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MEMORANDUM

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SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 3. F-50
First Annual Report dtd May 85
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Attachments

2

3. F-50

FIRST ANNUAL REPORT

ON

BIOLOGICAL CONTROL OF SCHISTOSOMIASIS TRANSMITTING SNAILS IN SOUTHEAST ASIA

SUBMITTED TO

OFFICE OF THE SCIENCE ADVISOR
PROGRAM IN SCIENCE AND TECHNOLOGY COOPERATION

BY

DR. SUCHART UPATHAM
DEPARTMENT OF BIOLOGY, FACULTY OF SCIENCE
MAHIDOL UNIVERSITY
RAMA 6 ROAD
BANGKOK 10400
THAILAND

GRANT NUMBER 936-5542
MAY 1985

FIRST ANNUAL REPORT

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PARTICIPANTS IN THE PROJECT

Principle investigator

Suchart Upatham, Ph.D.

Co-investigator

Vithoon Viyanant, Ph.D.

Maleeya Kruatrachue, Ph.D.

Nouwarath Sukhapanth, M.S.

Viroon Baidikul, M.S.

Suksiri Vichasri, M.S.

Laboratory assistants

Vimolsri Manasarn, B.S.

Jinrapa Phothikasikorn, B.S.

Boonsong Prayoonwiwat, B.S.

Kittisak Trisin

Surasak Kaewharn

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I. BACKGROUND

The award of the project entitled "Biological Control of Schistosomiasis-transmitting Snails in Southeast Asia" was presented by Mr.John Gunther Dean, Ambassador of the United States of America to Thailand, to Professor Dr. Natth Bhamarapravati, Rector of Mahidol University, at the Rockefeller Foundation Conference Room, Faculty of Science, Mahidol University, on March 5, 1984. The first payment of the grant was made available to the Faculty of Science in early April 1984.

II. PROJECT OBJECTIVE

The overall objective of this research project is as follows:

1. to study the efficacy of biological control of snails transmitting schistosomiasis in Southeast Asia using biological agents, and

2. to study genetic variability of snails transmitting schistosomiasis in Southeast Asia in relation to degrees of parasite compatibility and susceptibililty.

III. EXPERIMENTAL GOALS

1. Laboratory experiments. These studies will include the biological aspects of insects and snail agents and their efficacy against the target snail species. The life histories of insects and snail agents will be studied and various doses of biological agents and target species will be

tested. This phase of study will take approximately 16 months.

2. Simulated field experiments. Biological agents and target species will be tested in simulated field habitats. This phase of study will take approximately 8 months.

3. Genetic and susceptibility studies. Genetic variability of snails transmitting schistosomiasis in relation to degrees of parasite compatibility and susceptibility will be studied under laboratory conditions. This phase of study will take approximately 3 years.

4. Small scale field experiments. Pending the results of "goals" one and two as delineated above, a small scale field trial will be initiated. These experiments will include only local target species. This last stage of study will take approximately one year.

IV. RESEARCH FINDINGS

1. Biological control of snails

1.1 Mollusks

1.1.1 Biology of biological agents (mollusks)

Four species of snails from Thailand were used to test their efficacy against target snails; these were *Brotia costula costula* (Prosobranchiata : Thiaridae), *Tarebia (Thiara) granifera* (Prosobranchiata : Thiaridae), *Melanoides tuberculata* (Prosobranchiata : Thiaridae) and *Pila ampullacea* (Prosobranchiata : Ampullaridae).

Brotia costula costula

B.c. costula is the largest representative of the family in Thailand. They occur in Southeast Asia, in countries such as Thailand, Laos, Cambodia, Burma and India. They can be found in rivers and mountain creeks with running water, rarely in still water (Brandt, 1974).

B.c. costula is a very large snail, the largest of the family Thiaridae. Adults may vary in shell length from 5.5-7.4 cm. Figure 1 shows the photograph of adult *B.c. costula* collected from the field. The shell is elongately turreted, solid to thick, covered with a dark brownish or olive-brown periderm. The apex is generally truncate. The shell is sculptured with numerous spiral grooves which are weaker on the upper half of the body whorl. The animal ribs are more or less strongly developed. The shells are brown with 1-3 dark brown spiral bands. The operculum is almost circular, with 5 whorls. The animal is dark grey with orange or yellow pigment spots (Brandt, 1974).

B.c. costula is ovoviviparous and parthenogenetic. Males consist about 3% of the population. They are non-functional and the male reproductive organs seem to have no functional gonads. The females have many small, conical embryonic shells in the brood pouch. The young are reared in the brood pouch and are usually 0.2-0.3 cm long when born. Adult size or maturity is reached within 18-24 months. Several young are produced daily during the dry season

(December-April) and gradually decreased in number. *B.c. costula* feeds on diatoms, algae and detritus and does not harm growing vegetation. They prefer the flowing freshwater streams with the temperature around 25 ^{o}C.

B.c. costula shares many of the characteristics of *Tarebia (Thiara) granifera* and lives in the same type of habitat. It is considered to be another potential biological control agent. However, one species of *Brotia, B. asperata* had been reported to be the intermediate host of the human lung fluke, *Paragonimus westermani* in the Philippines (Cabrera and Vajrasthira, 1973).

Tarebia (Thiara) granifera

T. granifera is found in abundance in almost all provinces in Thailand. They occur in S.E. Asia, S. China, India, Sri Lanka, Indonesia, Philippines and numerous Western Pacific Islands. It is common in lakes, ponds, rivers, canals and creeks (Brandt, 1974).

T. granifera is a moderate-sized snails. Adults may vary from 1.5 to 3 cm. in shell length, with the common size of 2.5 cm. (Fig. 2). The shell is elongated, brownish or olive-colored, sculptured with several spiral rows of beads or blunt tubercles. The shell apex is pointed but often eroded. The body whorl is large and measures about half the length of the shell. The operculum is thin, opaque and blackish brown in color. The animal is grey with yellow and blackish pigmentation (Tucker Abbot, 1952).

T. granifera is ovoviviparous and parthenogenetic. Males are non-functional. The females have subhaemocoelic brood pouch. The young are reared in the brood pouch and is 0.2-0.3 cm. when born. The young reach maturity within 12-18 months. Several young are produced daily and the snail is capable of maintaining very high densities in a habitat over long periods of time. *T. granifera* feeds on diatoms, algae and detritus and does not harm growing vegetation.

T. granifera can act as the first intermediate host of the human lung fluke, *Paragonimus westermani*. However, man contracts parogonimiasis only incidentally by eating the raw or undercooked second intermediate host (various species of freshwater crustaceans) of *P. westermani*. However, this need not necessarily detract *T. granifera* from its usefulness as an effective biocontrol agent of snails. *T. granifera* has been reported to be an effective biocontrol agent for *Biomphularia glabrata* in Puerto Rico (Hairston et al., 1975), St. Lucia (WHO document, 1981). Laracuente et al. (1979) had done a preliminary study which showed that various species of aquatic snails could be used as decoys to intercept schistosome miracidia and prevented them from reaching their snail intermediate host. *T. granifera* has shown to be one of the slightly effective decoy for *Schistosoma mansoni* miracidia infecting *B. glabrata* especially in the laboratory i.e. caused slight reduction in the proportion of *B. glabrata* infected by miracidia of *S. mansoni*.

Melanoides tuberculata

M. tuberculata has a very wide geographical range, from E. Europe through Africa and Asia to the West Pacific Islands and Australia. This species is found in all provinces of Thailand in abundance. Its habitats range from lakes, ponds, rivers, brooks, mountain creeks and are abundant in the tidal areas (Brandt, 1974).

M. tuberculata is a small snail, with the average size of adult ranging from 1.5-2.0 cm. (Fig. 3). The shell is moderately thick, elongate, covered with a brownish, yellowish or olive periderm ; and sculptured with many narrow spiral ridges which are often crossed by obtuse ribs. The animal itself is dark grey with yellowish pigment spots.

M. tuberculata is ovoviviparous and parthenogenetic. The males contain reproductive organs without functional gonads. The young snails are reared in the brood pouch of the female and are 0.8 mm. long when born. Maturity is reached within 6-7 months. Young snails are produced daily but the number is much fewer than those produced by *T. granifera* or *B.c. costula*. *M. tuberculata* also feeds on diatoms, algae and detritus and does not harm growing vegetation.

M. tuberculata has been reported to act as intermediate host for *Paragonimus westermani*. However, no naturally infected snails have been found in Thailand. *M. tuberculata* has been reported to be the biological control agent of *B.*

glabrata on the island of Martinique, West Indies (Pointier, personal communication).

Pila ampullacea

P. ampullacea occurs widely in S.E. Asia such as Thailand, Malaysia, Indonesia, Borneo, Laos, Cambodia and Vietnam. In Thailand, it is found in almost all provinces, inhabitats in ponds, rivers, lakes and creeks (Brandt, 1974).

P. ampullacea is a very large snail, with the shell length ranges from 6-10 cm. Figure 4 shows the photograph of adult *P. ampullacea* with the shell length of 6 cm. The shell is subglobose with low conical, or almost flat spire. The body whorl is large and inflated. Periderm is unicolored of olive-green or with greenish or brown spiral bands. The operculum is thick, with greyish white nacre on the inner surface.

P. ampullacea is oviparous. The eggs are laid in white mass attached to mud or rock surface. The young reaches maturity within 12-18 months. *P. ampullacea* harbors metacercariae of *Schinostoma ilocanum*, serves as important intermediate host for the rat's lung worm *Angiostrongylus cantonensis*. People can be infected by eating raw or uncooked meat of *P. ampullacea*. However, it can be considered as a potential biological agent due to its large size and its ability to feed on wide range of food such as algae, detritus and fish food.

1.1.2 Cultivation of biological agents (molluska).

Four species of snails from Thailand were used to test their efficacy against target snails; these were *Brotia costula costula*, *Tarebia (Thiara) granifera*, *Melanoides tuberculata* (Prosobranchiata : Thiaridae), and *Pila ampullacea* (Prosobranchiata: Ampullaridae). These snails are being cultured in the laboratory of the Center for Applied Malacology and Entomology. In general, these snails are cultured with the same technique. *B.c. costula* were collected from Kanchanaburi Province, Northeast Thailand, while *T. granifera*, *M. tuberculata* and *P. ampullacea* were collected from Kasetsart University, Bangkok, Thailand.

The aquaria for maintenance of the four species of snails were round glass aquaria with the diameter of 24 cm. and with the height of 18 cm. They were equipped with aeration pumps, plastic tubes and air stones. Snail cultures were maintained under 8 hr. of 40-watt cool fluorescent lamps per day.

Aged tap water which had been left standing for at least 2 days, was used for rearing snails. The room and water temperatures were maintained at 27-28 ^{o}C. Water levels in snail containers, aeration, water and room temperatures were checked daily.

All four species of snails required substrate such as mud or sand. For *B.c. costula*, the fine sand brought from the snail collecting site was used as snail

substrate. The sand had been put in the oven and baked for 4 hr. at 160 ^{o}C before use. The thick layer of sand of about 1 inch deep was laid in the glass aquaria and then filled with aged tap water up to 1/2 of its height. Twenty adult snails were introduced into each aquarium. The snails were fed with lettuce leaves.

For *T. granifera* and *M. tuberculata*, dried mud powder which had been put in the oven at 160 ^{o}C for 4 hr. was used as snail substrate. The mud powder was put into an aquarium just to fill its bottom (about 1 cm. thick). The container was then filled with aged tap water up to 2/3 of its height. Thirty adult snails were introduced into each aquarium. The snails were fed with ground lettuce leaves.

For *P. ampullacea*, rich brown top soil collected from rice fields or river bank was used to make substrate on the bottom of the aquarium in the form of a mud slope of about 5-6 cm. in height. A few pieces of rocks were placed on the mud slope to serve as egg-laying sites for the snails. The container was filled with aged tap water approximately 2 cm. above the top of the mud slope. Ten adult snails were introduced into each aquarium. The snails were fed with fish food and fresh lettuce leaves.

