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MORPHOANATOMY OF *Achatina fulica* FERUSSAC AND THE EFFECTS OF ITS EXCISED CEREBRAL EXTRACT ON ITS GROWTH

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ABSTRACT

Achatina fulica Ferussac collected from the field were reared in the laboratory. They were fed with lettuce, yeast and chalk particles and frequently supplied with water to moisten the soil. Cerebral ganglion, a central club-shaped, brownish structure, dorsolateral to the esophagus was excised very carefully and was homogenized in a hand-operated glass homogenizer containing the extraction buffer. The homogenate was centrifuged until a crude extract was obtained. The total protein content (TPC) of the extracts was determined using the Bradford method. Extracts were obtained from both young and old snails.

Extracts were injected to young and adult live specimens of *A. fulica* Fer. The growth rates of both treated and control were monitored. The extracts have an inhibitory effect on the treated snails.

The extracts were run through a series of SDS-PAGE to assess the protein pattern of the extract.

Introduction

Achatina fulica Ferussac, the African giant snail, belongs to the higher Pulmonates (sub-order Stylommatophora) (Fig. 1). It is a terrestrial species and is characterized by the presence of two pairs of retractable tentacles with the eyes at the tips of the second, longer pair (Fig. 2). It is fully adapted for air-breathing, having a "lung" formed from a richly vascularized roof of the mantle cavity and no "true" or "false" gills (1 & 2).

A. fulica belongs to family Achatinidae. It is the only representative of the genus *Achatina* (Fig. 3) and native to the lowlands of tropical East Africa, including Zandivar Island, 25 miles off the coast of Tanhoyika, but had been subsequently introduced into other places (15).

KINGDOM Animalia
PHYLUM Mollusca
CLASS Gastropoda
SUBCLASS Pulmonata
ORDER Stylommatophora
FAMILY Achatinidae
GENUS Achatina
SPECIES fulica

Figure 1. Classification of *Achatina fulica* Ferussac.

Figure 2. *Achatina fulica* Ferussac showing two pairs of retractible tentacles with the eyes at the tips of the second longer pair.

African giant snails are of interest to researchers because of their tendency to become serious pests in any area where they become established. These soft, slimy, slow-moving creatures are voracious feeders, attacking many kinds of plants but with preference for succulent vegetables and ornamentals. The damages they cause have at times been as serious as those caused by certain insect pests (15). They also feed on decayed vegetation, garbage and dead snails and soil as demonstrated by the color

of their excreta (pale gray color). Soil favors their growth and also serves as their source of calcium (8). These snails attracted attention in the medical field because they are said to be the intermediate host of the rat lung worm *Angiostrongylus cantonensis,* the cause of parasitic meningoencephalitis in primates, man and several other mammals (13). Land snails, however, are generally less important than freshwater snails as carriers of helminth parasites, especially trematodes (2). These snails have been found to be positive for lectin (6, 10, & 17).

This investigation aims to determine: (1) the effect of the crude extracts obtained from cerebral ganglia extracts from young and adult snails on the development of *A. fulica* in terms of growth; and (2) if the crude extracts from young and adult snails produce the same reaction or effect on test organisms.

Findings of this investigation will contribute to knowledge on the basic biology of the species, specially in the area of molluscan neurophysiology. Since *A. fulica* can be a serious pest when established, control measures should be studied without employing pesticides. *A fulica* has lately been used as a source of nutrition for humans and domestic animals, especially in areas where it is a pest; hence, knowledge in the effective control of its growth is needed. The snail is also a good experimental animal for the study of the nervous system, particularly chemical neurotransmitters. This study will also contribute to the data on terrestrial snails in the Philippines. There are numerous works on Philippine freshwater and marine snails but almost none on terrestrial mollusks.

Figure 3. Dorsal view of an adult *Achatina fulica.*

Methodology

A. *Collection, Culture and Maintenance*

Achatina fulica Ferussac were collected around the U.P. Campus (Fig. 4). They were washed and sorted, according to sizes and placed in separate terraria covered with 1 cm. mesh expanded metal net and half-filled with soil (Fig. 5). These were then placed in the laboratory for observation and rearing. The snails were supplied with food daily. The terraria were cleaned once a week to remove excreta on walls. The soil was kept moist by watering and loosened with a fork to ensure that the snails could burrow. The soil was replaced at least quarterly to avoid accumulation of excreta.

Four trials were initiated to test the snail's food preference: (1) ground soil supplied with fresh gabi leaves; (2) ground soil plus dried leaves, supplied with fresh gabi leaves; (3) ground soil supplied with shredded lettuce (*Lactuca sativa* L.) (14); and (4) ground soil supplied with shredded lettuce plus chalk particles and yeast. The optimum number of snails per terrarium was determined by preparing a set-up with 2, 4, and 6 snails per cage. The set-up that gave the optimum condition continued to serve as the experimental set-up.

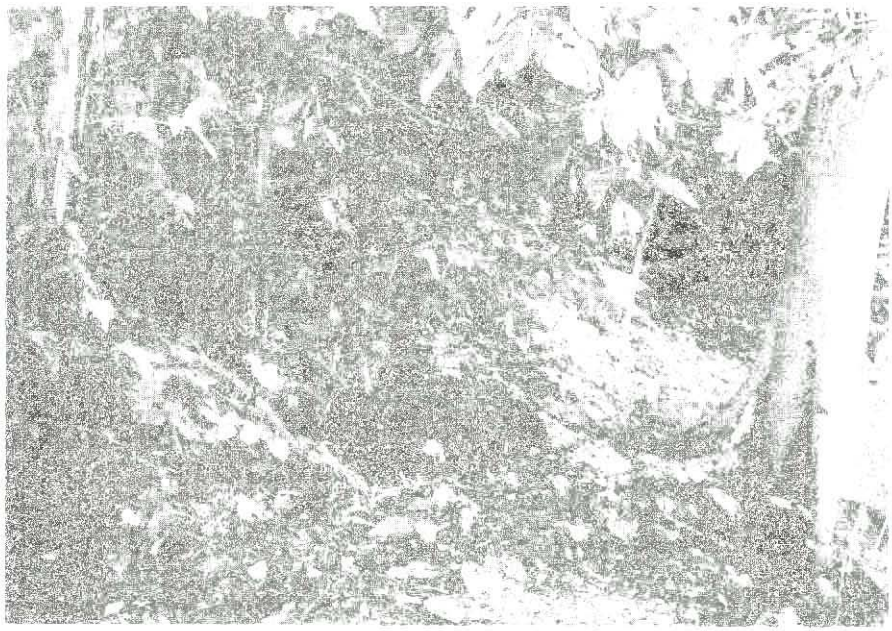


Figure 4. Collection site of *Achatina fulica* around the U.P. campus.

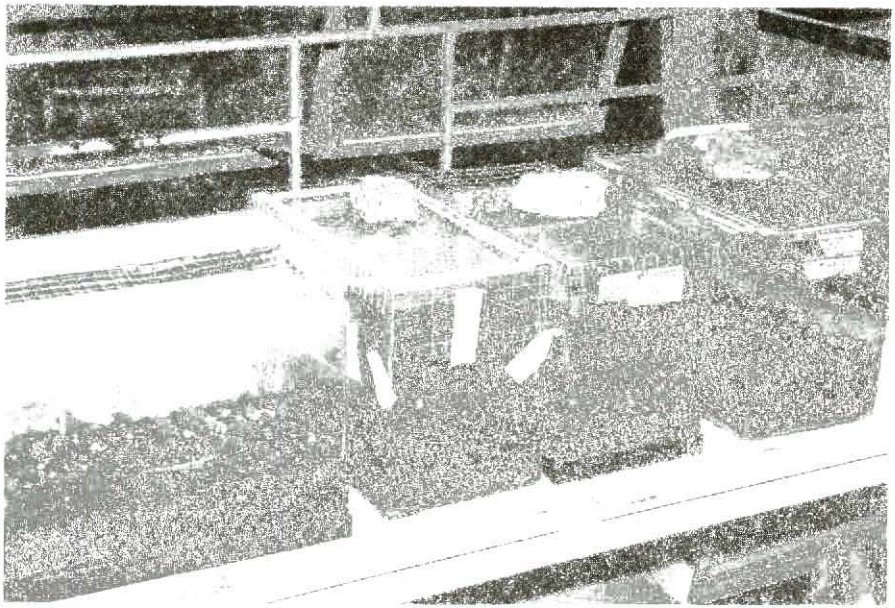


Figure 5. Rearing of *Achatina fulica* in terrarium supplied with lettuce and chalk particles. The soil is moistened every morning.

B. *Dissection of Cerebral Ganglia and Extraction*

The snails were frozen prior to dissection, then deshelled and pinned on a dissecting pan embedded in ice An incision was made starting at the medio-dorsal region of the head, and continuing anteriorly (Figs. 6 & 7). The cerebral ganglion was seen under the Will Stereozoom Microscope as a central club-shaped, brownish structure, dorsolateral to the esophagus (Fig. 8). It was excised very carefully and placed in eppendorf tubes (Fig. 9). Afterwards the ganglia were homogenized in a hand-operated glass homogenizer containing the extraction buffer. Temperature was maintained at 4°C by immersing the whole set-up in an ice bath. The homogenized ganglia were centrifuged at 1,000 rpm for five minutes using a refrigerated centrifuge (Beckman TL-100 Ultracentrifuge). The supernatant was again centrifuged at 4°C, 32,000 rpm for one hour using ultra-centrifuge (Fig. 10). The gross anatomy of *Achatina* was also studied.

