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10 A previous structure revision has been published[11]. Although our data do not result in the same conclusions reached by Livingston et al., the importance of understanding the correct structure of this toxin prompts us to report these results. A 2nd effort towards structure determination has been reported[12], and again some differences in proposed structures are found.

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13 *Note added in proof:* This residue correlates to an aglycone of formula $C_{15}H_{24}O_2$. In a recent abstract (Macko, V., Acklin, W., and Agrigoni, D., 183rd American Chemical Society National Meeting, Las Vegas, Nevada, 1982, abstract 252) the structures of 3 isomeric aglycones were reported. We thank Prof. Arigoni for bringing this work to our attention. Prof. Arigoni has also suggested that our toxin, although we thought it to be homogenous, may have also contained the 3 isomers.

Response of serum calcium to administration of an extract from Stannius corpuscles in the anurans

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Summary. The effect of i.p. injection of an aqueous extract of Stannius corpuscles (10 mg/ml/100 g b.wt) on serum calcium levels was investigated in *Rana cyanophlyctis* and *Bufo andersonii*. This treatment evokes in both species hypocalcemia over a period of 1–4 h, followed by hypercalcemia at 6 h, with a return to control at 8 h.

Amphibia occupy an important position in vertebrate phylogeny as they are the 1st class of vertebrates to occupy a terrestrial, as opposed to an aquatic environment. In amphibia, the parathyroid glands make their 1st phylogenic appearance and the corpuscles of Stannius (CS) (present in their piscine ancestors) disappear. However, they possess ultimobranchial bodies similar to those of their piscine ancestors.

It is now generally accepted that the CS produce the hypocalcemic hormones, hypocalcin[1] and teleocalcin[2]. The removal of this gland in teleosts leads to hypercalcemia[3-5] which can be corrected either by homotransplantation[6,7] or by the injections of corpuscular extract[3,7].

So far, there is no report concerning the possible effects of fish Stannius corpuscle extract on amphibian calcium regulation. The present report is, perhaps, the 1st attempt to describe such an effect in anurans, *Rana cyanophlyctis* and *Bufo andersonii*.

Materials and methods. The CS used in this study were obtained from both sexes of adult freshwater catfish, *Heteropneustes fossilis*. The glands were stored in ice and then used almost immediately. The glands were weighed wet and homogenized in ice-cold 0.6% sodium chloride solution (saline). The homogenate was centrifuged (5000 rev/min for 10 min) and the supernatant was collected. The final volume of the supernatant was made up so that 1 ml of the solution contained the extract from 10 mg of wet CS.

60 male *Rana cyanophlyctis* (b.wt 15–20 g) and 60 male *Bufo andersonii* (b.wt 30–45 g) were acclimatized under laboratory conditions for 1 week prior to use. They were then divided into 2 numerically equal groups - a) saline-injected (control); and b) CS extract-injected (experimental).

The experimental specimens were injected i.p. with CS extract at a dosage of 10 mg/ml/100 g b.wt. The control specimens were injected i.p. with 1 ml/100 g b.wt of saline. Blood samples from both the groups were collected by cardiac puncture under ether anesthesia at 0.5, 1, 2, 4, 6, and 8 h following the onset of the treatment. The analysis of serum calcium was made by Trinder's[8] method.

Effect of Stannius corpuscle extract on serum calcium level (mg/100 ml) of *Rana cyanophlyctis* and *Bufo andersonii*

h	*Rana cyanophlyctis*		*Bufo andersonii*	
	Saline	CS extract	Saline	CS extract
0.5	10.61 ± 0.19	10.00 ± 0.33[a]	17.23 ± 0.15	15.45 ± 0.21[c]
1	10.78 ± 0.22	9.28 ± 0.33[b]	16.64 ± 0.16	15.05 ± 0.12[c]
2	10.86 ± 0.17	9.13 ± 0.34[c]	16.85 ± 0.32	14.44 ± 0.19[c]
4	10.74 ± 0.19	9.42 ± 0.41[b]	17.07 ± 0.28	12.17 ± 0.49[c]
6	10.68 ± 0.23	11.26 ± 0.28[a]	17.67 ± 0.09	18.58 ± 0.23[b]
8	10.59 ± 0.15	10.40 ± 0.28	17.80 ± 0.05	18.01 ± 0.34

The values are mean ± SD of 5 determinations. [a,b,c] Indicate significant responses. $p < 0.025$, $p < 0.005$ and $p < 0.001$, respectively.