1.1.3 Growth rates of snail agents

B.c. costula, *T. granifera* and *M. tuberculata* are ovoviviparous (bear young). Approximately 1-2 days after the introduction of adult snails to the breeding

cultures, the young snails emerged and they were removed to another culture to prevent the aquarium from being overcrowded. The young snails were fed with ground lettuce leaves supplemented with diatoms. The shell lengths of the young snails were measured once a week. These measurements provided data necessary for the growth rates of the snails.

P. ampullacea are oviparous (bear eggs). The white egg masses were laid on rocks or attached to the inner side of the aquaria. The eggs hatched and the young snails emerged in 12-14 days. The shell lengths of the young snails were measured once a week.

For *M. tuberculata*, the data for snail growth has been completed. The newly emerged young snails were 0.8 mm in shell length, and the full grown snails were 12.8-13 mm long. The average shell growth was 0.5 mm per week and it had taken the young snails 25 weeks to become fully mature and start to reproduce (Table 1).

For *T. granifera*, *B.c. costula* and *P. ampullacea*, the data for snail growth have been completed for the 12 month period and are still in progress. For *T. granifera*, the newly emerged young snails were 0.2 cm in shell length. The mean shell growth per month for the 12-month period was 0.14 cm (Table 2).

For *B.c. costula*, the newly emerged young snails were 0.25 cm in shell length and the mean shell growth per month for the 12-month period was 0.2 cm (Table 3). For

P. ampullacea, the newly emerged young snails were 0.4 mm in shell length and the mean shell growth per week for the 12-month period was 0.36 cm (Table 4).

1.1.4 Experimental work on biological control of mollusks in the laboratory.

The study is now being in progress in the laboratory. Various numbers of mollusk biological agents and target species are being tested to determine the efficacy of the agents in terms of competition, predation or suppression of reproductive potential of the target species.

The 24-cm. diameter glass aquaria are used with the water depths of 15 cm. The ratio between biological agent and target species is as follows:

Control agent	Target species
0	40
10	30
20	20
30	10
40	0

Control agents are *T. granifera* and *M. tuberculata*

For B.c. costula and *P. ampullacea* being control agents, the following ratio has been used.

Control agent	Target species
0	20
5	15

10	10
15	5
20	0

The general procedure, observation on mortality rates of both control and target species is done daily. The original snails are marked with finger nail polish so that the offspring can be distinguished from them. The number and shell growth of offspring were counted and measured once every two weeks.

The following lists of control and target species of snails on the biological control study have been completed in the laboratory.

Control agent	Target species
Tarebia (Thiara) granifera	*Oncomelania h. quadrasi*
	Bithynia s. goniomphalos
	Tricula aperta (beta race)
	Indoplanorbis exustus
	Robertsiella kaporensis
Melanoides tuberculata	*O.h. quadrasi*
	T. aperta
	R. kaporensis
Brotia c. costula	*B. glabrata*
	O.h. quadrasi
	B.s. goniomphalos
	T. aperta

I. exustus

Pila ampullacea *O.h. quadrasi*

Tarebia granifera

The biological control study between *T. granifera*, being the control agent, and *O.h. quadrasi* (Prosobranchiata : Hydrobiidae), being the target species has shown that (Table 5, Fig. 13) (1) During the first 6 weeks, *T. granifera* has increased remarkably in number due to the ovoviviparity of the snails. (2) After the sixth week, the number of young *T. granifera* has dropped which may be due to the overcrowding of snails in one container. (3) While the target species *O.h. quadrasi* alone reproduced a large number of offsprings, those living with *T. granifera* did not reproduce and the mortality rates seemed to increase. (4) Within 16 weeks from the beginning of the experiment, there is larger number of control species, *T. granifera* over the target species, *O.h. quadrasi.*

The biological control study between *T. granifera*, being the control agent and *B.s. goniomphalos* (Prosobranchiata : Bithyniidae), being the target species has shown that (Table 6, Fig. 14), (1) During the first six weeks, the number of *T. granifera* has increased remarkably, decreased after the sixth week and remained relatively constant until the end of the experiment. (2) Container with *B.s. goniomphalos* alone yielded few young snails while those with *T. granifera* and

B.s. goniomphalos did not yield any young *B.s. goniomphalos*. (3) Within 16 weeks from the beginning of the experiment, there was larger number of control species, *T. granifera* over the target species, *B.s. goniomphalos*.

The biological control study between *T. granifera*, being the control agent, and *R. kaporensis* (Prosobranchiata : Pomatiopsidae) revealed the following results (Table 7, Fig. 15). (1) The number of *T. granifera* has remarkably increased upto the sixth week, then decreased and remained contant until the end of the experiment. (2) The target species, *R. kaporensis* did not have any eggs. (3) Within 16 weeks, there was larger number of control species, *T. granifera* over the target species, *R. kaporensis*.

The biological control study between *T. granifera*, being the control agent, and *T. aperta* (Prosobranchiata : Pomatiopsidae) has revealed very similar results to those with *R. kaporensis* (Table 8, Fig. 16) in the forementioned paragraph. The number of *T. granifera* has fluctuated during the whole experiment, but still remained in much larger number over the target species, *T. aperta*, at the end of the experiment.

The biological control study between *T. granifera*, being the control agent, and *I. exustus* (Pulmonata : Planorbidae), being the target species revealed the following results (Table 9, Fig. 17), (1) During the first four weeks, the number of control agent, *T. granifera* has increased

remarkably. (2) After ten weeks, the number of *T. granifera* has dropped considerably and almost died out at the end of the experiment. (3) The number of target species, *I. exustus* also increased up to the sixth week, then started to decline and remained constant except for the container of 10 *T. granifera* and 30 *I. exustus*, in which *I. exustus* were all dead after ten weeks. (4) There was larger number of the target species, *I. exustus*, over the control species, *T. granifera*.

Based on the results obtained from these experiments, it is apparent that the pulmonate snails are more difficult to control than the prosobranch snails since the pulmonates are hermaphrodite and could lay large numbers of egg masses while the prosobranchs have separate sexes. *T. granifera* is very difficult to colonize in the laboratory. They usually could produce a large number of youngs for a certain period of time but the young snails have very high mortality rates. This may be due to the limitation of space or overcrowding of snails. It should be noted that in the experiments betweeen *T. granifera* and the target prosobranch snails (*O.h. quadrasi*, *B.s. goniomphalos*), the target species living together with the control did not reproduce any offsprings. There may be a suppression in reproductivity exerted on the target species by the control species, *T. granifera*. This phenomenon will be investigated in the field simulated experiments, in which larger number of snails will be used and a longer period of time will be required.

Melanoides tuberculata

The biological control study between *M. tuberculata*, being the control species and *O.h. quadrasi*, being the target species, revealed the following results (Table 10, Fig. 18), (1) During the first six weeks, there was a great increase in numbers of offsprings of *M. tuberculata*. After that, the number has dropped slightly and remained about the same until the end of the experiment. (2) Only the container with *O.h. quadrasi* alone produced offsprings while those with *M. tuberculata* did not reproduce. (3) Within 16 weeks, there was larger number of the control species, *M. tuberculata* over the target species, *O.h. quadrasi*.

The biological control study between *M. tuberculata* and *T. aperta* revealed the following results (Table 11, Fig. 19), (1) The number of *M. tuberculata* has gradually increased upto the tenth week, then decreased or fluctuated in some containers until the end of the experiment. (2) The number of *T. aperta* was more or less constant during the whole experiment. (3) The number of control species, *M. tuberculata* was larger than that of *T. aperta* after 16 weeks.

The biological control study between *M. tuberculata* and *R. kaporensis* revealed the following results (Table 12, Fig. 20), (1) Neither the control nor target species had produced any offsprings. (2) During the whole experiment, no sign of competition or interaction was observed.

Further studies to complete the laboratory investigation on *M. tuberculata* and other target species are underway. In addition, the experiments using field simulated conditions will be investigated. It will be toc early to draw any conclusion at this stage.

Brotia costula costula

For the biological control experiments with *B.c. costula* as the control agent, we had decided to use young *B.s. costula* of 3-4 cm in size (the adult *B.c. costula* is 6-7 cm) due to the very small size of the target species. Thus, there would be no reproductivity of *B.c. costula* during the whole experimental period.

The biological control study between *B.c. costula* and *O.h. quadrasi* (Table 13, Fig. 21) has revealed that there seem to be no interference between the control and target species. This may be due to the difference in habitats of the two species; *B.c. costula* are aquatic and are at the bottom of the container while *O.h. quadrasi* are amphibious and usually attached to the damp filter paper which lined the sides of containers.

The biological control study between *B.c. costula* and *B.s. goniomphalos* (Table 14, Fig. 22) has shown that there appeared to be no competition between the control and target species. However, only the container of *B.s. goniomphalos* alone produced few offsprings, while those with *B.c. costula* did not produce any offspring.

The biclogical control study between *B.c. costula* and *T. aperta* (Table 15, Fig. 23) has revealed very similar result to those with *O.h. quadrasi* except that *T. aperta* had higher mortality rate than *O.h. quadrasi*.

The biological control study between *B.c. costula* and *B. glabrata* (Pulmonata : Planorbidae) revealed the following results (Table 16, Fig. 24), (1) During the first six weeks, no sign of competition was observed between the control and target species except that the control species, *B.c. costula* started to decrease in number. At the same time, the target species, *B. glabrata* started to lay large numbers of egg masses. (2) Between 5-8 weeks, the eggs were hatched and the young snails started to increase in large numbers. (3) Within 16 months, the number of the target in each container was larger than that of the control species.

The biological control study between *B.c. costula* and *I. exustus* revealed very similar result as that between *B.c. costula* and *B. glabrata* (Table 17, Fig.25) except that the numbers of offspring produced by *I. exustus* were fewer than those produced by *B. glabrata*.

It is apparent that we could not draw any conclusive results from these experiments since we used snails too young to reproduce. However, *B.c. costula* in nature or raised in cement tanks with sand substrate could produce several young daily. These experiments will be repeated using the field simulated conditions. Larger number and full-grown snails

will be used and the experimental period will be much longer than that in the laboratory.

From the results, it is apparent that the pulmonate snails (*I. exustus*, *B. glabrata*) are more difficult to control than the prosobranch snails. This is due to the hermaphroditic nature of pulmonates, their ability to lay large numbers of eggs daily and shorter period of time to become mature. *B.c. costula*, even though could produce large numbers of young, requires 1 -2 years to become fully mature due to the very large size of the snails. In addition, there is a difference in the habitats of the control and target snails. The pulmonate snails float on the surface of water while *B.c. costula* remain at the bottom of the container most of the time. However, the experiments using field simulated control conditions are now being investigated.

Pila ampullacea

For the biological control experiments with *P. ampullacea* as the control agent, young *P. ampullacea* (3-4 cm in size) were used. Hence, there would be no reproductivity of *P. ampullacea* during the whole experiment.

The biological control study between *P. ampullacea* and *O.h. quadrasi* revealed the following results (Table 18, Fig. 26), (1) *P. ampullacea* appeared to have lower mortality rate than *O.h. quadrasi*. (2) *O.h. quadrasi* did not produce any offspring. This may be due to the very low number of snails which was insufficient to produce any eggs.

Another biological control study between *P. ampullacea* and the pulmonate snail, *B. glabrata* is now being investigated under the laboratory conditions. It appeared that the effect of *P. ampullacea* on *B. glabrata* could be as an incidental predator on the egg masses of *B. glabrata*. Further study especially using the field simulated conditions is underway to investigate these interactions.

1.2 Insects

1.2.1 Cultivation and growth rates

Adults of *Sepedon plumbella* were collectd from natural habitats. A pair of male and female sciomyzid flies was reared in a 2000-ml glass jar containing a batch of rice plants. These rice plants were used as resting and oviposition sites in the breeding jars. Cotton wool was used in place of sphagnum moss to provide a moisture-retaining substrate in the breeding jars. 20 pairs were used for this study.

The adult insects were fed with 3 types of food : (1) crushed flesh of *Radix rubiginosa* snail, (2) crushed flesh of *R. rubiginosa* snail and honey bee syrup, (3) crushed flesh of *Indoplanorbis exustus*, and (4) crushed flesh of *I. exustus* and honey bee syrup.