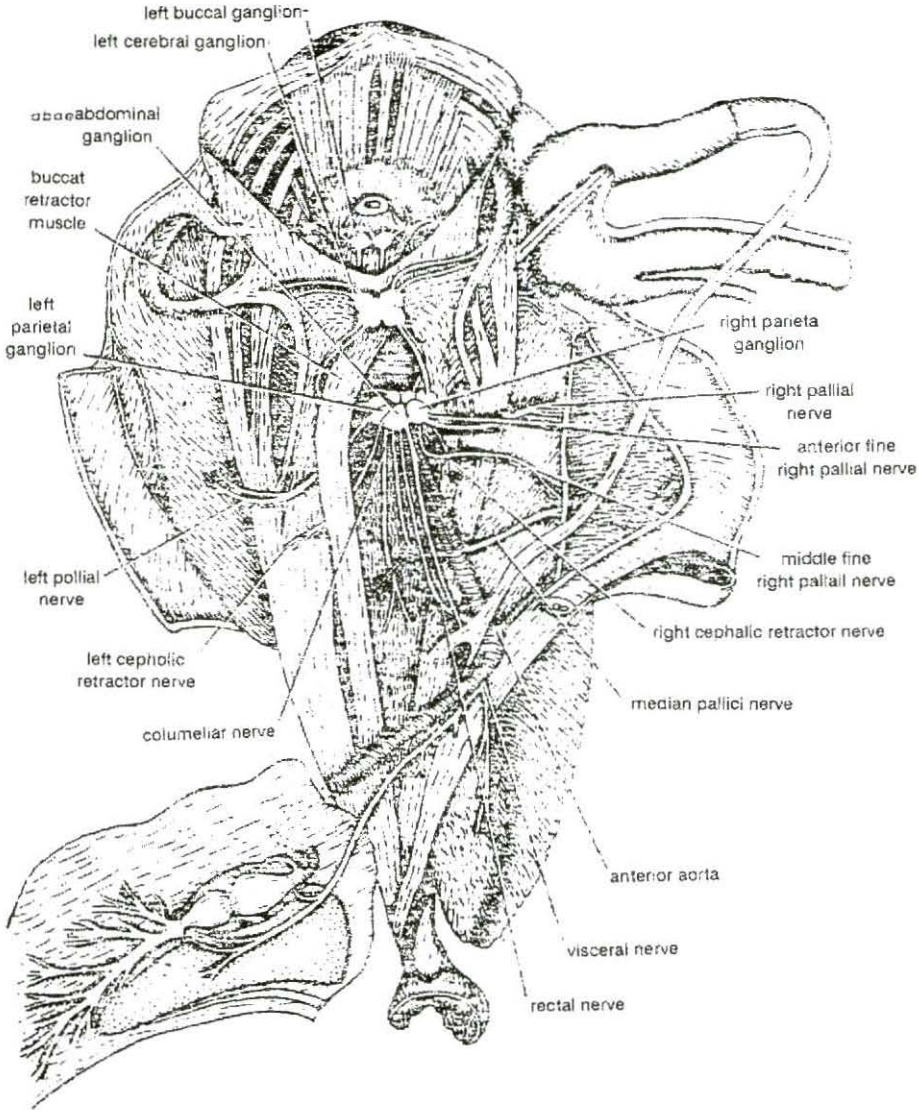


Figure 6. Dorsal dissection with visceral hump, oesophagus and most of the genital system removed, to show ganglia and relevant nerves, including the innervation of the heart.

Figure 7. Dissected snail with an incission made starting at the medio-dorsal region of the head and continuing anteriorly.

Figure 8. A club-shaped and brownish cerebral ganglion observed under Will Stereozoom Microscope.

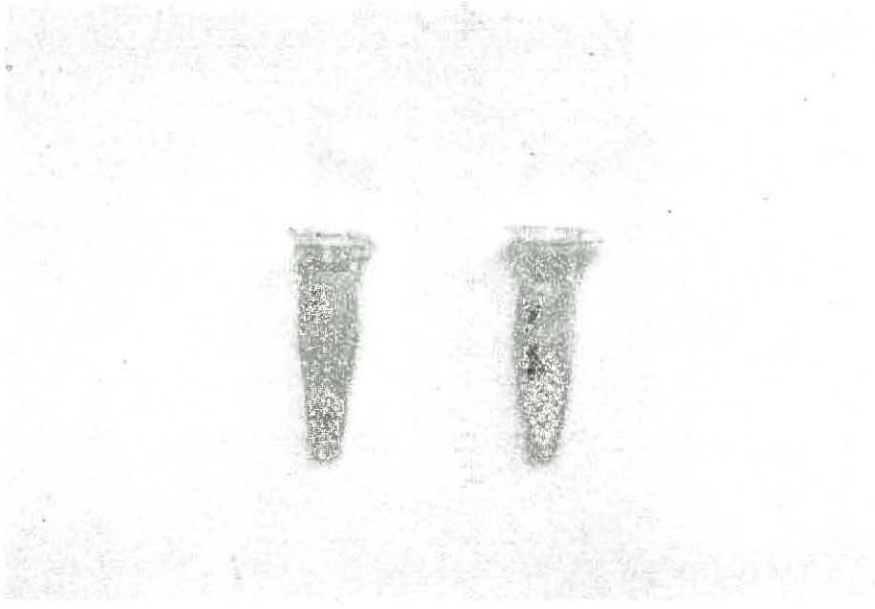


Figure 9. Excised ganglia placed in eppendorf tubes.

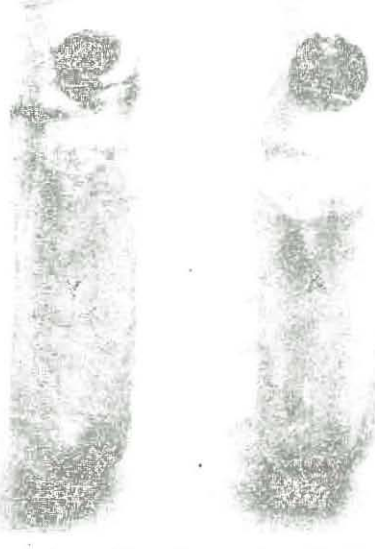


Figure 10. Homogenized ganglia with an extraction buffer.

C. *Total Protein Content Determination*

The Bradford method was used to determine the total protein content (TPC) of the crude extract. A standard curve was generated by reacting 3.0 ml each of the different bovine serum albumin (BSA) solutions (0.5 mg/ml, 1.0 mg/ml, 2.0 mg/ml, 4.0 mg/ml, 8.0 mg/ml, and 10 mg/ml) with 0.3 ml of Bradford's reagent. The optical density was read at 595 nm after every two minutes using a spectrophotometer (Beckman DU-65 Spectrophotometer) (3). The excess extract was dispensed in an aliquot of 50 µl and stored in a freezer for further application

D. *Electrophoretic Study*

Electrophoretic patterns of the extracts were studied using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) (Fig. 11). The slab gel was prepared using 12% separation gel and 3% stacking gel. The amount of sample loaded per well was 30 µg and the extracts were diluted with sample buffer to obtain 60 µl which was the total volume loaded in the wells. The voltage used was 50 V, and when the bromphenol blue dye had reached the separation gel, the voltage was increased to 150 V (Bio-Rad Model 1000/500 Power Supply) until the dye had flowed almost to the end of the running gel which was about 0.5 cm from the base. It was stained by the silver-staining method which is more sensitive than other staining methods. The 3rd and 7th wells were read under the Laser Densitometer (LKB Bromma 202 Ultroscan).

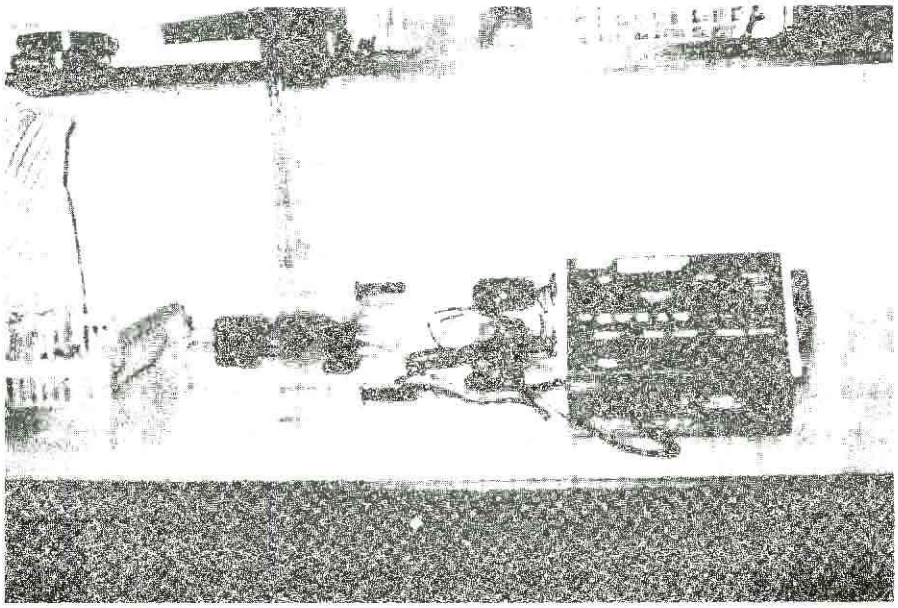


Figure 11. Set-up of the electrophoretic study of cerebral ganglia extract showing:
EK – electrophoretic kit **VR – voltage regulator**

E. *Application*

The crude extracts from young (I) and adult (II) snails were applied twice a month into young (y) and adult (a) snails. Two concentrations were administered: 0.5 mg/ml (V_1) and 1 mg/ml (V_2) concentration of ganglia extract. A certain amount (young extract - 0.75 ml; adult extract - 1.5 ml) was injected slowly at about 10 to 20 seconds into the dorsal cephalic region of the snail. For the control, 0.50 ml of saline was injected.