Differences between saline-injected and CS extract-injected specimens were evaluated using Student's t-test.

Results. On injection of CS extract the serum calcium level decreases at 0.5 h in both the species (table). This response intensities progressively up to 2 h in *Rana cyanophlyctis* ($p < 0.001$) and up to 4 h in *Bufo andersonii* ($p < 0.001$). At 6 h higher calcemic values have been recorded (*R. cyanophlyctis*, $p < 0.025$; *B. andersonii*, $p < 0.005$). By 8 h serum calcium returns to normal levels.

Discussion. The hypocalcemic effect of CS extract has been reported earlier in eels[3] and in *Fundulus heteroclitus*[1]. The present study clearly indicates that CS extract induces hypocalcemia in anurans as well. The hypocalcemic effect of CS extract in non-piscine vertebrates receives further support from the recent studies in rats[9] and parrots[10]. However, reports of the effect of CS extract administration on serum calcium of rats are contradictory. Milet et al.[11] have reported hypercalcemia whereas Pang (cited by Leung and Fenwick[9]) and Copp (cited by Leung and Fenwick[9]) have failed to obtain any response.

The hypercalcemia observed in the present study at 6 h may be due to the activity of the parathyroid glands in response to the CS extract-induced hypocalcemia.

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Embryonic diapause in annual fishes: evaporative water loss and survival

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Summary. The effects of partial desiccation on the survival of diapause I, diapause II, and pre-hatching embryos of the annual fish *N. guentheri* were investigated. Embryos at diapause II were found to be the most resistant stage. Prolonged exposure of diapause II embryos to 92 and 95% relative humidities retarded the termination of diapause II.

The East African annual fish, *Nothobranchius guentheri* (Pisces; Cyprinodontidae), inhabits isolated bodies of fresh water that are normally subject to periodic drought[1,2]. The evaporative water loss during the dry season results in the death of adult and juvenile fishes. The population escapes extinction in the form of thickly chorionated embryos encased in the bottom substrate which enter a state of developmental arrest or diapause at specific stages of their normal ontogeny[2,3].

A variety of factors, such as temperature[4], photoperiod[5,6] and oxygen tension[3,7], have been demonstrated to control the onset and the duration of diapause. But knowledge of the role of partial desiccation on the survival and development of diapausing embryos have been lacking. The present work was therefore undertaken to determine the influence of partial desiccation on diapause I, diapause II, and pre-hatching embryos by exposure to a series of controlled relative humidities.

Materials and methods. The source of fish, husbandry conditions, and method of embryo collection have already been described in detail for *N. guentheri*[4,8]. Breeding pairs were maintained at a photoperiod of 14L:10D at 20±1 °C. Fertilized embryos were collected and incubated at 20 °C in 125 ml Ehrlenmeyer flasks containing 100 ml of aquarium water. This temperature has been shown to induce longer sojourn in diapause I and entry into diapause II.

A series of controlled relative humidities (RH) was achieved by using graded solutions of potassium hydroxide as described by Solomon[9]. Groups of 20 to 25 embryos were selected, blotted until no water was visible, and placed on a Whatman filter paper cut to fit inside a 60-mm petri dish. The embryos were immediately transferred to a 160-mm glass desiccator containing 100 ml of the appropriate potassium hydroxide solution. After the replacement of the lid, faster equilibration of the humidity level was achieved by the use of a magnetic stirring bar for 5 min to agitate the solution at the bottom. The humidified chamber was then placed in an incubator at a constant temperature of 25 °C. Aquarium water was added to the petri dishes and the number of surviving embryos were counted 24 h later.

Embryos were exposed to nonlethal RH for extended periods to determine the effect of partial desiccation on the duration of diapause. The embryos were removed from the humidity chamber at selected time points and water was added immediately to the petri dishes to prevent total desiccation. The nomenclature established by Wourms[10] to describe the stages of development of the annual fish *Austrofundulus myersi* Dahl was used. After the determination of the developmental stages under a dissecting microscope the embryos were returned to the humidity chamber by the procedure described above. Controls represent those embryos placed on filter paper saturated with water inside a tightly sealed petri dish and incubated at 25 °C.

The frequency distribution of the stages of development among diapause II embryos maintained for 10 days in various relative humidities (RH) at 25 °C

		Stages of development			
	(n)	Diapause II	33–34	36–37	39–40
Control	54	18.5	20.4	46.3	14.8
97% RH	23	43.5	34.8	21.7	–
92% RH	31	45.2	38.7	16.1	–