The results are shown in Tables 19 and 20. The life durations of eggs, larvae, pupae and adults were 2.5, 13.5, 9.0 and 33.0 days respectively when they were fed

with crushed flesh of *R. rubiginosa* and honey bee syrup. The oviposition period was 139.5 days, and a female laid approximately 1069.8 eggs. The life span of male and female flies was 138 and 129 days respectively (Table 19). The mean numbers of eggs laid per female fly were 489.5, 1069.8, 392.7 and 395.7 respectively when they were fed with flesh of *R. rubiginosa* alone, with flesh of *R. rubiginosa* and honey bee syrup, with flesh of *I. exustus* alone, and with flesh of *I. exustus* and honey bee syrup (Table 20). The survival rates from egg to adult were higher in groups of flies fed with flesh of snails and honey bee syrup than those fed with flesh of snails alone (Table 20).

1.2.2 Experimental work on biological control of mollusks in the laboratory

Sciomyzid fly larvae were used as biological control agent against pulmonate and operculate snails in the ratios of 4:0, 3:1, 2:2, 1:3, and 0:4, as follows:

Ratios of sciomyzid fly larvae to snails				
4:0	3:1	2:2	1:3	0:4
Numbers of sciomyzid fly larvae to snails				
40:0	30:10	20:20	10:30	0:40

Each experiment was replicated three times.

Table 21 shows results of biological control study between sciomyzid fly larvae and snails. It can be summarized as follows: (1) the sciomyzid fly larvae

were effective in controlling pulmonate snails only, (2) it was better to use the third stage sciomyzid fly larvae because its survival rate was higher than the second stage, and (3) the ratio of 3 sciomyzid fly larvae to 1 snail rendered the best result.

1.3 Invertebrate (unknown)

An unknown invertebrate species was found in north Thailand. Preliminary observation has indicated that they predate on both pulmonate and operculate snails (Fig. 31). Detail experimental work is in progress.

2. Enzyme analyses of various populations of *Oncomelania hupensis quadrasi* in comparison with *O.h. hupensis*, *Tricula aperta* and other related species.

O.h. quadrasi snails were collected from Irosin, Mindoro and Leyte Islands in the Philippines. *O.h. hupensis* were obtained from Anhui Province, People's Republic of China. *T. aperta* alpha and gamma races were collected from Mekong River along the Thai-Laos border and beta race from Mun River in Ubon Ratchathani Province, Thailand. *T. bollingi* and *Robertsiella kaporensis* were collected from Chiangmai Province, Thailand and from Pahang State, Malaysia respectively. Two additional species of *Tricula*-like collected from Phitsanuloke and Kanchanaburi Provinces, Thailand were also included in this study. These snail species have been found to be the host of human or animal schistosomes or both.

2.1 Preparation of specimens for enzyme electrophoresis

Only field collected snails of similar size were used in this study. Snails were ground individually in 0.1% glycine in ice-bath using small tissue grinder and sonicated 3 times at 75 W for a total of 60 sec (Lab-Line Instrument, Inc.). The snail suspensions were centrifuged at 5000 rpm for 30 min. at 4-6 ^{o}C. Only fresh preparations of the clear snail suspensions were used in the study.

2.2 Isoelectricfocusing of the enzyme types

The LKB multiphor system (LKB-Produkter, Sweden) was used in this study. Isoelectricfocusing of the enzyme types was performed on thin layers of 4.8% polyacrylamide gel (0.2 mm) using Ampholine as carrier ampholytes. The enzymes were separated on either pH 3.5 to 20 or 5 to 8 gels. The homogenate of individual snails was absorbed on 4 x 10 mm Whatman-fliter paper No. 3 and placed on the gel slab with about 5 mm apart. Snail extracts were electropheresed on the same gel for good accuracy in comparison between population and species. The gel was pre-run at 50 mA for 45 min. before filter papers were removed. Electrofocusing was performed at 1200 V for 3 hr. The pH gradient of the gel was measured by means of a surface electrode. The total number of snails used for analyses from each species and subspecies were between 24 to 30.

Twelve enzymes were analysed. They were phosphoglucomutase (PGM), phosphoglucoisomerase (PGI), aldehyde oxidase (ALD), malate dehydrogenase (MDH), acid phosphatase (AcP), alkaline phosphatase (ALK), hydroxybutyrate dehydrogenase (HBDH), hexokinase (HK), glutamate dehydrogenase (GDH), leucine aminopeptidase (LAP), esterase (EST) and lactic dehydrogenase (LDH). The enzyme types were distinguished by the patterns of their activities in the stained gel. The isoenzymes are localized at their pI values, thus comparison between species and subspecies was based on the differing pI values of the bands of enzyme activities. Of the twelve enzymes examined, consistent and interpretable patterns of isoenzymes were observed in the first eight systems (Figs. 32-39).

2.3 Preliminary analysis of results

O.h. quadrasi

Slight variation of the isoenzyme patterns was detected within these subspecies and populations of snails examined. The isoenzyme types clearly distinguished *O.h. quadrasi* from *O.h. hupensis* and other species.

O.h. hupensis

The isoenzymes of *O.h. hupensis* were found to have characteristic patterns distinct from *O.h.quadrasi* and other species in most of the enzymes studied.

T. aperta

The isoenzyme patterns of *T. aperta* were clearly distinct from other species. Of the three races of *T. aperta*, the beta race was found to have enzyme patterns different from the alpha and gamma races, *T. bollingi*, *R. kaporensis* and *Tricula*-like species.

All of these snail species have their own characteristic isoenzyme patterns different from each other and from other species studied.

The results of this study indicate differences of the isoenzyme patterns between each snail species and subspecies. Slight variation of the enzyme types was detected between the populations of *O.h. quadrasi* and between the three races of *T. aperta*. More studies on the other enzyme types and the use of more snail samples would help to clarify this variation. Attempts to correlate between enzyme types and parasite susceptibility rates are in progress.

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TABLE 1

Growth table for *Melanoides tuberculata*.

Week	Ave. shell length per week (mm.)	Ave. shell growth per week (mm.)
1	0.8	0.4
2	1.2	0.3
3	1.5	0.3
4	1.8	0.4
5	2.2	0.3
6	2.5	0.5
7	3.0	0.6
8	3.6	0.4
9	4.0	0.5
10	4.5	0.6
11	5.1	0.8
12	5.9	0.6
13	6.5	0.5
14	7.0	0.5
15	7.5	0.5
16	8.0	0.6
17	8.5	0.5
18	9.0	0.5
19	9.5	0.4
20	10.0	0.4
21	10.3	0.7
22	11.0	0.6
23	11.5	0.5
24	12.0	0.8
25	12.8	

Mean shell growth per week = 0.5 mm.

TABLE 2

Growth table for *Tarebia granifera*

Month	Ave. shell length per month (cm.)	Ave. shell growth per month (cm.)
1	0.30	0.31
2	0.61	0.14
3	0.75	0.07
4	0.82	0.14
5	0.96	0.02
6	1.08	0.05
7	1.13	0.28
8	1.41	0.15
9	1.56	0.05
10	1.61	0.09
11	1.70	0.19
12	1.89	

Mean shell growth per month = 0.14 cm.

TABLE 3

Growth table for *Brotia costula costula*

Month	Ave. shell length per month (cm.)	Ave. shell growth per month (cm.)
1	0.29	0.16
2	0.45	0.18
3	0.63	0.28
4	0.91	0.21
5	1.20	0.38
6	1.58	0.03
7	1.61	0.30
8	1.91	0.19
9	2.10	0.16
10	2.26	0.67
11	2.33	0.25
12	2.58	

Mean shell growth per month = 0.20 cm.

TABLE 4

Growth table for *Pila ampullacea*

Month	Ave. shell length per month (cm.)	Ave. shell growth per month (cm.)
1	0.42	0.33
2	0.75	0.36
3	1.11	0.31
4	1.42	0.44
5	1.86	0.45
6	2.31	0.73
7	3.04	0.30
8	3.34	0.14
9	3.48	0.34
10	3.82	0.34
11	4.16	0.23
12	4.39	

Mean shell growth per month = 0.36 cm.

TABLE 5

Biological control study between *Tarebia granifera* (control) and *Oncomelania hupensis quadrasi* (target).

Week	Number of snails									
	1		2		3		4		5	
	Tg	Oq	Tg	Oq	Tg	Oq	Tg	Oq	Tg	Oq
0	40	0	30	10	20	20	10	30	0	40
2	127	0	109	9	73	18	28	30	0	40
4	351	0	314	9	248	17	172	30	0	38
6	689	0	634	8	573	17	364	28	0	44
8	287	0	34	7	369	17	301	26	0	55
10	388	0	34	6	369	15	301	26	0	77
12	430	0	57	5	489	15	424	26	0	117
14	430	0	77	5	379	15	297	25	0	350
16	394	0	79	5	329	10	270	23	0	224

Tg = *Tarebia granifera*

Oq = *Oncomelania hupensis quadrasi*

Ratio of *T. granifera* : *O.h. quadrasi*

1 = 40 : 0

2 = 30 : 10

3 = 20 : 20

4 = 10 : 30

5 = 0 : 40

TABLE 6

Biological control study between *Tarebia granifera* (control) and *Bithynia siamensis goniomphalos* (target).

Week	Number of snails									
	1		2		3		4		5	
	Tg	Bsg	Tg	Bsg	Tg	Bsg	Tg	Bsg	Tg	Bsg
0	40	0	30	10	20	20	10	30	0	40
2	287	0	211	10	117	20	63	30	0	40
4	388	0	286	10	157	20	97	29	0	46
6	430	0	332	10	170	20	144	12	0	47
8	394	0	153	10	184	19	139	11	0	47
10	279	0	97	10	187	19	120	10	0	47
12	357	0	89	10	173	19	125	7	0	47
14	282	0	98	10	166	18	149	7	0	40
16	305	0	108	10	152	18	160	7	0	40

Tg = *Tarebia granifera*

Bsg = *Bithynia siamensis goniomphalos*

Ratio of *T. granifera* : *B.s. goniomphalos*

1 = 40 : 0

2 = 30 : 10

3 = 20 : 20

4 = 10 : 30

5 = 0 : 40

TABLE 7

Biological control study between *Tarebia granifera* (control) and *Robertsiella kaporensis* (target).

Week	Number of snails									
	1		2		3		4		5	
	Tg	Rk	Tg	Rk	Tg	Rk	Tg	Rk	Tg	Rk
0	40	0	30	10	20	20	10	30	0	40
2	95	0	61	9	37	20	20	29	0	40
4	135	0	75	9	43	20	26	22	0	40
6	130	0	78	9	26	20	34	22	0	40
8	98	0	49	9	23	11	50	17	0	37
10	82	0	40	9	20	11	52	17	0	37
12	76	0	32	9	20	10	48	17	0	37
14	84	0	23	3	22	10	42	13	0	37
16	81	0	27	3	20	9	39	11	0	30

Tg = *Tarebia granifera*

Rk = *Robertsiella kaporensis*

Ratio of *T. granifera* : *R. kaporensis*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

TABLE 8

Biological control study between *Tarebia granifera* (control) and *Tricula aperta* (target).

Week	Number of snails									
	1		2		3		4		5	
	Tg	Ta	Tg	Ta	Tg	Ta	Tg	Ta	Tg	Ta
0	40	0	30	10	20	20	10	30	0	40
2	158	0	49	9	36	20	86	28	0	40
4	147	0	67	9	34	20	89	27	0	40
6	162	0	75	9	49	20	109	27	0	37
8	146	0	76	9	65	20	117	27	0	37
10	152	0	65	9	71	20	100	27	0	37
12	92	0	52	9	49	18	96	27	0	37
14	123	0	86	9	81	18	88	25	0	37
16	168	0	88	9	87	15	126	25	0	31

Tg = *Tarebia granifera*

Ta = *Tricula aperta* (beta race)

Ratio of *T. granifera* : *T. aperta*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

TABLE 9

Biological control study between *Tarebia granifera* (control and *Indoplanorbis exustus* (target).