F. *Data Gathering*

The shell length was measured with a Vernier Caliper; it was determined as the maximum distance from the apex to the outer edge of the aperture (Fig. 12).

The growth rates of both treated and untreated young and adult snails were observed by measuring their length every after 15 to 16 days. The data were gathered for a period of eight weeks from the months of May to September which represent most of the wet or rainy season (Trial I) and from the months of December to April which represent most of the dry season (Trial II). There were two snails per set-up.

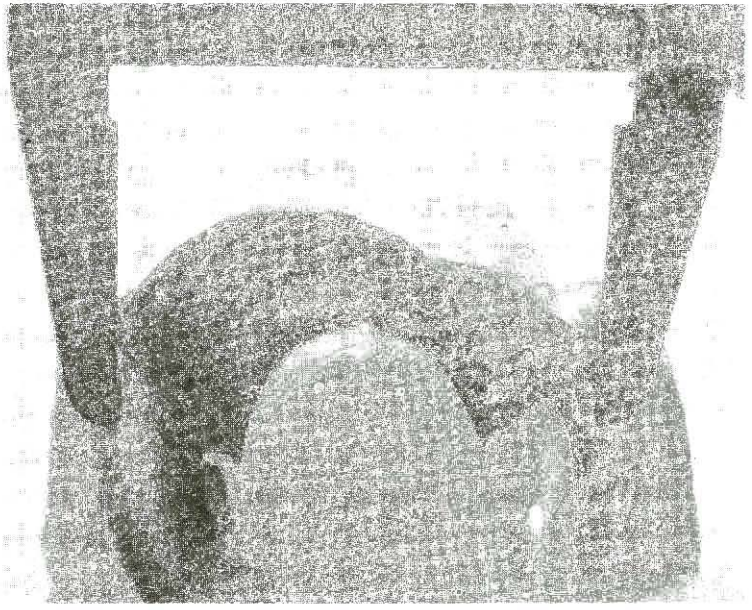


Figure 12. Measuring the lenght of *Achatina fulica* using a Vernier caliper.

Results and Discussion

A. *Biology of Achatina fulica* Fer.

Achatina fulica Ferussac are abundant in the wet season; thus collection is easier than during the dry season when they withdraw into their shells and go into temporary seclusion. They aestivate by burrowing and closing their aperture (Fig. 13) with an epiphragm which is a hardened mucous secretion (Fig. 14). With this kind of adaptation, they can survive for long periods without food; this increases the threat of their introduction to a certain area (5 & 15).

Shredded lettuce with chalk particles and yeast were found to be preferred by *Achatina*. The chalk particles and yeast were the sources of their calcium and vitamins, respectively. The optimum number of snails per terrarium was two because the cage with only two snails persisted and the growth rate was higher than the rest.

A mass of 27 up to 135 eggs laid per snail were obtained from the primary cultures. It had an initial reading, ranging from 3.5 mm - 5.5 mm in length and 3.6 mm - 4.9 mm in width. These eggs had been laid one by one an inch deep into the substratum. They were oval, opaque and were surrounded by a hard shell. At first they were light yellow, but as the time of hatching approached, they became white (Fig. 15). Incubation ranged from 7-15 days (4 & 15).

Among the dissected snails, some were found to have eggs inside the spermoviduct (Fig. 16). In such cases, the spermoviduct was found to be distended. The eggs receive the brittle calcareous shell as they pass through the lower part of the egg channel since eggs found in the upper convoluted part are soft (2).

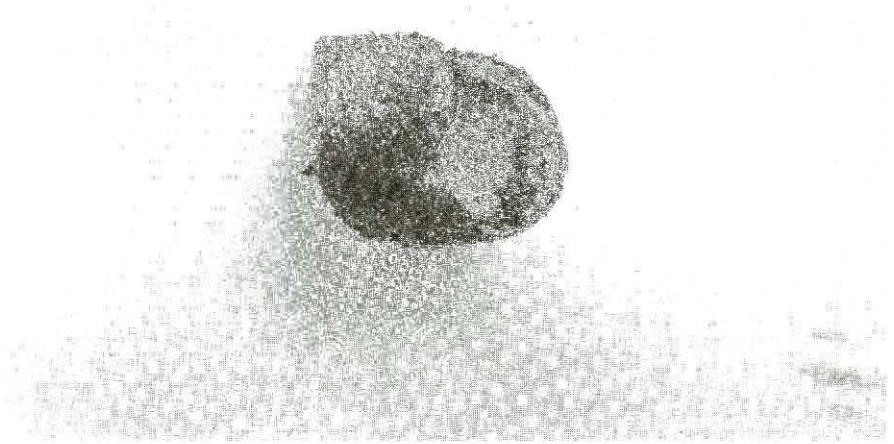


Figure 13. Young *Achatina fulica* on its hibernating stage. The opening is covered with an epiphragm.

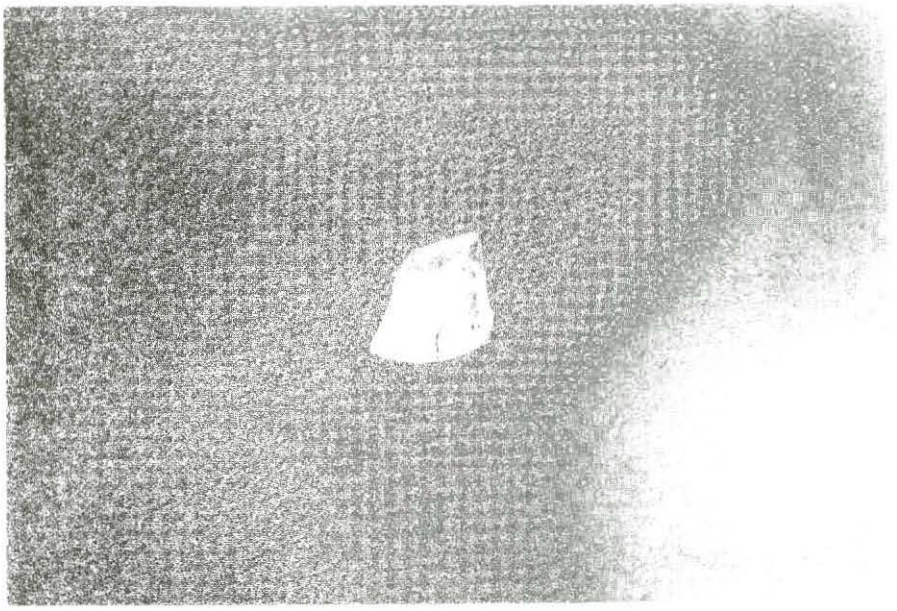


Figure 14. An epiphragm obtained from hibernating snail.

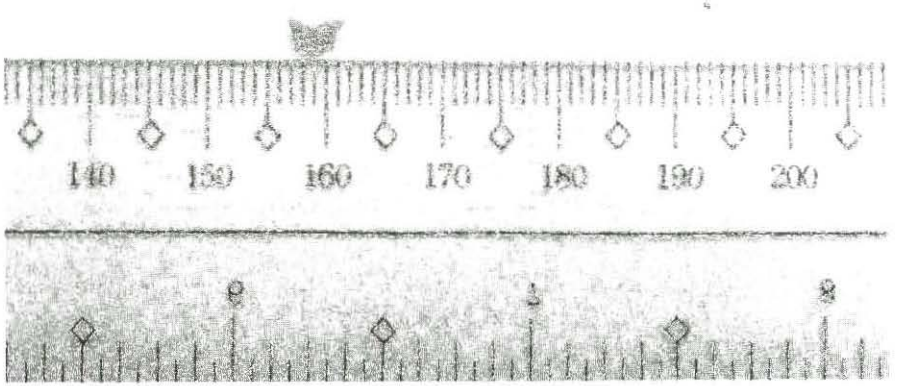


Figure 15. Picture of an egg of *Achatina fulica*.

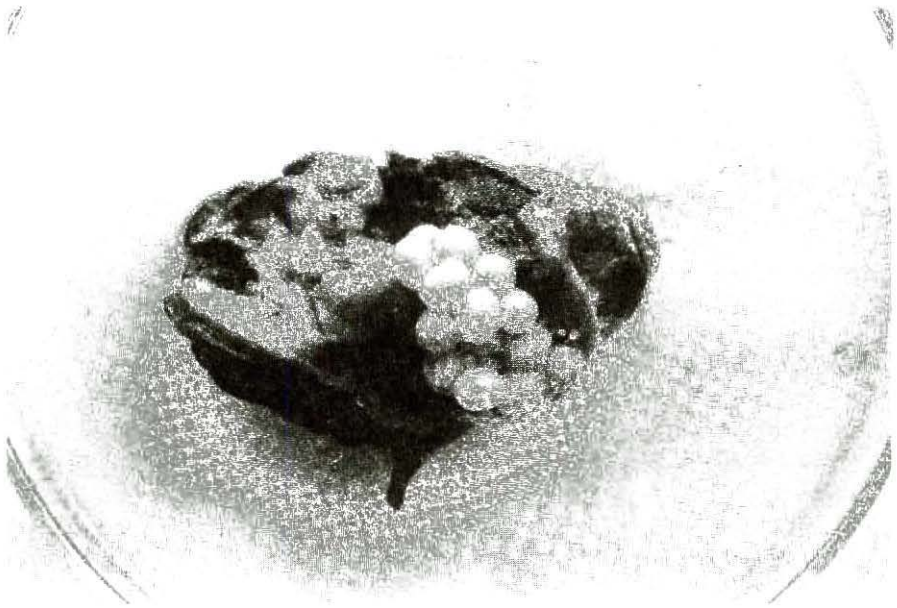


Figure 16. Dissected *Achatina fulica* with eggs found inside the spermoviduct.

