergi* than in *B. peltocephalus.* 4. *Digitalis-like substances.* A 50-mg sample of each secretion was extracted with methanol for one week under frequent agitation. The extract was filtered and reduced to 10 ml. This was

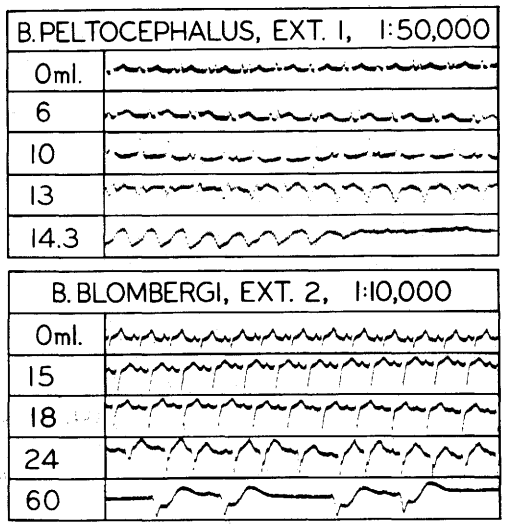

FIG. 1. Action of cardiotonic substances on electrocardiogram. Both cats under ether anesthesia. Electrocardiograms taken continuously from Lead II. Rate of intrav. inj.: 1 ml/min. Note difference of bottom tracings: cat receiving extract from Cuban toad died of ventricular fibrillation, whereas the one receiving that of Colombian toad showed slow ventricular beats almost indefinitely.

called extract No. 1. For *B. blombergi* a second extract, called extract No. 2, had to be made with twice the quantity of secretion because of its low potency. When extract No. 1 of *B. peltocephalus* was diluted to 1:50,000 and infused at the rate of 1 ml/min into the femoral vein of an etherized cat, characteristic electrocardiographic changes were observed (Fig. 1). The cat died of ventricular fibrillation. Two other cats succumbed in the same manner. With extract No. 2 of *B. blombergi,* a 1:10,000 dilution produced slowing of sinus rhythm, P-R prolongation and A-V dissociation, but failed to cause cardiac stoppage with as much as 60 ml (Fig. 1). Two additional animals were studied in the same manner and similar results were obtained. This may indicate low potency of cardiotonic substances. Convulsant movements as observed resembled those produced by 5-anhydro-periplogenone(17).

In frogs, 0.4 to 1.1 ml of undiluted extract No. 1 from either toad, injected into the ventral lymph sac, did not induce systolic ventricular standstill. This could have been due to lack of absorption of the active substances.

Summary. Dried parotoid secretions of *B.*

peltocephalus and *B. blombergi* have been studied for presence of substances known to occur in secretions of other toads. Catecholamines and indolethylamine derivatives were found in both samples, more in *B. peltocephalus* than in *B. blombergi*. Cholesterol was also detected in both species. The digitalis-like substances of *B. peltocephalus* were more potent in cats than those of *B. blombergi*.

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Effect of Thiamine Deficiency on Glutathione Contents of Erythrocytes and Tissues in the Rat. (25771)

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Thiamine-deficient rats excrete a significant amount of methyl glyoxal which is not found in the urine of treated ones. This phenomenon is probably attributable to reduction of glyoxlase activity in liver(1). The mechanism for the decrease of this enzymatic action is, however, not clear. Since glutathione is known as an essential co-factor for glyoxlase activity(2), it becomes important to ascertain whether reduction of glyoxlase activity is related to changes of glutathione content of thiamine-deficient animals. Experiments, therefore, were undertaken to determine the influence of thiamine deficiency on glutathione levels of erythrocytes and tissues in the rat.

Materials and methods. Twenty-five-day-old male rats weighing from 32 to 41 g were used for this study. They were randomly distributed into 2 groups of 6 each. One group received the basal diet supplemented with all known vitamins, except thiamine. The percentage composition of the basal diet has been shown elsewhere(3). The other group was kept on the same basal diet supplemented with thiamine HCl (0.22 mg/100 g basal diet). All animals were housed individually in screen-bottom cages and were offered drinking water *ad libitum*. The feeding was continued for 4 weeks in the first experiment and 8 weeks in the second experiment. Animals were fasted overnight before being sacrificed. Heparinized blood samples were drawn by direct cardiac puncture. A small portion of liver (approximately 0.5 g) was quickly excised in the first experiment; and, in addition, liver tissue, one left kidney and whole heart were removed from each rat in the second experiment. Glutathione content of blood and tissues were measured by the nitroprusside test as described previously(4).