Week	Number of snails									
	1		2		3		4		5	
	Tg	Ie	Tg	Ie	Tg	Ie	Tg	Ie	Tg	Ie
0	40	0	30	10	20	20	10	30	0	40
2	270	0	256	10	151	20	47	30	0	40
4	351	0	253	9	214	20	61	30	0	39
6	309	0	200	64	40	61	29	60	0	65
8	159	0	122	48	55	46	12	20	0	43
10	56	0	47	47	24	22	14	0	0	43
12	52	0	14	48	18	29	16	0	0	43
14	32	0	18	20	6	45	8	0	0	43
16	26	0	24	42	6	43	2	0	0	54

Tg = *Tarebia granifera*

Ie = *Indoplanorbis exustus*

Ratio of *T. granifera* : *I. exustus*

1 = 40 : 0

2 = 30 : 10

3 = 20 : 20

4 = 10 : 30

5 = 0 : 40

TABLE 10

Biological control study between *Melanoides tuberculata* (control) and *Oncomelania hupensis quadrasi* (target).

Week	Number of snails									
	1		2		3		4		5	
	Mt	Oq	Mt	Oq	Mt	Oq	Mt	Oq	Mt	Oq
0	40	0	30	10	20	20	10	30	0	40
2	113	0	82	10	89	20	67	30	0	39
4	186	0	138	10	137	20	100	29	0	42
6	187	0	100	10	115	18	112	29	0	49
8	150	0	105	9	122	15	114	25	0	50
10	142	0	80	8	104	13	111	21	0	43
12	141	0	74	7	108	13	111	18	0	39
14	137	0	130	7	102	12	111	16	0	44
16	133	0	124	7	100	12	148	16	0	40

Mt = *Melanoides tuberculata*

Oq = *Oncomelania hupensis quadrasi*

Ratio of *M. tuberculata* : *O.h. quadrasi*

1 = 40 : 0

2 = 30 : 10

3 = 20 : 20

4 = 10 : 30

5 = 0 : 40

TABLE 11

Biological control study between *Melanoides tuberculata* (control) and *Tricula aperta* (target)

Week	Number of snails									
	1		2		3		4		5	
	Mt	Ta	Mt	Ta	Mt	Ta	Mt	Ta	Mt	Ta
0	40	0	30	10	20	20	10	30	0	40
2	55	0	53	8	37	20	9	27	0	40
4	46	0	51	8	50	20	22	27	0	40
6	44	0	50	8	67	20	24	27	0	38
8	31	0	47	8	71	20	23	27	0	38
10	129	0	63	8	83	19	30	27	0	38
12	116	0	49	8	84	19	33	27	0	37
14	122	0	46	8	104	19	33	24	0	37
16	117	0	44	8	120	19	41	23	0	37

Mt = *Melanoides tuberculata*

Ta = *Tricula aperta* (beta race)

Ratio of *M. tuberculata* : *T. aperta*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

TABLE 12

Biological control study between *Melanoides tuberculata* (control) and *Robertsiella kaporensis* (target).

Week	Number of snails									
	1		2		3		4		5	
	Mt	Rk	Mt	Rk	Mt	Rk	Mt	Rk	Mt	Rk
0	40	0	30	10	20	20	10	30	0	40
2	39	0	29	10	20	20	10	30	0	40
4	36	0	21	10	18	20	9	30	0	40
6	33	0	14	6	14	20	9	30	0	40
8	33	0	13	6	13	20	12	26	0	37
10	34	0	16	6	17	20	28	26	0	37
12	36	0	19	6	15	18	20	26	0	37
14	36	0	19	6	15	18	20	26	0	37
16	38	0	21	4	14	18	27	25	0	30

Mt = *Melanoides tuberculata*

Rk = *Robertsiella kaporensis*

Ratio of *M. tuberculata* : *R. kaporensis*

1 = 40 : 0

2 = 30 : 10

3 = 20 : 20

4 = 10 : 30

5 = 0 : 40

TABLE 13

Biological control study between *Brotia costula costula* (control) and *Oncomelania hupensis quadrasi* (target).

Week	Number of snails									
	1		2		3		4		5	
	Bcc	Oq	Bcc	Oq	Bcc	Oq	Bcc	Oq	Bcc	Oq
0	20	0	15	5	10	10	5	15	0	20
2	20	0	15	5	10	10	5	15	0	20
4	20	0	15	5	10	10	5	15	0	20
6	20	0	15	5	10	10	5	15	0	20
8	20	0	15	5	10	10	5	15	0	20
10	20	0	15	4	10	10	5	15	0	20
12	20	0	14	3	10	9	5	15	0	20
14	20	0	14	3	10	9	5	15	0	20
16	20	0	14	3	10	9	5	15	0	20

Bcc = *Brotia costula costula*

Oq = *Oncomelania hupensis quadrasi*

Ratio of *B.c. costula* : *O.h. quadrasi*

1 = 20 : 0

2 = 15 : 5

3 = 10 : 10

4 = 5 : 15

5 = 0 : 20

TABLE 14

Biological control study between *Brotia costula costula* (control) and *Bithynia siamensis goniomphalos* (target).

Week	Number of snails									
	1		2		3		4		5	
	Bcc	Bsg	Bcc	Bsg	Bcc	Bsg	Bcc	Bsg	Bcc	Bsg
0	20	0	15	5	10	10	5	15	0	20
2	20	0	14	5	10	10	5	15	0	20
4	20	0	13	5	10	10	5	15	0	20
6	20	0	13	5	10	10	5	15	0	20
8	20	0	13	4	9	9	5	14	0	17
10	20	0	13	4	8	7	5	14	0	23
12	20	0	12	4	7	7	5	14	0	23
14	20	0	12	4	7	7	5	14	0	27
16	20	0	11	4	7	5	5	14	0	25

Bcc = *Brotia costula costula*

Bsg = *Bithynia siamensis goniomphalos*

Ratio of *B.c. costula* : *B.s. goniomphalos*

1	=	20	:	0
2	=	15	:	5
3	=	10	:	10
4	=	5	:	15
5	=	0	:	20

TABLE 15

Biological control study between *Brotia costula costula* (control) and *Tricula aperta* (target).

Week	Number of snails									
	1		2		3		4		5	
	Bcc	Ta	Bcc	Ta	Bcc	Ta	Bcc	Ta	Bcc	Ta
0	20	0	15	5	10	10	5	15	0	20
2	20	0	15	5	10	10	5	15	0	20
4	20	0	15	5	10	10	5	14	0	19
6	20	0	15	5	10	6	5	14	0	18
8	20	0	15	5	10	6	5	14	0	18
10	20	0	15	4	9	4	5	13	0	18
12	20	0	15	4	9	4	5	13	0	18
14	20	0	15	3	9	4	5	12	0	15
16	19	0	14	3	9	3	5	12	0	13

Bcc = *Brotia costula costula*

Ta = *Tricula aperta* (beta rače)

Ratio of *B.c. costula* : *T. aperta*

1	=	20	:	0
2	=	15	:	5
3	=	10	:	10
4	=	5	:	15
5	=	0	:	20

TABLE 16

Biological control study between *Brotia costula costula* (control) and *Biomphalaria glabrata* (target).

Week	Number of snails									
	1		2		3		4		5	
	Bcc	Bg	Bcc	Bg	Bcc	Bg	Bcc	Bg	Bcc	Bg
0	20	0	15	5	10	10	5	15	0	20
2	20	0	15	5	9	10	5	15	0	20
4	19	0	14	5	8	10	5	15	0	19
6	18	0	14	6	1	29	5	15	0	19
8	17	0	14	12	1	192	5	39	0	67
10	17	0	12	55	1	159	5	21	0	77
12	16	0	12	11	1	78	5	46	0	74
14	15	0	1	39	1	73	3	34	0	74
16	14	0	1	89	0	102	3	177	0	76

Bcc = *Brotia costula costula*

Bg = *Biomphalaria glabrata*

Ratio of *B.c. costula* : *B. glabrata*

1 = 20 : 0

2 = 15 : 5

3 = 10 : 10

4 = 5 : 15

5 = 0 : 20

TABLE 17

Biological control study between *Brotia costula costula* (control) and *Indoplanorbis exustus* (target).

Week	Number of snails									
	1		2		3		4		5	
	Bcc	Ie	Bcc	Ie	Bcc	Ie	Bcc	Ie	Bcc	Ie
0	20	0	15	5	10	10	5	15	0	20
2	20	0	15	4	10	9	5	14	0	19
4	20	0	15	10	9	11	5	15	0	55
6	20	0	15	2	9	10	5	11	0	19
8	20	0	15	9	7	7	4	14	0	25
10	20	0	15	9	7	7	4	39	0	57
12	20	0	13	11	5	8	3	23	0	26
14	20	0	13	11	5	8	3	23	0	26
16	20	0	11	34	4	8	3	6	0	50

Bcc = *Brotia costula costula*

Ie = *Indoplanorbis exustus*

Ratio of *B.c. costula* : *I. exustus*

1	=	20	:	0
2	=	15	:	5
3	=	10	:	10
4	=	5	:	15
5	=	0	:	20

TABLE 18

Biological control study between *Pila ampullacea* (control) and *Oncomelania hupensis quadrasi* (target).

Week	Number of snails									
	1		2		3		4		5	
	Pa	Oq	Pa	Oq	Pa	Oq	Pa	Oq	Pa	Oq
0	20	0	15	5	10	10	5	15	0	20
2	20	0	15	5	10	10	5	15	0	20
4	18	0	15	5	10	10	5	15	0	20
6	18	0	15	5	10	10	5	15	0	20
8	15	0	13	3	10	6	4	10	0	20
10	15	0	10	1	9	1	4	8	0	18
12	15	0	10	1	9	1	4	6	0	18
14	15	0	10	1	9	1	4	6	0	18
16	15	0	10	1	8	1	4	2	0	17

Pa = *Pila ampullacea*

Oq = *Oncomelania hupensis quadrasi*

Ratio of *P. ampullacea* : *O.h. quadrasi*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

TABLE 19

Life history for *Sepedon plumbella* (reared with crushed flesh of *Radix rubiginosa* snail and honey bee syrup.

Developmental stages	Life duration (days)	Average size (mm)	
	Mean ± S.D. (range)	Length ± S.D. (range)	Width ± S.D. (range)
Eggs	2.5 ± 1.29 (1-4)	1.08 ± 0.09 (0.95-1.2)	0.34 ± 0.15 (0.32-0.36)
First stadium	4.5 ± 1.29 (3-6)	1.13 ± 0.09 (1.00-1.25)	0.31 ± 0.01 (0.30-0.33)
Second stadium	4.5 ± 1.29 (3-6)	3.25 ± 0.16 (3.0-3.5)	0.8 ± 0.15 (0.6-1.1)
Third stadium	4.5 ± 1.29 (2-7)	8.5 ± 0.39 (8.0-9.0)	2.25 ± 0.18 (2.0-2.5)
Pupae	9.0 ± 3.31 (4-14)	5.5 ± 0.3 (5.0-6.0)	2.25 ± 0.18 (2.0-2.5)
Adults : Female	32.0 ± 4.47 (25-39)	10.5 ± 0.25 (9.0-12.0)	1.97 ± 0.35 (1.8-2.2)
Male	34.0 ± 3.89 (28-40)	12.0 ± 1.58 (10.0-14.0)	2.14 ± 0.17 (1.90-2.35)
One complete generation	33.6 ± 2.58 (25-45)		
Pre-oviposition period	13.5 ± 5.33 (9-22)		
Oviposition period	139.5 ± 9.66 (9-270)		
Frequency of egg laying/female	53.2 ± 4.07 (20-75)		
No. of eggs/batch	15.1 ± 7.67 (3-27)		
No. of eggs/female	1069.8 ± 69.2 (128-2450)		
Adult longevity : Female	30.8 ± 3.12 (25-68)		
Male	47.4 ± 2.94 (32-60)		
Life span : Female	129 ± 7.43 (32-210)		
Male	138 ± 8.39 (32-270)		

TABLE 20

Life history for *Sepedon plumbella*, reared with various types of food

Developmental stage	Types of food			
	Flesh of *R. rubiginosa*		Flesh of *I. exustus*	
	alone	with honey bee syrup	alone	with honey bee syrup
Oviposition rate of female with a complete cycle of development (mean number of eggs per female ± S.D.)				
	489.5 ± 151.25	1069.8 ± 69.2	392.75 ± 12.5	395.75 ± 32.7
Survival rate of each developmental stage, reared from eggs to adults (%)				
Eggs	100	100	100	100
First instar	71	94	65	81
Second instar	55	78	51	70
Third instar	54	64	48	67
Pupae	42	59	32	59
Adults	31	57	25	51

TABLE 21

Biological control study between sciomyzid fly larvae (control) and snails (target). L = sciomyzid fly larvae, S = snails.