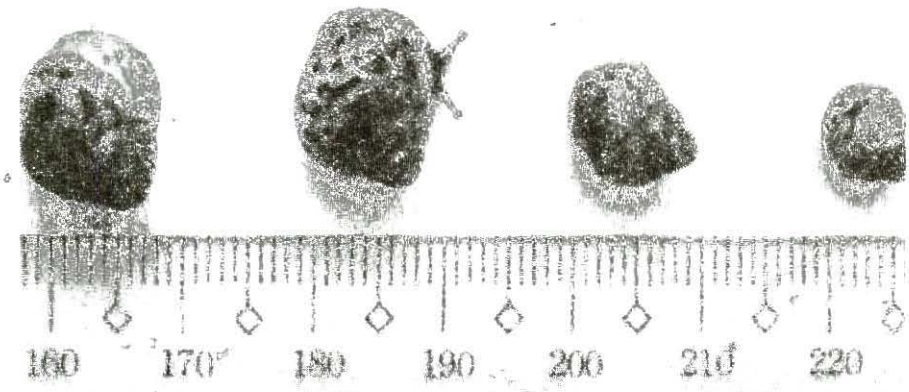


Figure 17. Newly hatched and two-week old *Achatina fulica*.

The newly hatched snails are provided with a shell of more than one whorl, silvery white and semitransparent. They are different from the adult only in size, frailty and markings in the shell (Fig. 17). They usually remain a few days in the nest before they search for food (15).

B. *Gross Anatomy and Morphology*

The snails' shell ranges from a moderate to very large size, usually ovately conical, rarely cylindrical, often with feeble striations and without internal lamella (7). A full-grown shell has from 6 to 7 whorls. The shell is that of dextral or right-hand spiral. The first and large whorl is of a chestnut-brown color with longitudinal bands of light brown and yellow. The second and third whorls from the above are chestnut-brown with wavy bands of yellow. The other whorls are brown with longitudinal bands of light brown. The columella is either straight or concave, usually truncated below, seldom dilated at the lower margin. The body is soft light gray to dark gray measuring from 6 cm to 9 cm long when fully extended. The mouth is surrounded by fleshy lips. On the upper part of the mouth is a sharp, cutting mandible or radula. The pneumostome opens at the right side of a mantle rim lining the anterior margin of the shell. Within it are the anus and kidney aperture. There is a single genital aperture near the right eye-bearing tentacle (15).

Upon dissection, the mouth opens to the buccal mass with jaws, radula and long salivary glands. The esophagus passes back to the simple stomach and is expanded to form a crop. The stomach is muscular but has no style or distinct compartments. The two large masses of digestive gland occupy most of the visceral coil in the apical part of the shell. The intestine emerges from the anterior region of the stomach, which is an "S"-shaped course, through the anterior lobe of the digestive gland and passes to the rectum along the right edge of the mantle cavity. The kidney duct originates from the posterior end of the large kidney and lies close to the rectum, its aperture being confluent with the anus and close to the pneumostome (Fig. 18) (2).

A. fulica is hermaphroditic; that is, each snail has both male and female organs. However, it takes two snails to produce eggs. In these snails courtship and copulation are reciprocal, both animals acting as males and females at the same time (12). Dissection revealed that they are indeed hermaphroditic; the gonad is a whitish ovotestis embedded in the posterior part of the digestive gland on its inner, columellar surface. From near the anterior end of the gonad arises the little hermaphroditic duct which is convoluted and swollen with stored spermatozoa throughout much of its length. The duct is followed by a short "duct" of the albumen gland. The albumen gland is often large, especially in mature snail, and creamy-yellow. The spermoviduct consists of two halves, a female part or egg channel and a male part or seminal groove. It appears as a large tube which may be round or flattened with a gutter-like seminal channel set at one side. Large lobes of prostate gland open into the seminal groove through its floor and these prostatic lobes are attached to the male side of the spermoviduct throughout its length. The spermoviduct divides into a non-glandular oviduct and a vas deferens. The oviduct connects with the duct to the bursa copulatrix

and passes on as the vagina to open at the genital atrium. The vas deferens also passes forward and makes a "hairpin" turn backward to enter the penis which opens alongside the vagina at the genital atrium (Fig. 19). There is a single genital aperture near the right eye-bearing tentacle (2).

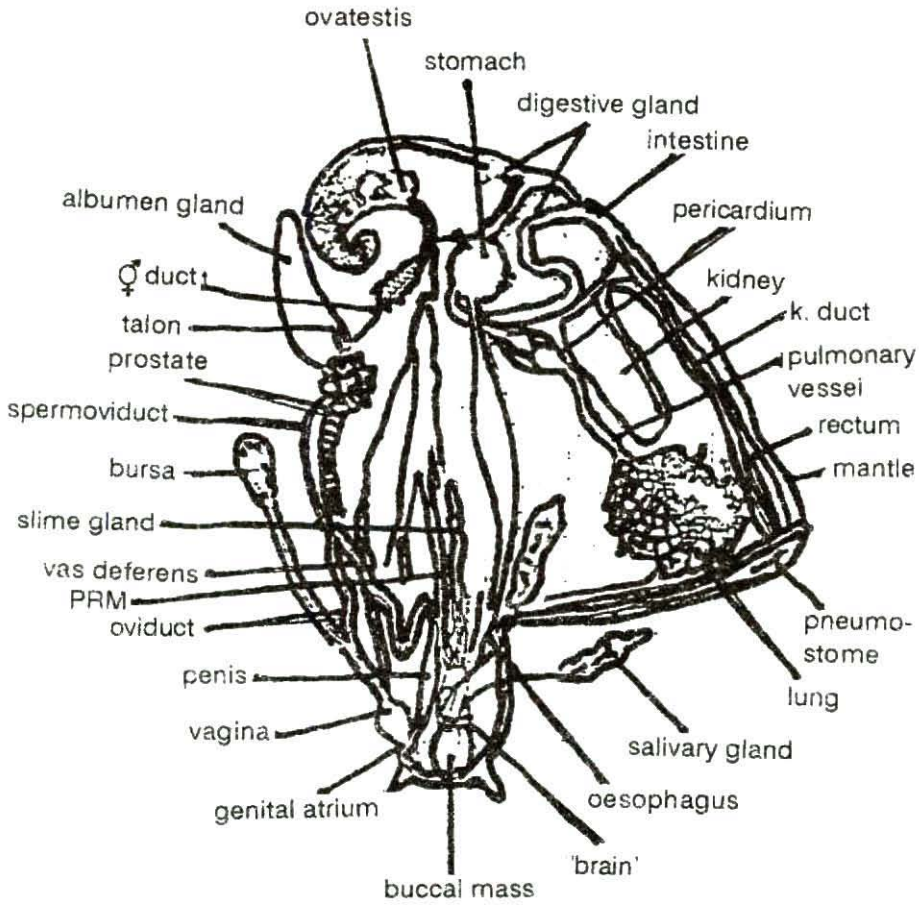


Figure 18. Gross anatomy of *Achatina fulica*.

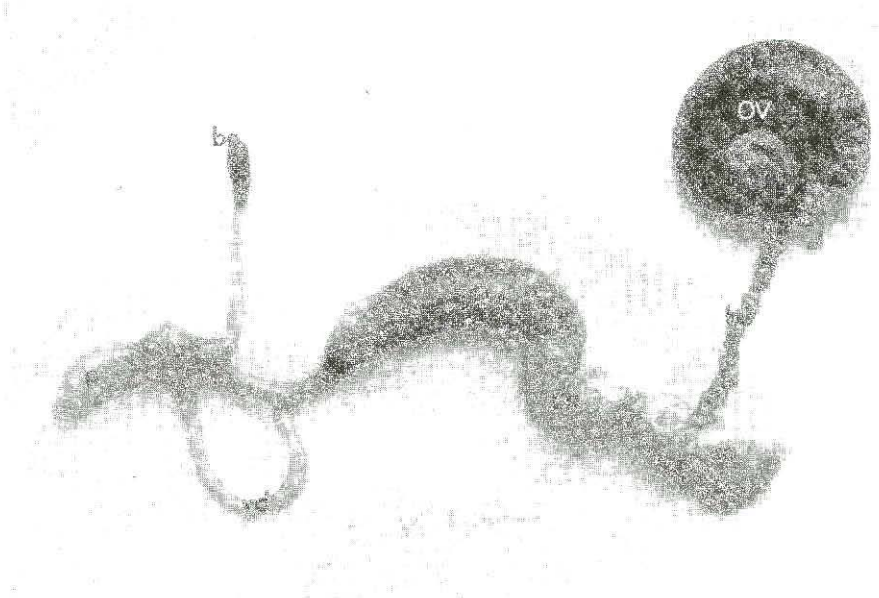


Figure 19. Reproductive system of *Achatina fulica* showing:

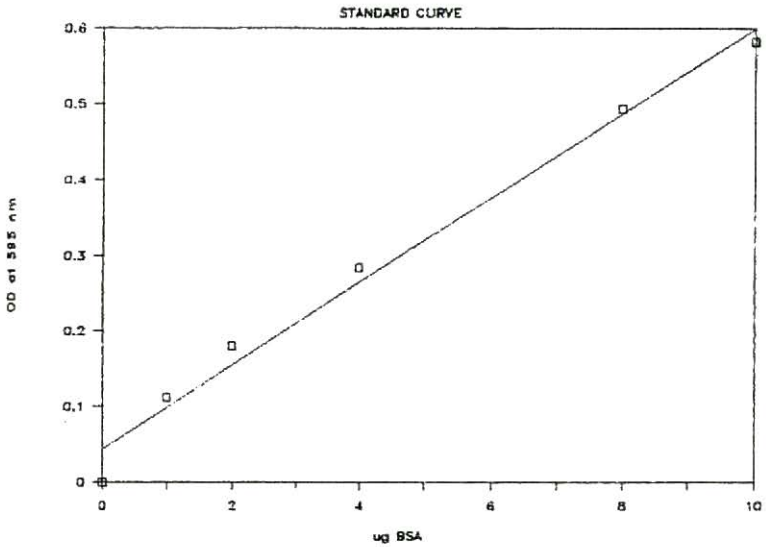
va	**– vagina**	**so**	**– spermoviduct**
p	**– penis**	**pg**	**– prostate gland**
bc	**– bursa copulatrix**	**ag**	**– albumen gland**
vd	**– vas deferens**	**hd**	**– hermaphroditic duct**
ov	**– ovotestis**		

C. *Total Protein Content*

Total protein content (TPC) of the extracts was determined using the Bradford method which was better than Lowry's method which was previously used in the preliminary analysis of the extracts. Extract from the cerebral ganglia of adult snails had a higher protein content (3.12 µg/ml) than the extract from young snails (0.67 µg/ml) (Fig. 20).

D. *Electrophoretic Pattern of the Extracts*

The protein profile as discerned by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis has no difference, whether the extracts were from the cerebral ganglia of the young or adult snails (Fig. 21). Densitometric readings of both young and adult extracts conform to these. There are eight (8) major bands (Figs. 22 & 20). The only major difference is the higher peak of the first three major bands in the adult extract. The higher total protein content and the higher peak in the densitometric reading of the adult extract possibly mean that there are proteins in the cerebral ganglia extract that are developmentally regulated; that is, a greater amount of the protein is synthesized as the snail matures.



STANDARD

X:UG BSA	y: OD (actual)	y: OD (expected)	Regression Output:	
			Constant	0.043914
			Std Err of Y Est	0.029232
0.0	0.0000	0.0439	R Squared	0.966483
1.0	0.1127	0.0995	No. of observations	6
2.0	0.1800	0.1550	Degrees of Freedom	4
4.0		0.2843	0.2661	
8.0	0.4940	0.4883	Slope	0.055553
10.0	0.5813	0.5995	Std Err of Slope	0.003251

SAMPLES

DILUTION	ABS 595	AVERAGE	µg AS 595	µG x DIL. EXTRACT	µg/ul FACTOR (µg/. 1 ml)
Young					
1:10	0.470 0.360	0.415	6.68	66.80	0.67
Mature					
1:100	0.219	0.217	3.12	311.56	3.12

Figure 20. Bradford Analysis: Achatina extracts

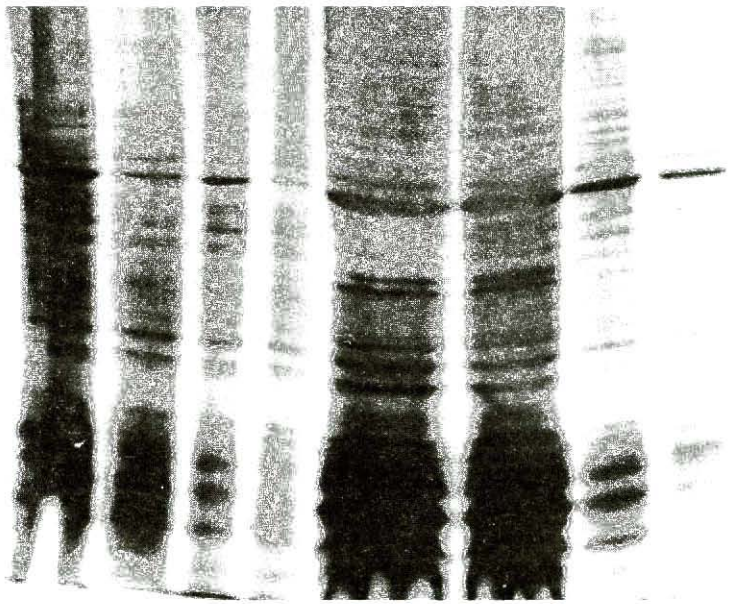


Figure 21. Protein pattern of the extracts from Achatina fulica. First four wells extracts from adult snails and the rest were extracts from young snails.

E. *Growth Increment*

Figures 24-25 and Tables 1 and 3 show the total growth increment of young snails (y) treated with young (I) and adult (II) extracts with two concentrations: V_1 - 0.5 mg/ml and V_2 = 1.0 mg/ml. Trial 1 (Fig. 24 & Table 1) represents the greater part of the rainy or wet season while trial 2 (Fig. 25 & Table 3) represents the greater part of the dry season. The two concentrations had an inhibitory effect on young snails treated with young extract, V_1 being more effective during the wet season and V_2 = during the dry season.

The total growth increment of adult snails (a) treated with young and adult extracts are shown in Fig. 26 & Table 2 (Trial 1) and Fig. 27 & Table 4 (Trial 2). V_1 inhibited the growth of the snails more effectively during the wet and dry season; V_2, on the other hand, enhanced the growth of the snails during wet season and inhibited their growth during the dry season. In the adult snails treated with adult extract, both concentrations inhibited the growth of the snails, V_2 = being more effective.

Table 5 and Fig. 28 (Trial 1) and Table 6 and Fig. 29 (Trial 2) show the average growth increment of the young (y) and adult (a) snails treated with young extract (I) of 2 concentrations (V_1 - 0.5 mg/ml; V_2 - 1.0 mg/ml). V_1 and V_2 both inhibited the growth increment of young snails, V_1 also inhibited the growth of adult snails while V_2 enhanced their growth during the wet or rainy season (Fig. 28). During the dry season V_1 and V_2 inhibited the growth of both young and adult snails, V_2 being a more effective inhibitor for young snails and V_1 for adult snails (Fig. 29).

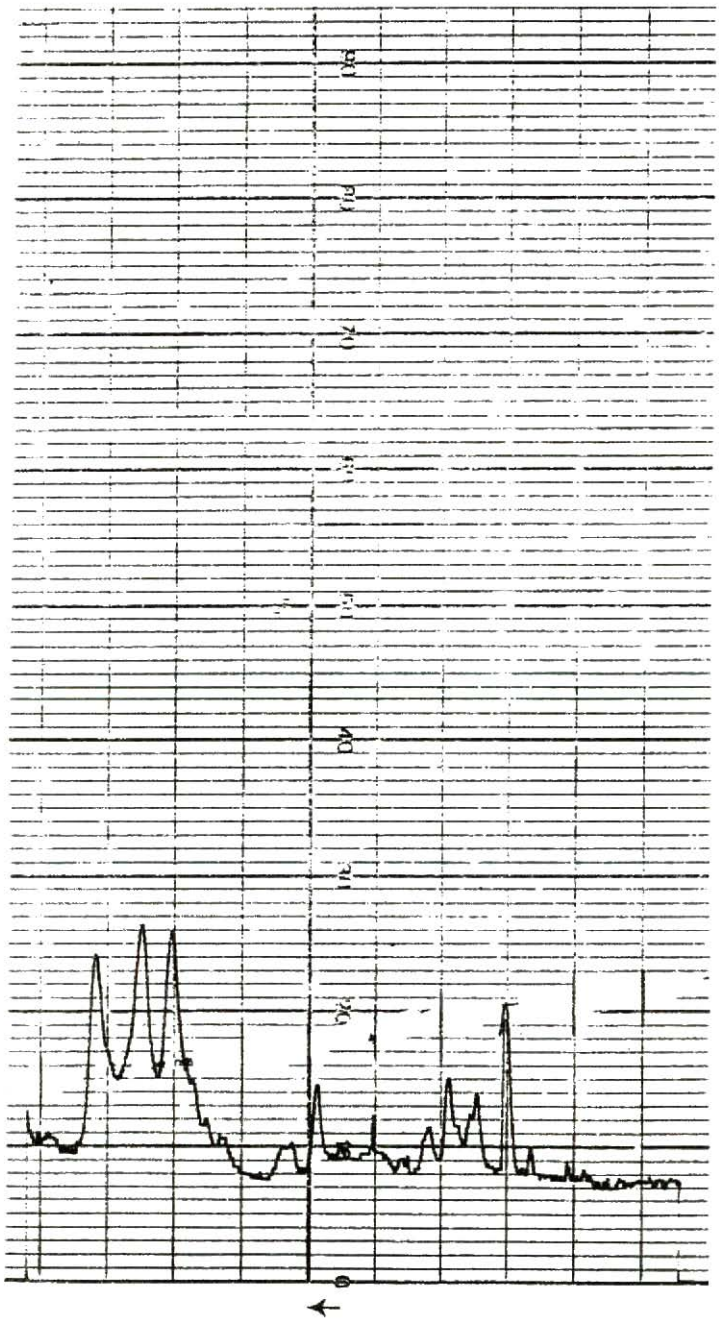


Figure 22. Densitometric reading of the protein profile of young extract.