Snail species	Stage of sciomyzid fly larvae	Death (days)	Mortality rates (%) Ratio of sciomyzid fly larvae (L) to snails (S)									
			L : S		L : S		L : S		L : S		L : S	
			4	0	3	1	2	2	1	3	0	4
Pulmonate snails												
Biomphalaria glabrata	2nd	5	27.5	–	25.0	75.0	27.5	57.5	2.5	47.5	–	0
	3rd	4	0	–	10.0	100	4.7	81.8	9.1	72.8	–	0
Bulinus (P.) abyssinicus	2nd	4	27.5	–	25.0	100	20.0	75.0	25.0	50.0	–	0
	3rd	3	0	–	0	100	0	75.0	25.0	83.3	–	0
Indoplanorbis exustus	2nd	4	10.0	–	14.0	80.0	20.0	62.5	22.5	49.1	–	0
	3rd	3	0	–	0	100	0	87.5	0	66.7	–	0
Radix rubiginosa	2nd	4	0	–	8.3	25.0	0	77.3	9	56.0	–	0
	3rd	4	25.0	–	15.0	100	2.5	87.5	0	58.3	–	0
Operculate snails												
Bithynia s. goniomphalos	2nd	7	0	–	5.0	15.0	1.5	4.0	0	2.0	–	0
	3rd	9	0	–	9.0	22.0	2.0	8.0	0	5.0	–	0
Oncomelania h. quadrasi	2nd	3	27.5	–	13.3	0	25.0	0	30.0	0	–	0
	3rd	5	6.3	–	16.7	0	20.0	0	10.0	0	–	0
Tricula aperta	2nd	3	25.0	–	23.3	0	27.5	0	30.0	0	–	0
	3rd	5	25.0	–	16.7	0	50.0	0	10.0	0	–	0
Robertsiella kaporensis	2nd	3	18.8	–	20.0	0	22.5	0	20.0	0	–	0
	3rd	5	12.5	–	13.3	0	25.0	0	15.0	0	–	0

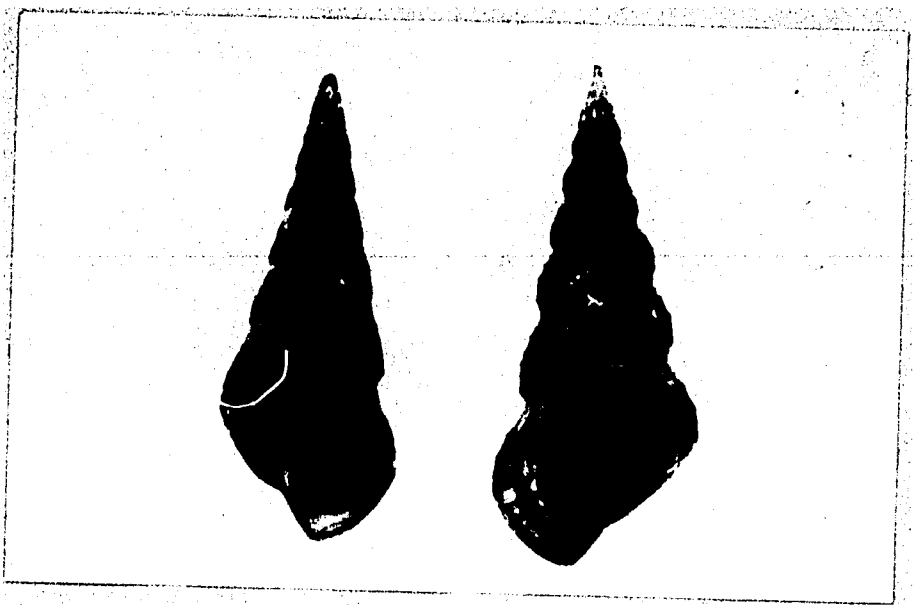

Fig. 1. *Brotia costula costula* (as is)

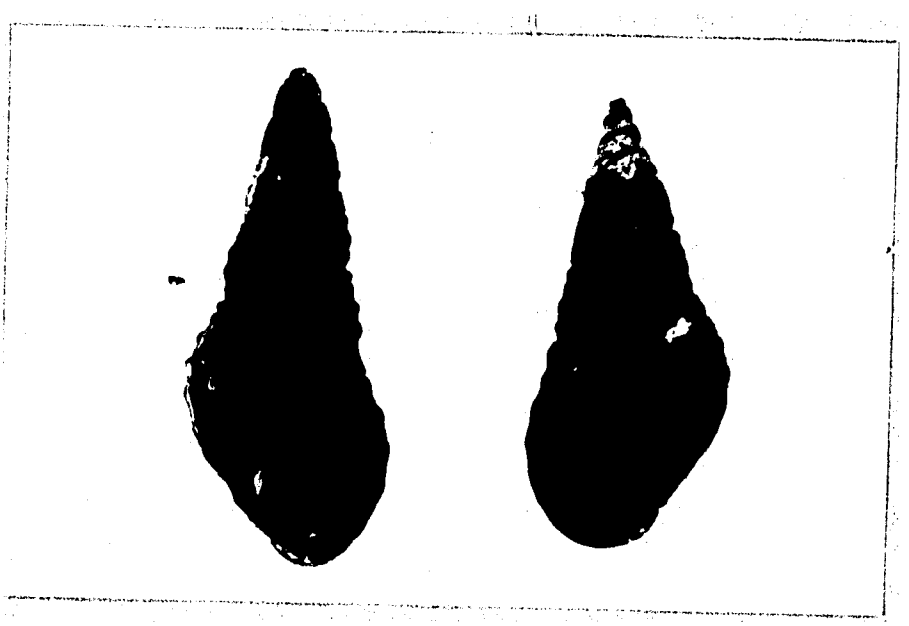

Fig. 2. *Tarebia (Thiara) granifera* (x 2.5)

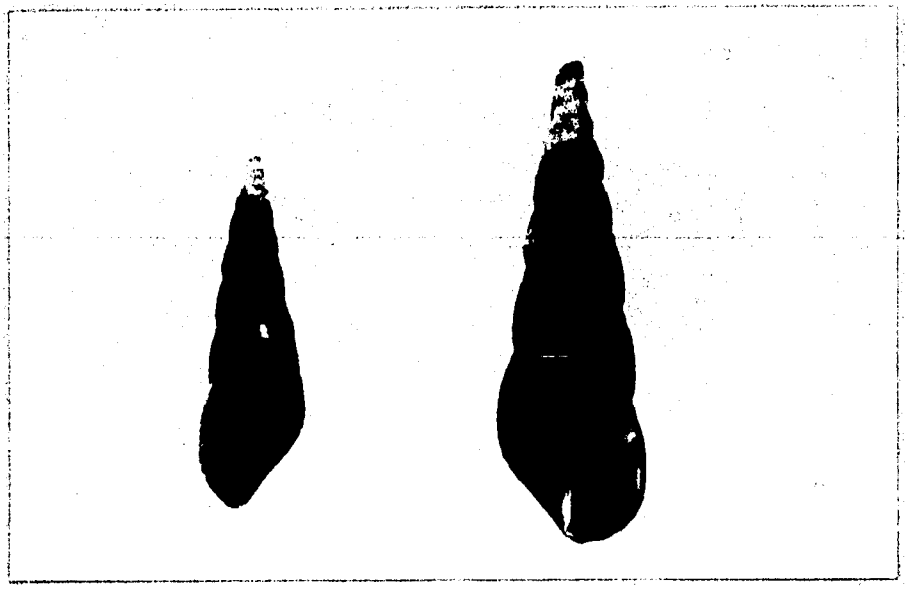

Fig. 3. *Melanoides tuberculata* (x 2)

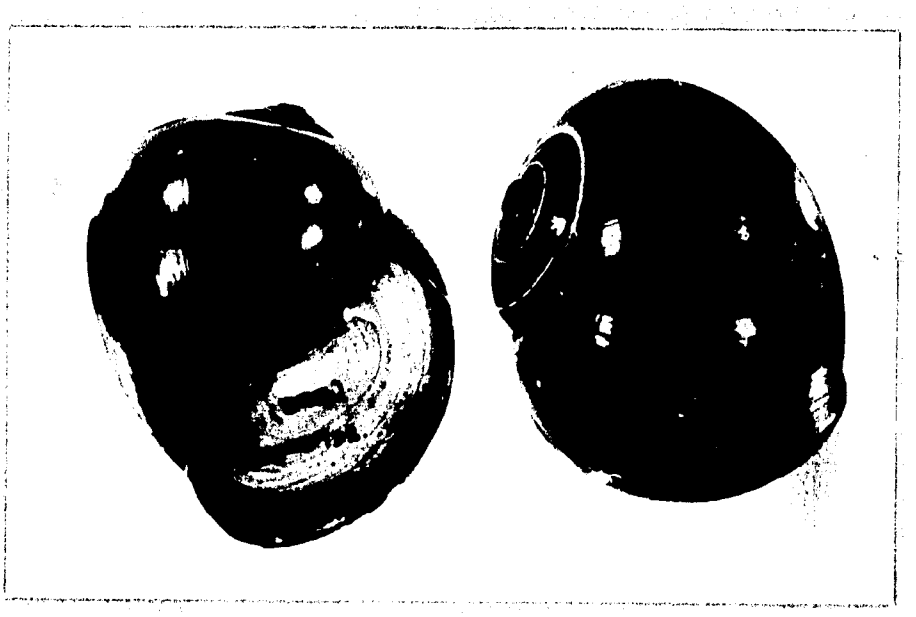

Fig. 4. *Pila ampullacea* (as is)

Fig. 5. *Tricula aperta* (beta race) (x 16)

Fig. 6. *Robertsiella kaporensis* (x 32)

Fig. 7. *Oncomelania hupensis quadrasi* (x 17)

Fig. 8. *Bithynia siamensis goniomphalos* (x 12)

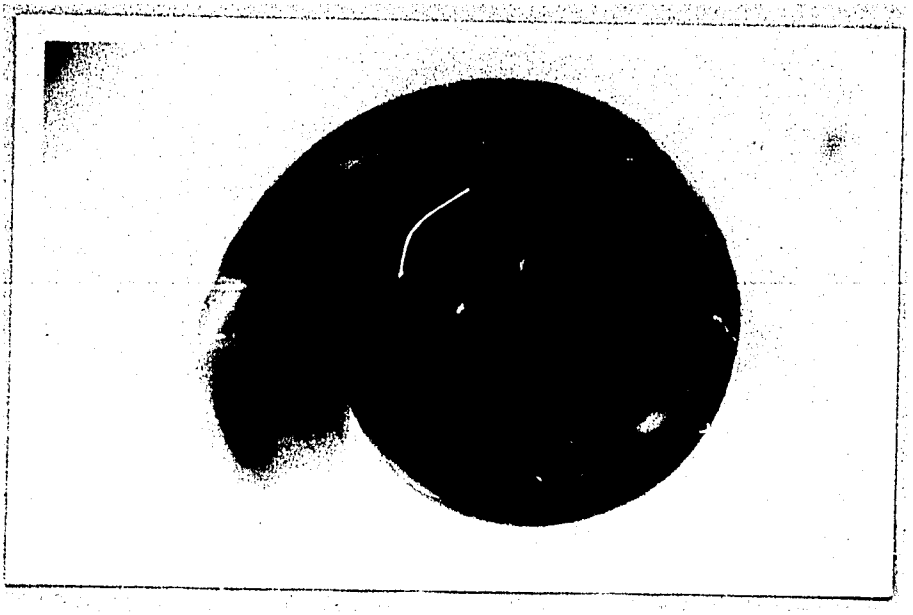

Fig. 9. *Biomphalaria glabrata* (x 4.5)