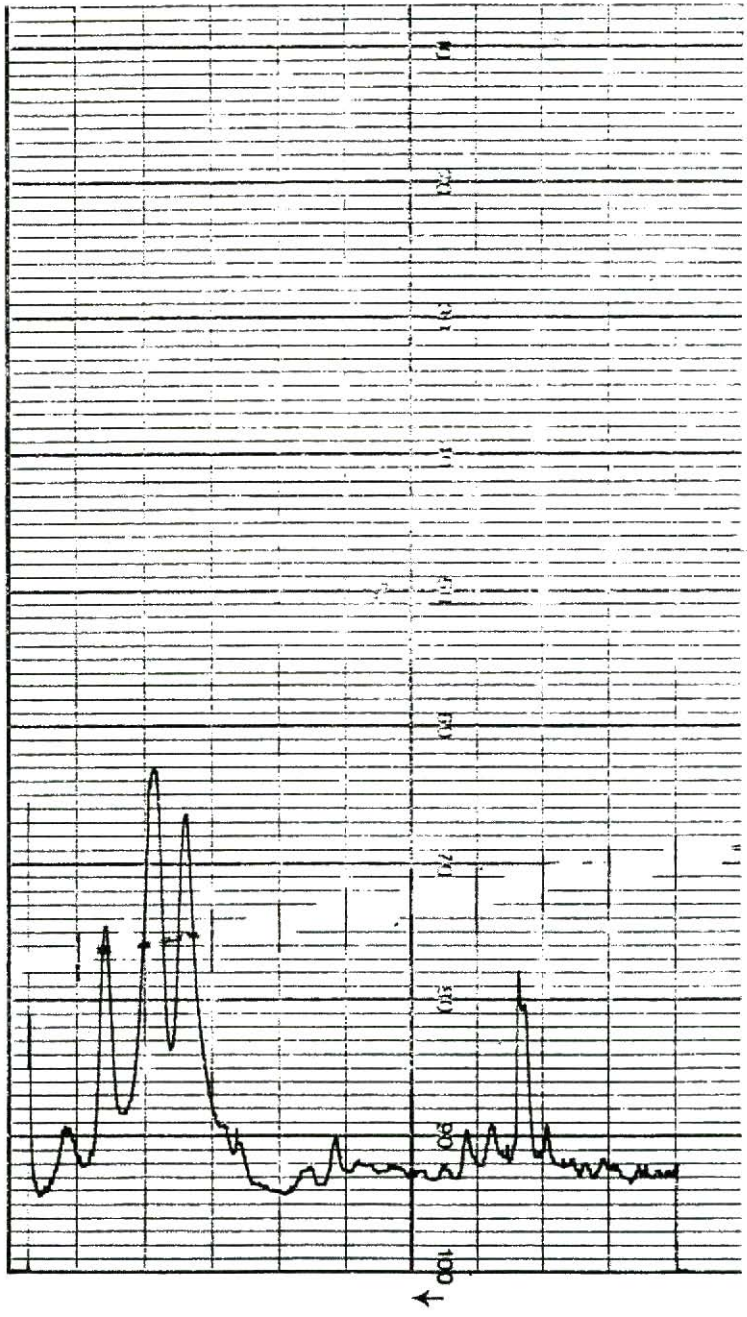


Figure 23. Densitometric reading of the protein profile of adult extract.

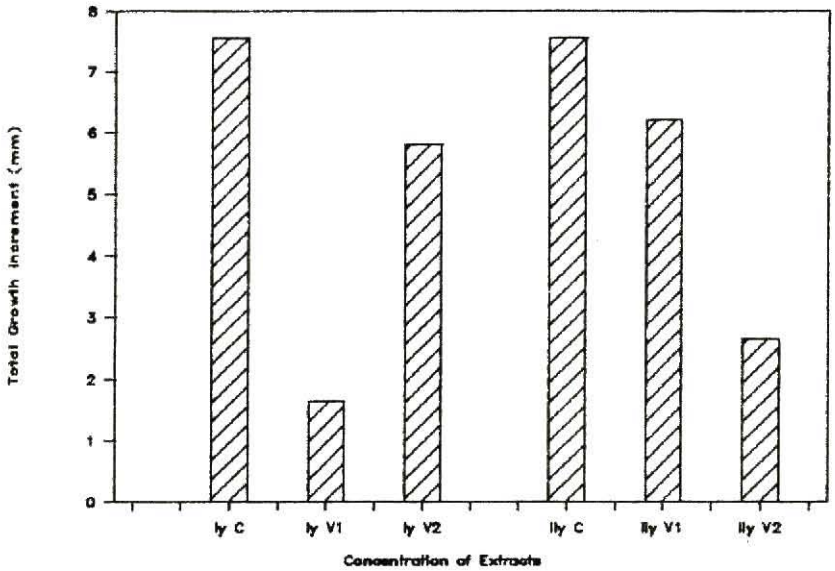


Figure 24. Total growth increment of young (y) snails treated with young (I) and adult (II) extracts (V_1 - 0.5 mg/ml; V_2 - 1.0 mg/ml) Trial 1 (May - September).

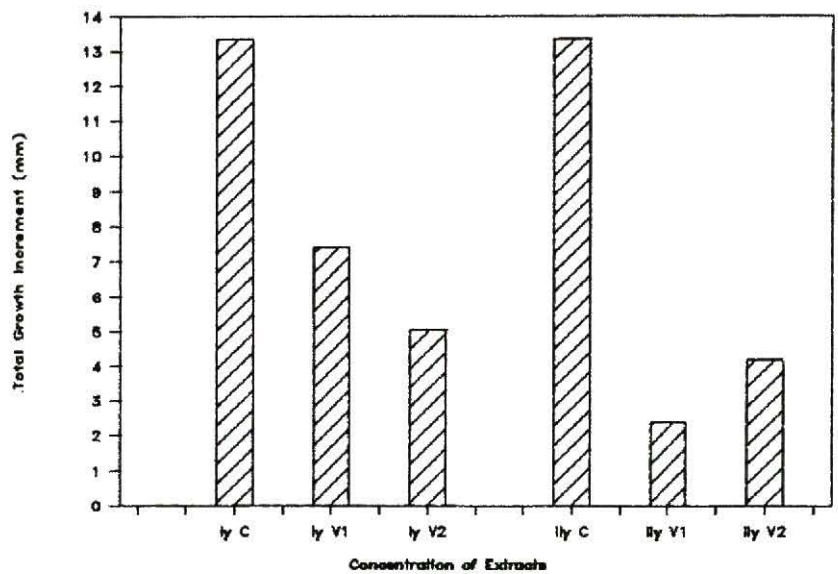


Figure 25. Total growth increment of young (y) snails treated with young (I) and adult (II) extracts (V_1 - 0.5 mg/ml; V_2 - 1.0 mg/ml) Trial 2 (December - April).

Table 1. Effects of Extracts from Young and Adult *A. fulica* (I - extract from young snails; II - extract from adult snails) on Young Snails (y - young snails) Trial 1.

Extract	**Date**							
	June 13, '91	June 28, '91	July 15, '91	Aug. 2, '91	Aug. 30, '91	Sept. 16, '92	Sept. 30, '91	Total
Iy C	2.3	2.6	1.9	0.3	0.2	0.2	0.2	7.6
Iy V1	0.6	0.2	0.3	0.3	0.2	0.2	0.1	1.7
Iy V2	2.2	1.2	2.2	0.2	0.1	0.0	0.1	5.8
	June 5, '91	June 20, '91	July 6, '91	July 22, '91	Aug. 30, '91	Sept. 16, '91	Sept. 30, '92	Total
Ily C	2.3	2.6	1.9	0.3	0.2	0.2	0.2	7.6
Ily V1	1.1	0.8	1.9	1.2	0.7	0.3	0.4	6.2
Ily V2	0.5	0.5	0.5	0.6	0.6	0.0	0.2	2.7

Table 2. Effects of Extracts from Young and Adult *A. fulica* (I - extract from young; II - extract from adult) on Adult Snails (a - adult snails) Trial 1.

Extract	**Date**							
	June 13, '91	June 28, '91	July 15, '91	Aug. 2, '91	Aug. 30, '91	Sept. 16, '91	Sept. 30, '91	Total
Ia C	1.0	0.1	0.7	0.6	0.6	0.3	0.3	3.5
Ia V1	0.3	0.3	0.2	0.3	0.1	0.2	0.1	1.4
Ia V2	1.8	1.7	1.3	0.2	0.2	0.2	0.1	5.4
	June 5, '91	June 20, '91	July 6, '91	July 22, '91	Aug. 30, '91	Sept. 16, '91	Sept. 30, '91	Total
IIa C	1.0	0.1	0.7	0.6	0.6	0.3	0.3	3.5
IIa V1	0.5	0.2	0.5	0.6	0.4	0.2	0.1	2.4
IIa V2	0.2	0.0	0.0	0.7	0.3	0.2	0.1	1.5

Table 3. Effects of Extracts from Young and Adult *A. fulica* (I - extract from young snails; II - extract from adult snails) on Young Snails (y - young snails) Trial 2.

Extract	**Date**							
	Jan. 3, '92	Jan. 16, '92	Feb. 3, '92	Feb. 17, '92	Mar. 3, '92	Mar. 25, '92	Apr. 10, '92	Total
Iy C	3.85	3.20	3.25	0.40	0.65	1.90	0.10	13.35
Iy V1	0.65	1.00	2.00	0.45	0.65	1.35	1.30	7.40
Iy V2	0.30	0.60	0.95	0.45	0.95	0.90	0.90	5.05
Ily C	3.85	3.20	3.25	0.40	0.65	1.90	0.10	13.35
Ily V1	0.35	0.35	0.20	0.25	0.50	0.30	0.45	2.40
Ily V2	0.80	0.75	0.35	0.40	0.65	0.85	0.40	4.20

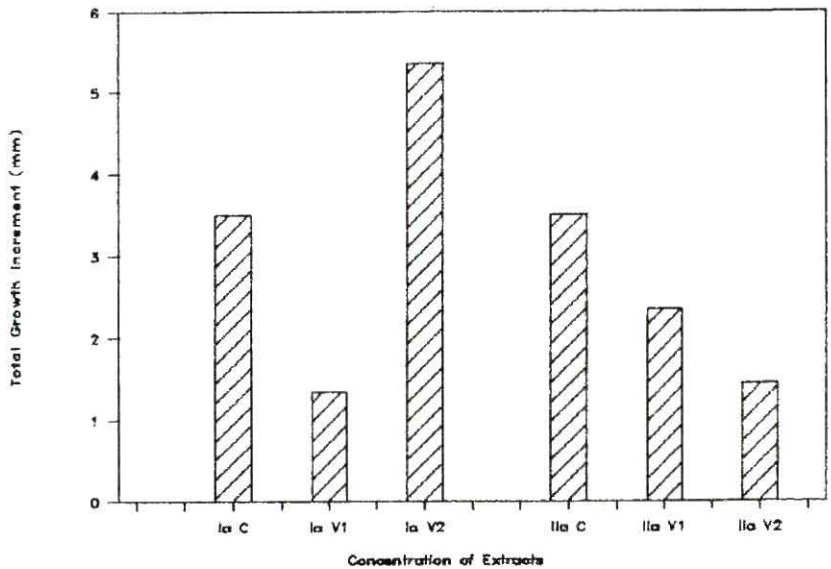


Figure 26. Total growth increment of adult (a) snails treated with young (I) and adult (II) extracts (V_1 - 0.5 mg/ml; V_2 - 1.0 mg/ml) Trial 1 (May - September).

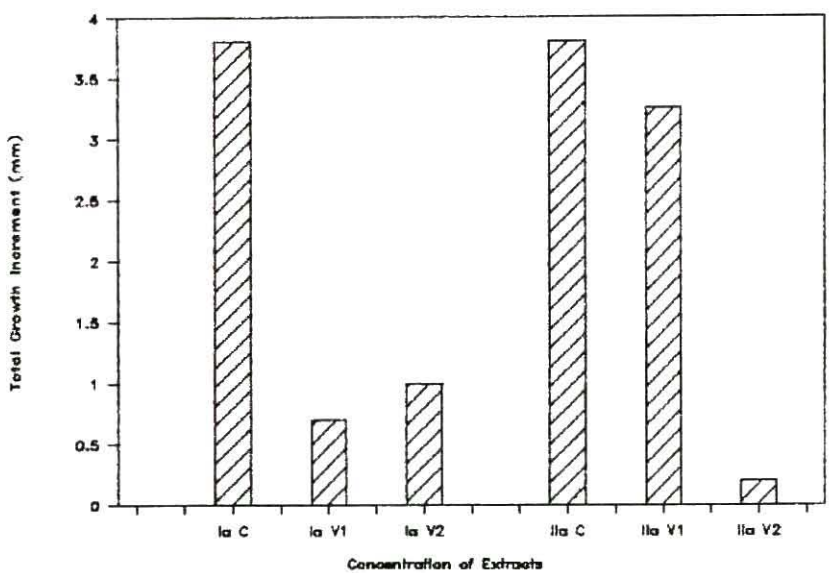


Figure 27. Total growth increment of adult (a) snails treated with young (I) and adult (II) extracts (V_1 - 0.5 mg/ml; V_2 - 1.0 mg/ml) Trial 2 (December - April).

Table 4. Effects of Extracts from Young and Adult *A. fulica* (I - extract from young snails; II - extract from adult snails) on Adult Snails (a - adult snails) Trial 2.

Extract	**Date**							
	Jan. 3, '92	Jan. 16, '92	Feb. 3, '92	Feb. 17, '92	Mar. 3, '92	Mar. 25, '92	Apr. 10, '92	Total
Ia C	1.15	0.65	0.80	0.20	0.30	0.45	0.25	3.80
Ia V1	0.15	0.10	0.20	0.15	0.10			0.70
Ia V2	0.30	0.25	0.20	0.10	0.00	0.15	0.00	1.00
IIa C	1.15	0.65	0.80	0.20	0.30	0.45	0.25	3.80
IIa V1	0.80	0.80	0.45	0.20	0.15	0.35	0.50	3.25
IIa V2	0.10	0.05	0.00	0.05	0.00	0.00	0.00	1.00

Table 5. Average Growth Increment (mm) of *A. fulica* Treated with 2 Concentrations (V_1 - 0.5 mg/ml; V_2 - 1.0 mg/ml of Cerebral Ganglia Extract from Young *A. fulica* (y - young; c - control; a - adult) Trial 1.

Extract	**Date**							
	June 13, '91	June 28, '91	July 15, '91	Aug. 2, '91	Aug. 30, '91	Sept. 16, '91	Sept. 30, '91	Total
Iy C	2.30	2.60	1.85	0.25	0.20	0.20	0.15	7.55
Iy V1	0.55	0.20	0.25	0.25	0.20	0.15	0.05	1.65
Iy V2	2.15	1.20	2.15	0.15	0.10	0.00	0.05	5.80
Ia C	1.00	0.05	0.70	0.60	0.55	0.30	0.30	3.50
Ia V1	0.30	0.30	0.20	0.25	0.05	0.20	0.05	1.35
Ia V2	1.75	1.70	1.30	0.20	0.20	0.15	0.05	5.35

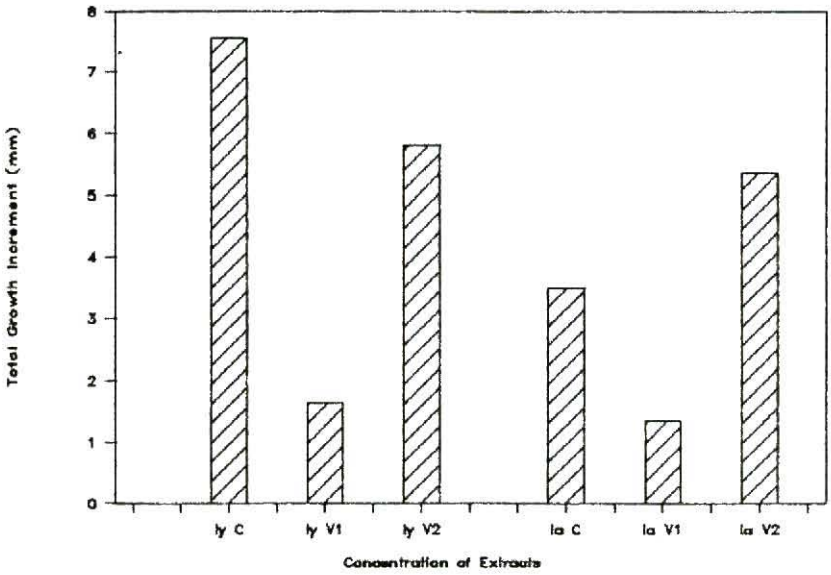


Figure 28. Total growth increment of young (y) and adult (a) snails treated with young (I) extract (V_1 - 0.5 mg/ml; V_2 - 1.0 mg/ml) Trial 1 (May - September).

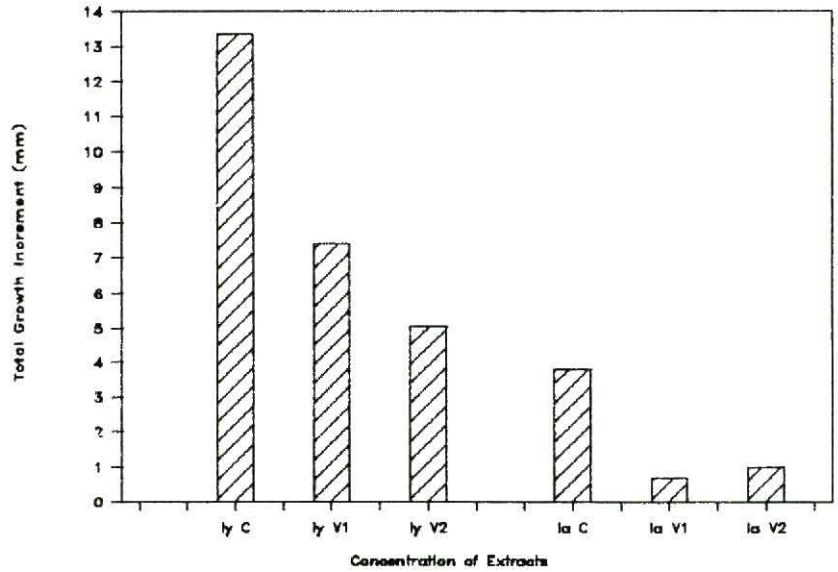


Figure 29. Total growth increment of young (y) and adult (a) snails treated with young (I) extract (V_1 - 0.5 mg/ml V_2 - 1.0 mg/ml) Trial 2 (December - April).

Table 6. Average Growth Increment (mm) of *A. fulica* Treated with 2 Concentrations (V_1 - 0.5 mg/ml; V_2 - 1.0 mg/ml) of Cerebral Ganglia Extract from Adult *A. fulica* (y - young; c - control; a - adult) Trial 1.