Fig. 10. *Indoplanorbis exustus* (x 7)

Fig. 11. *Bulinus abyssinicus*

(x 20)

Fig. 12. *Radix rubiginosa*

(x 8)

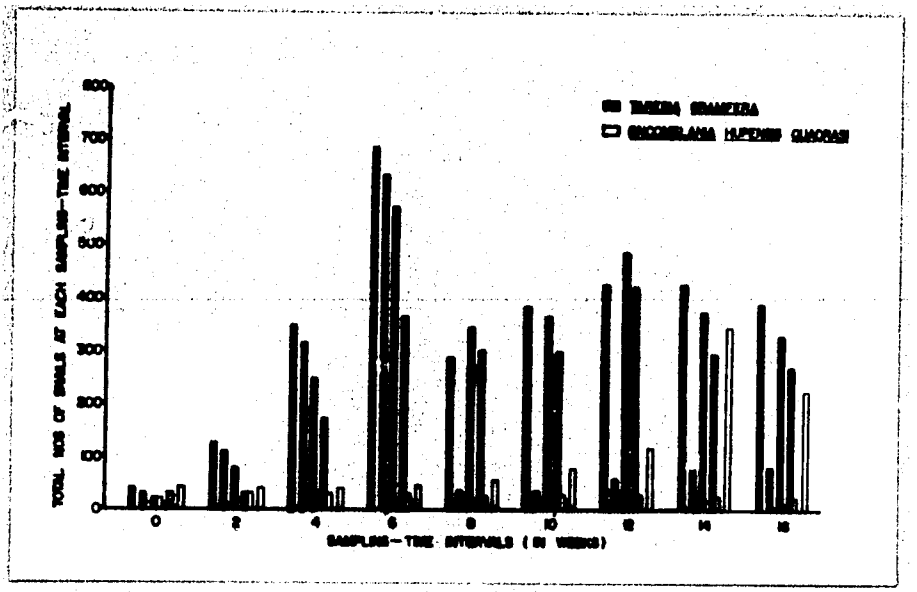

Fig. 13. Biological control study between *T. granifera* (control) and *O.h. quadrasi* (target).

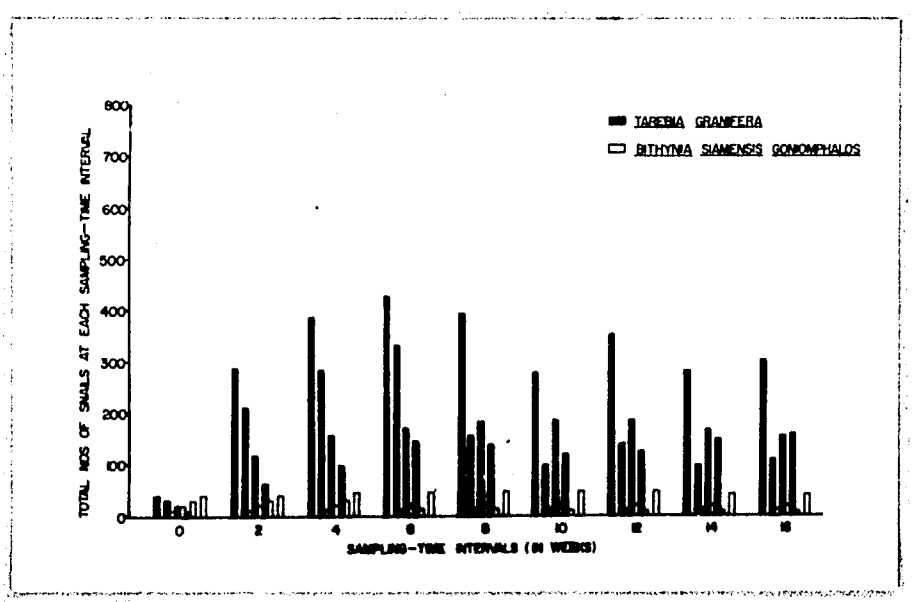

Fig. 14. Biological control study between *T. granifera* (control) and *B.s. goniomphalos* (target).

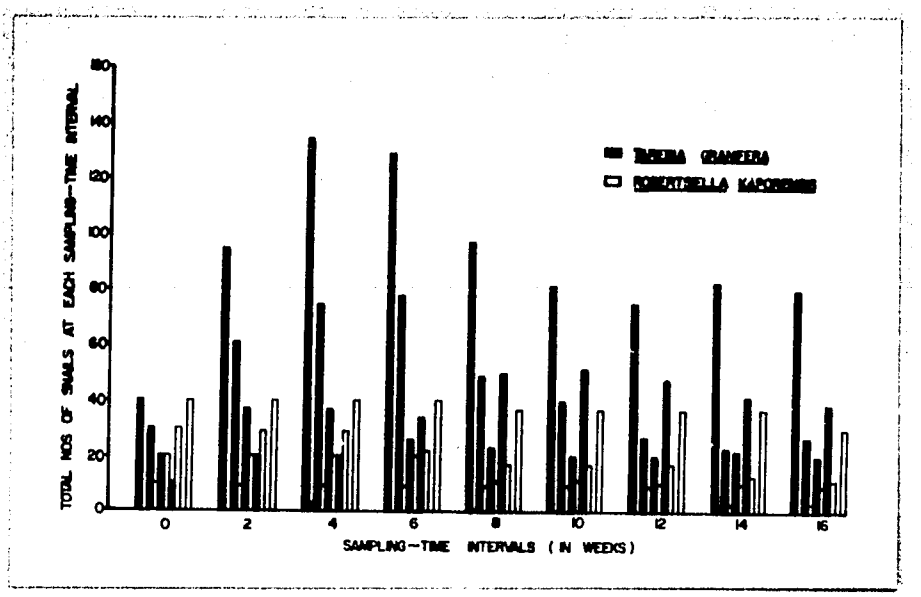

Fig. 15. Biological control study between *T. granifera* (control) and *R. kaporensis* (target).

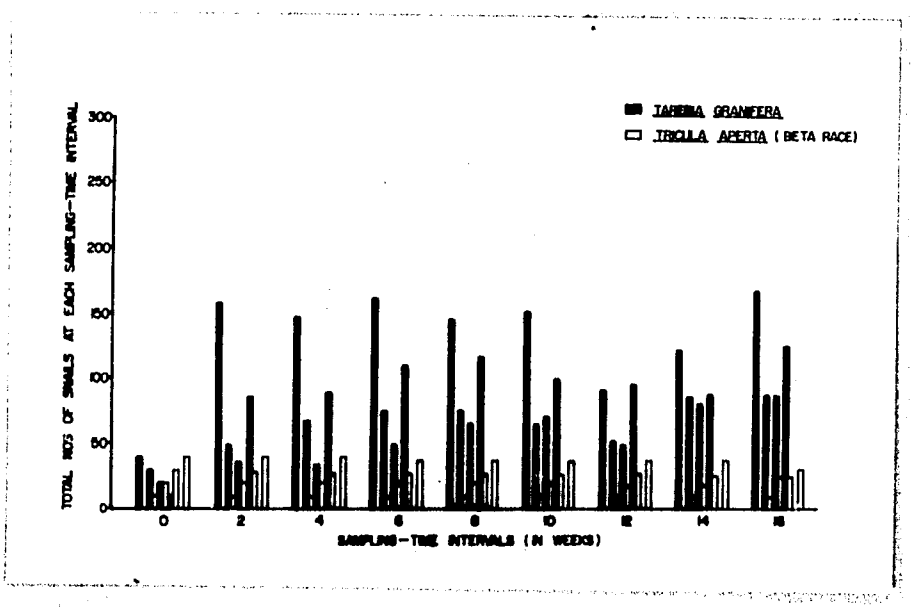

Fig. 16. Biological control study between *T. granifera* (control) and *T. aperta* (target).

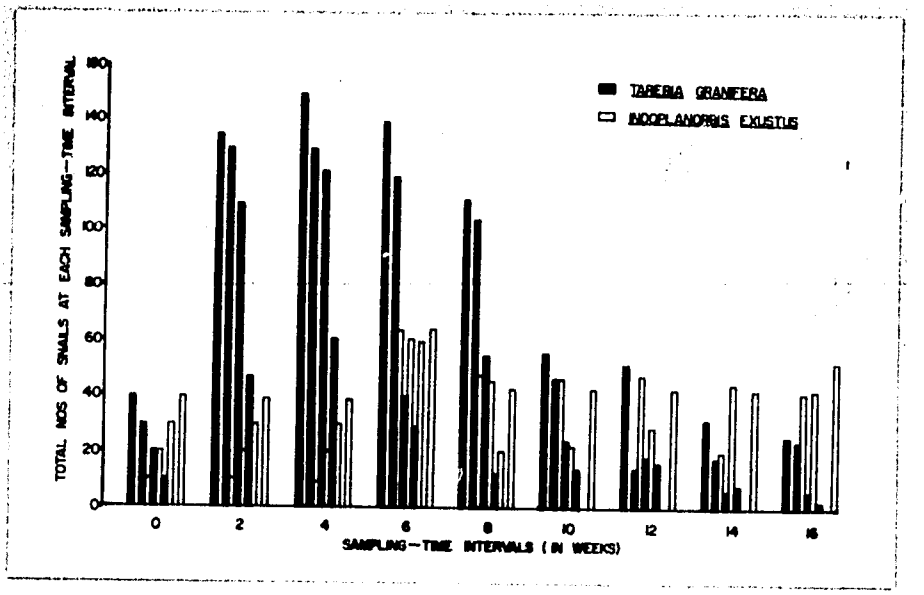

Fig. 17. Biological control study between *T. granifera* (control) and *I. exustus* (target).

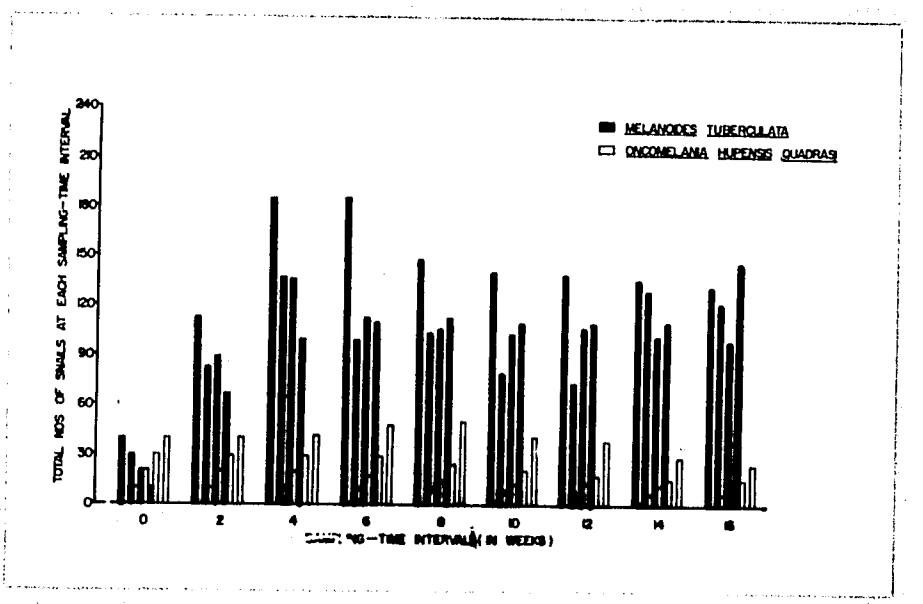

Fig. 18. Biological control study between *M. tuberculata* (control) and *O.h. quadrasi* (target).

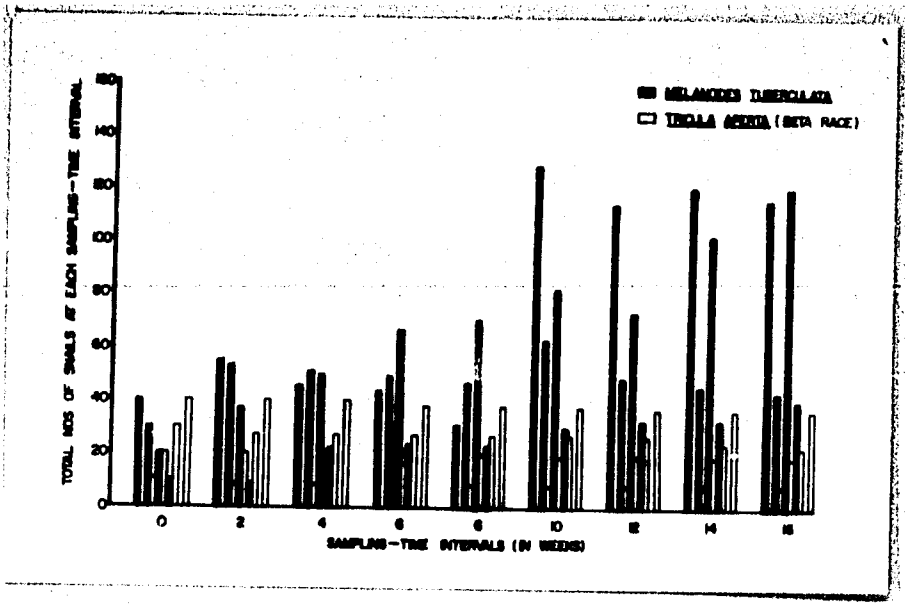

Fig. 19. Biological control study between *M. tuberculata* (control) and *T. aperta* (target).

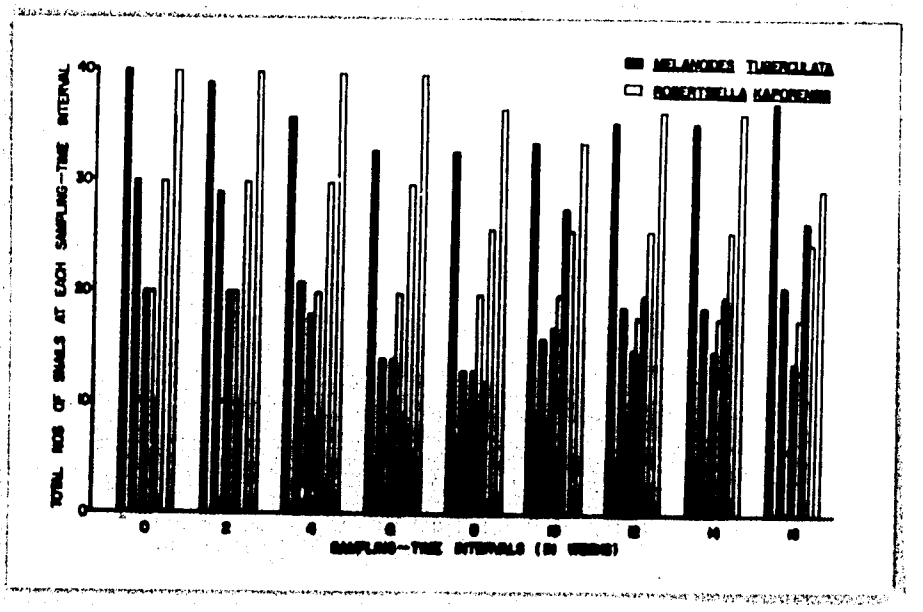

Fig. 20. Biological control study between *M. tuberculata* (control) and *R. kaporensis* (target).

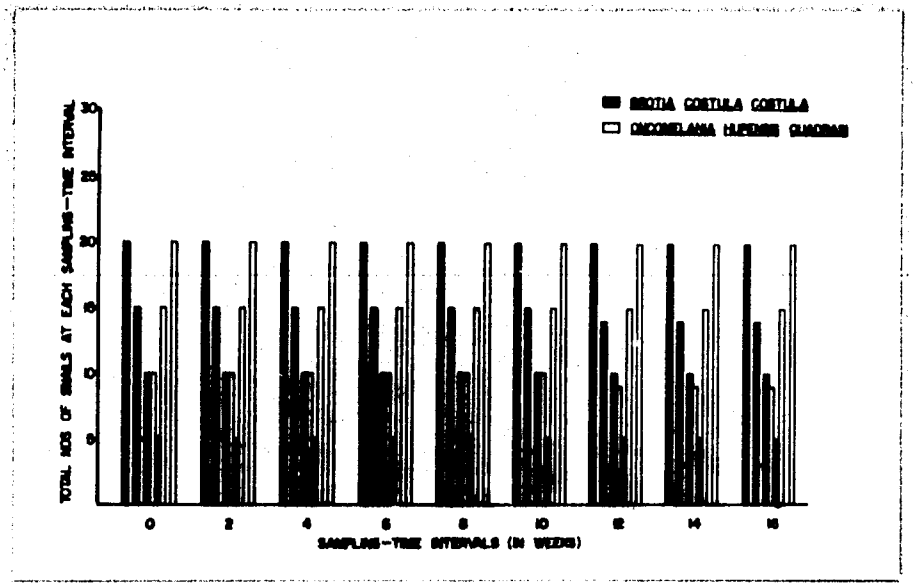

Fig. 21. Biological control study between *B.c. costula* (control) and *O.h. quadrasi* (target).

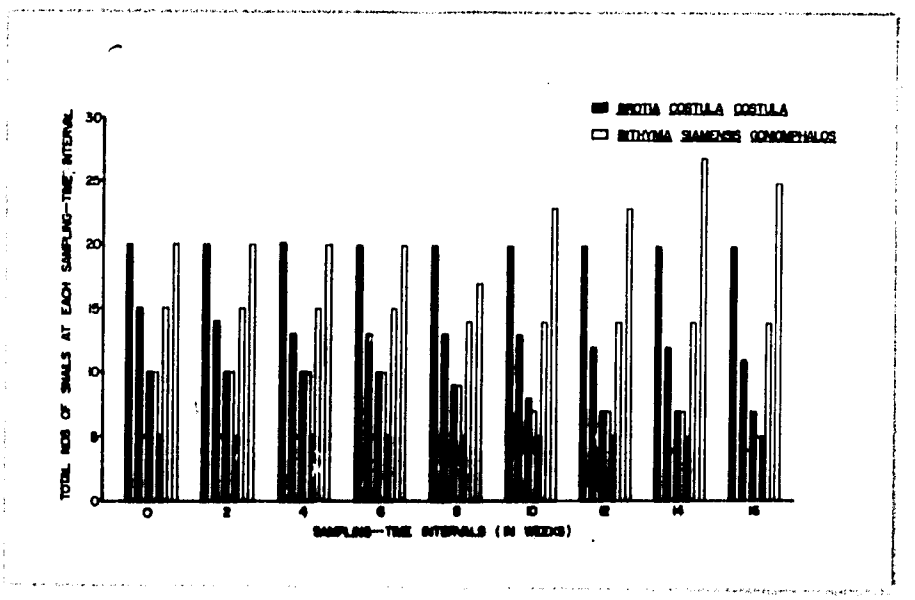

Fig. 22. Biological control study between *B.c. costula* (control) and *B.s. goniomphalos* (target).

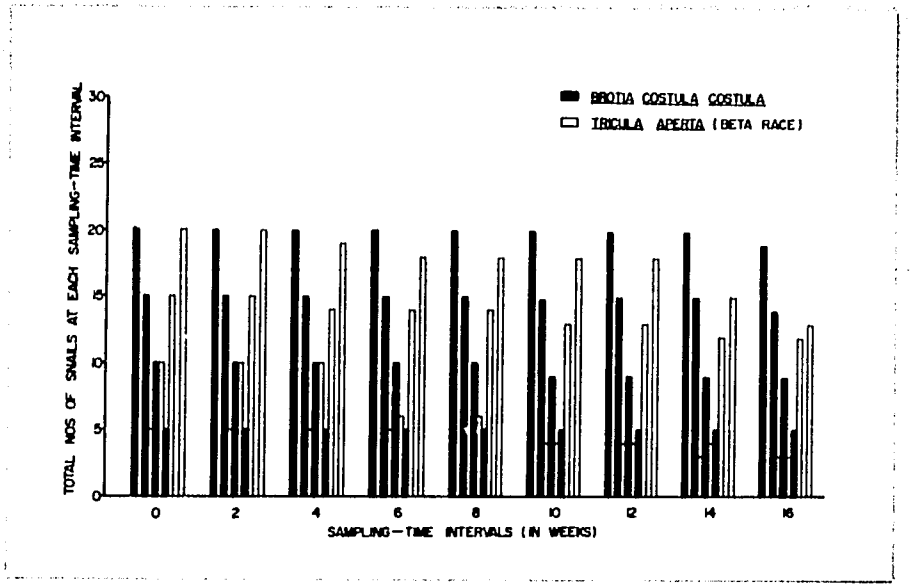

Fig. 23. Biological control study between *B.c. costula* (control) and *T. aperta* (target).

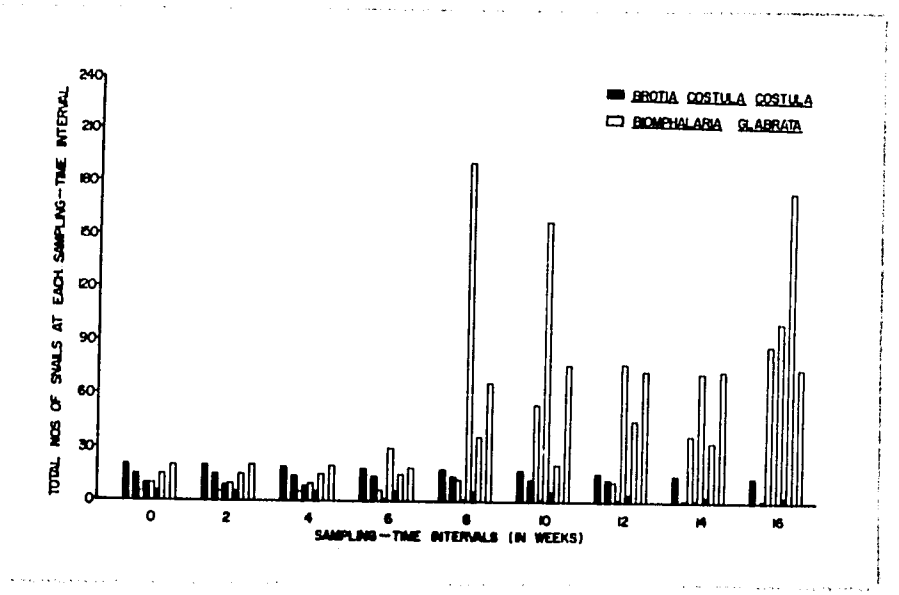

Fig. 24. Biological control study between *B.c. costula* (control) and *B. glabrata* (target).

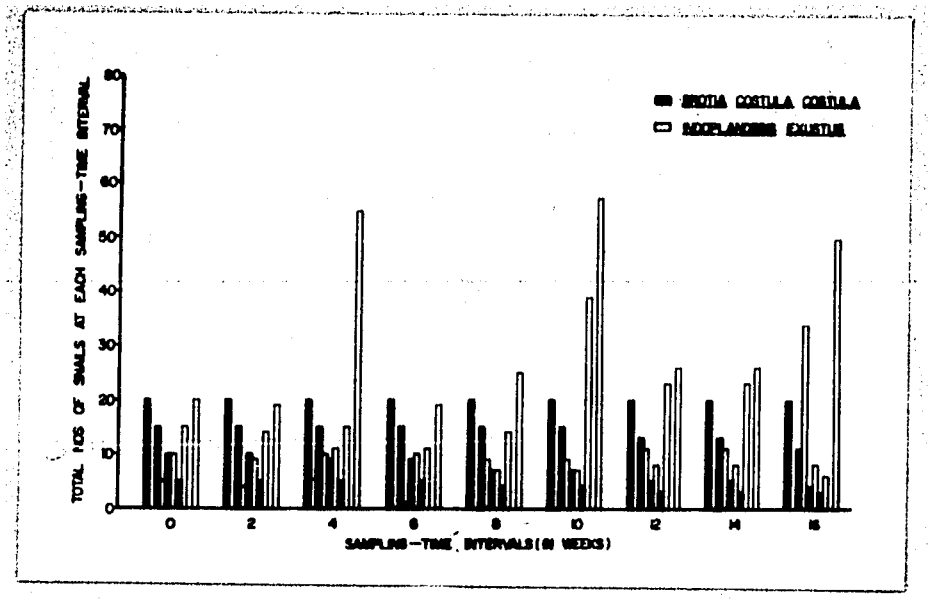

Fig. 25. Biological control study between *B.c. costula* (control) and *I. exustus* (target).