Extract	**Date**							
	June 6, '91	June 20, '91	July 6, '91	July 22, '91	Aug. 30, '91	Sept. 16, '91	Sept. 30, '91	Total
Ily C	2.30	2.60	1.85	0.25	0.20	0.20	0.15	7.55
Ily V1	1.10	0.75	1.90	1.20	0.65	0.25	0.35	6.20
Ily V2	0.50	0.45	0.45	0.55	0.55	0.30	0.15	2.95
Ila C	1.00	0.05	0.70	0.60	0.55	0.30	0.30	3.50
Ila V1	0.50	0.20	0.45	0.55	0.40	0.15	0.10	2.35
Ila V2	0.20	0.00	0.00	0.70	0.30	0.15	0.10	1.45

Table 7. Average Growth Increment (mm) of *A. fulica* Treated with 2 Concentrations (V_1 - 0.5 mg/ml; V_2 - 1.0 mg/ml) of Cerebral Ganglia Extract from Young *A. fulica* (y - young; c - control; a - adult) Trial 2.

Extract	**Date**							
	Jan. 3, '92	Jan. 16, '92	Feb. 3, '92	Feb. 17, '92	Mar. 3, '92	Mar. 25, '92	Apr. 10, '92	Total
Iy C	3.85	3.20	3.25	0.40	0.65	1.90	0.10	13.35
Iy V1	0.65	1.00	2.00	0.45	0.65	1.35	1.30	7.40
Iy V2	0.30	0.60	0.95	0.45	0 95	0.90	0.90	5.05
Ia C	1.15	0.65	0.80	0.20	0.30	0.45	0.25	3.80
Ia V1	0.15	0 10	0.20	0.15	0.10			0.70
Ia V2	0.30	0.25	0.20	0.10	0.00	0.15	0.00	1.00

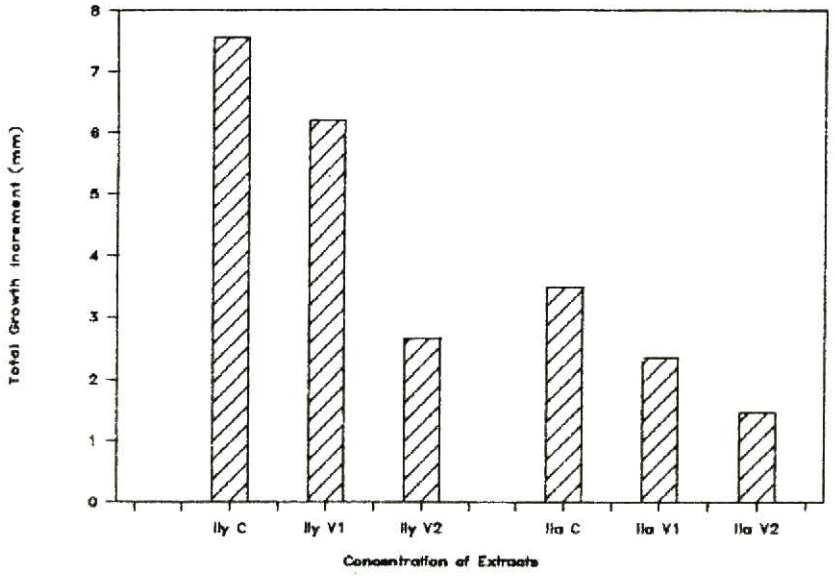


Figure 30. Total growth increment of young (y) and adult (a) snails treated with adult (II) extract (V_1 - 0.5 mg/ml; V_2 - 1.0 mg/ml) Trial 1 (May - September).

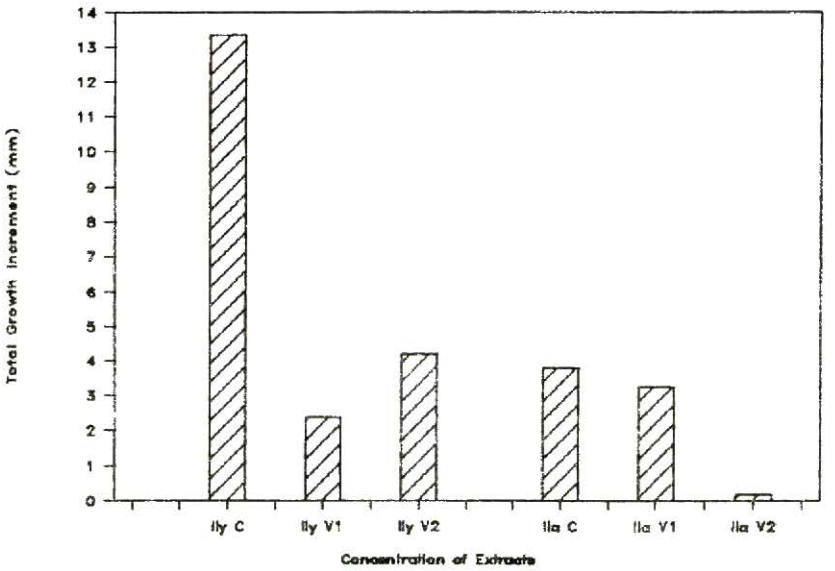


Figure 31. Total growth increment of young (y) and adult (a) snails treated with adult (II) extract (V_1 - 0. 5mg/ml; V_2 - 1.0 mg/ml) Trial 2 (December - April).

Table 8. Average Growth Increment (mm) of *A. fulica* Treated with 2 Concentrations (V_1 - 0.5 mg/ml; V_2 - 1.0 mg/ml) of Cerebral Ganglia Extract from Adult *A. fulica* (y - young; c - control; a - adult) Trial 2.

Extract	**Date**							
	Jan. 3, '92	Jan. 16, '92	Feb. 3, '92	Feb. 17, '92	Mar. 3, '92	Mar. 25, '92	Apr. 10, '92	Total
Ily C	3.85	3.20	3.25	0.40	0.65	1.90	0.10	13.35
Ily V1	0.35	0.35	0.20	0.25	0.50	0.30	0.45	2.40
Ily V2	0.80	0.75	0.35	0.40	0.65	0.85	0.40	4.20
IIa C	1.15	0.65	0.80	0.20	0.30	0.45	0.25	3.80
IIa V1	0.80	0.80	0.45	0.20	0.15	0.35	0.50	3.25
IIa V2	0.10	0.05	0.00	0.05	0.00	0.00	0.00	0.20

Figure 30 & Table 7 (Trial 1) and Figure 31 & Table 8 (Trial 2) show the total growth increment of young and adult snails treated with adult extract. V_1 and V_2 inhibited the growth of both young and adult snails with V_2 having a greater inhibitory effect during the rainy or wet season (Fig. 30). During dry season, V_1 and V_2 also inhibited the growth of young and adult snails, V_1 being more effective for young snails and V_2 for adult snails (Fig. 30).

In the natural environment, behavioral activities of terrestrial pulmonates are controlled by some factors like temperature, humidity or water in the environment and light. Of the three factors, humidity or water in the environment is intimately related to the behavioral activity of the snails. They are known to have an enormous variability in their water content which is greatly affected by the water content of the environment. During wet season or hydrated conditions such as cool weather, their water content increases with a decrease in hemolymph osmolality while during dehydrated conditions such as dry weather it decreases with an increase in hemolymph osmolality. In general, water content in the hemolymph in active pulmonates is greater than that of inactive, aestivating pulmonates. Extreme dehydration causes higher hemolymph concentration and induces hibernation (9, 16 & 19). Growth rate of snails is affected by hydration and dehydration because increase in shell length stops during times of inactivity (15).

The central nervous system of *Achatina fulica* (brain) is made up of buccal, cerebral and subesophageal ganglion-complexes which are connected with interganglional connectives. The cerebral ganglia are situated between the buccal and the subesophageal ganglia and are composed of the left and right cerebral ganglia and are connected to the subesophageal ganglia with the cerebro-pleural and cerebro-pedal connectives. Eyes and tentacles are innervated by the cerebral ganglia (21). The central nervous system is thought to control the behavioral activities of snails according to hydration and dehydration by causing drastic ionic or osmotic changes. A preliminary study done by Takeda and Ozaki (19) suggested that the osmo-receptor neurons in the central nervous system of *Achatina fulica* Ferussac change their activity according to the hemolymph osmotic pressure.

Total growth increment of young snails is higher than that of the adult snails in both control and treated (Figs. 24-27 & Tables 1-4), because the growth of snails slows down progressively as they grow older (18). The growth rate of treated snails was generally inhibited by the extracts from young and adult snails but is not dose-dependent.

The seasons (wet and dry) played a minimal role in the growth rate of both control and treated snails because they were maintained in the laboratory; thus the environment was controlled. The time of the year and the age of the snails as source of the extracts applied did not serve as a parameter for variation. The lack of consistency in the results seems to indicate that both neuroexcitatory and neuroinhibitory substances are present in the crude extracts. There may be factors other than the snail age of the source of the extract and the time of the year of treatment that influence enhancement or inhibition of growth.

Conclusion and Recommendation

Cerebral ganglia extract generally inhibited the growth of *A. fulica.* The not-so-consistent result may be due to the fact that the extract was not purified. It is possible that the bioactive substance may not be effective if not purified and applied directly to the neurons. The amount of the extract might be too little to elicit such effects, in the isolation of a tetrapeptide from the cerebral ganglia of *A. fulica* they were able to isolate only 5 mg of Achatin-I from 30,000 snails (11).

It is recommended that the extract be purified before it is applied to live specimens and further characterization of the extract must be employed.

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