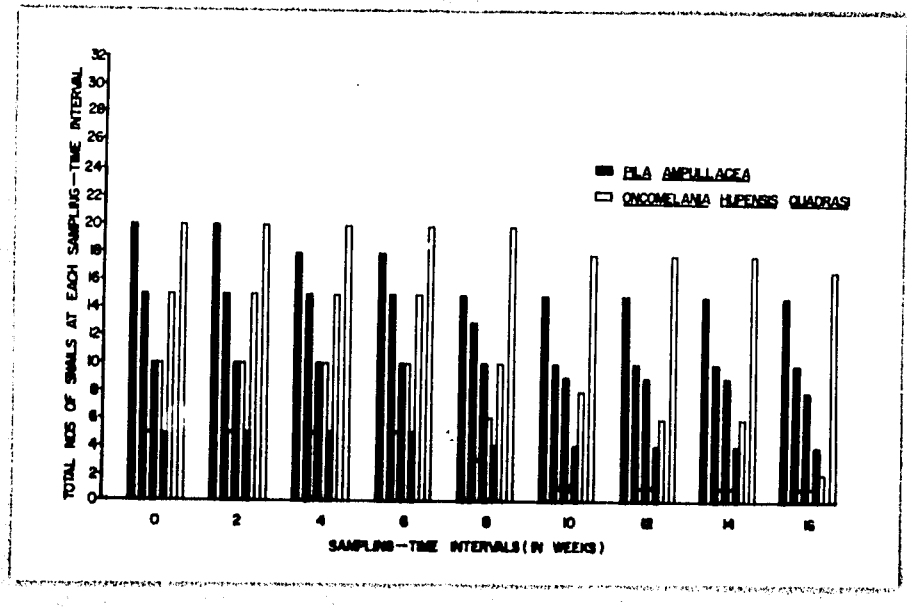

Fig. 26. Biological control study between *P. ampullacea* (control) and *O.h. quadrasi* (target).

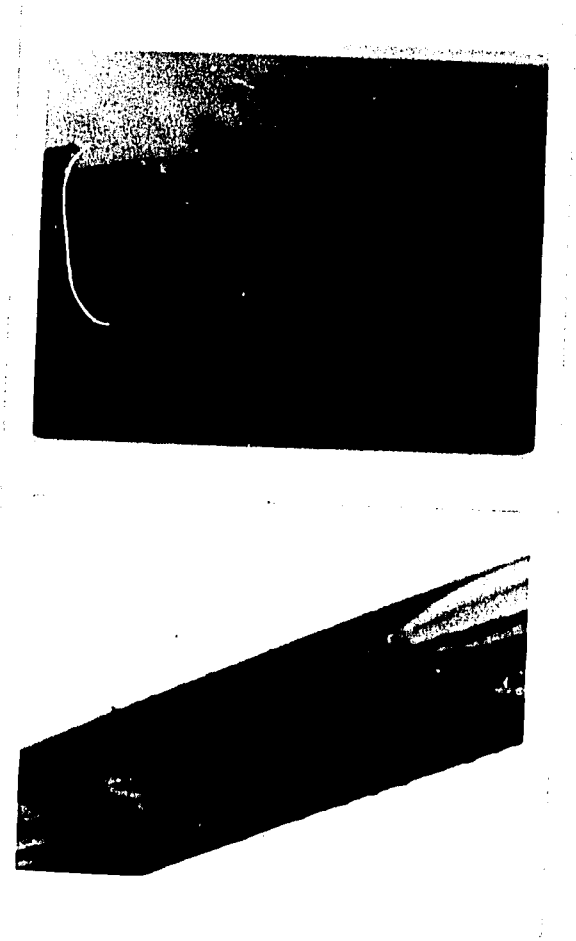

Fig. 27. Eggs of sciomyzid fly *(Sepedon plumbella)* on rice plant.

Fig. 28. Larvae of sciomyzid fly *(Sepedon plumbella)*

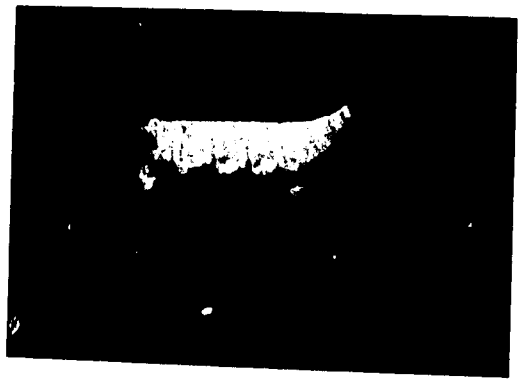

Fig. 29. Pupae of sciomyzid fly *(Sepedon plumbella)*

Fig. 30. Adults of sciomyzid fly *(Sepedon plumbella)* (male-left, female-right).

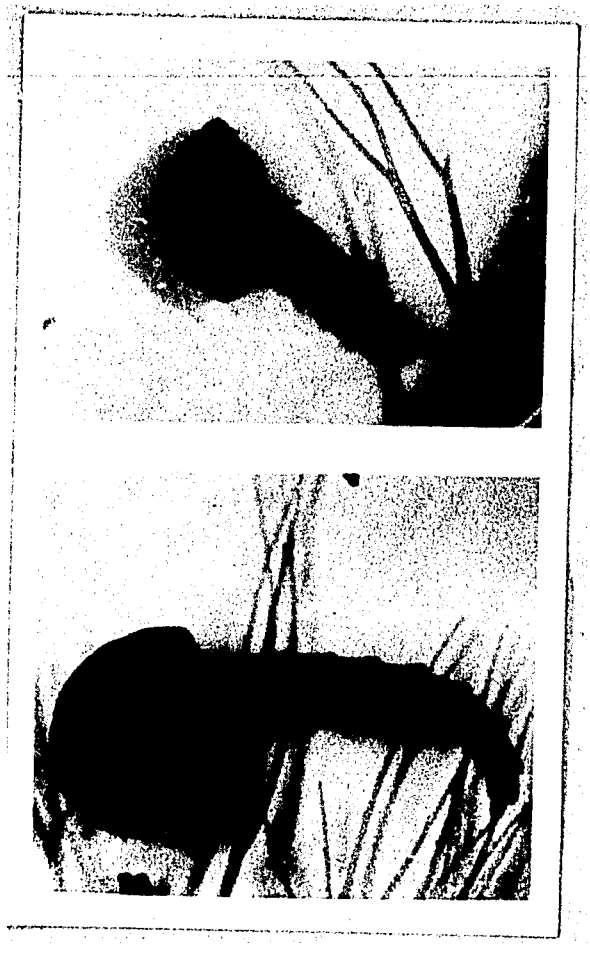

Fig. 31. Larvae of the unknown invertebrate feeding on the snails, *Bithynia siamensis goniomphalos* (operculate snail) (top), and *Indoplanorbis exustus* (pulmonate snail) (bottom).

Explanation of the Figures:-

A = *O.h. quadrasi* from Irosin

B = *O.h. quadrasi from Mindoro*

C, D, E = three population of O.h. quadrasi from Leyte

F = *O.h. hupensis*

G = *T. aperta* (alpha race)

H = *T. aperta* (beta race)

I = *T. aperta* (gamma race)

J = *T. bollingi*

K = *Robertsiella kaporensis*

L = *Tricula*-like species from Phitsanuloke

M = *Tricula*-like species from Kanchanaburi

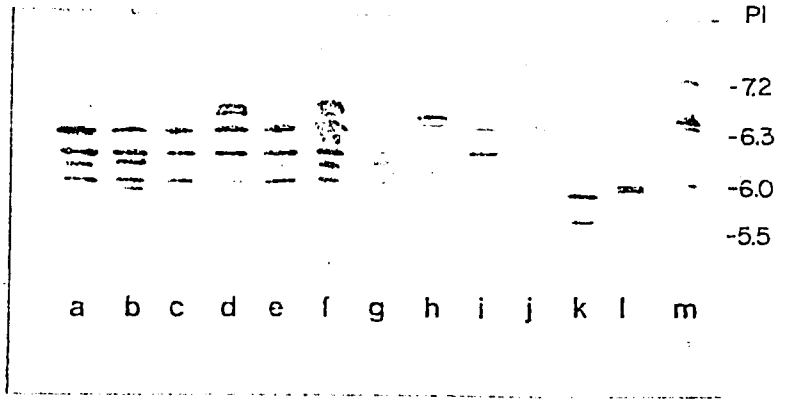

Fig. 32. Photograph of isoelectric focusing of the isoenzyme patterns of phosphoglucomutase.

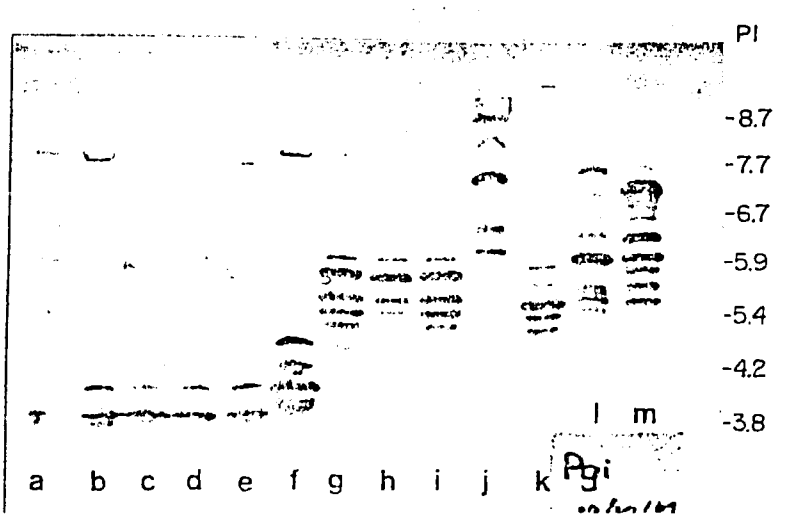

Fig. 33. Photograph of isoelectric focusing of the isoenzyme patterns of phosphoglucoisomerase.

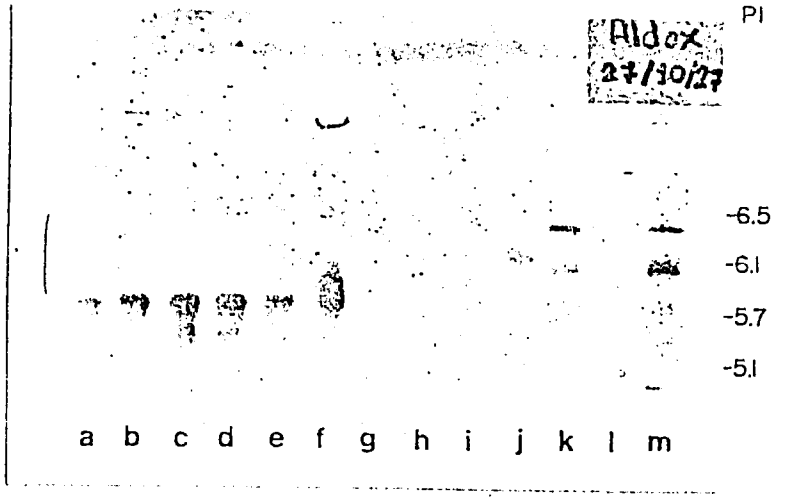

Fig. 34. Photograph of isoelectric focusing of the isoenzyme patterns of aldehyde oxidase.

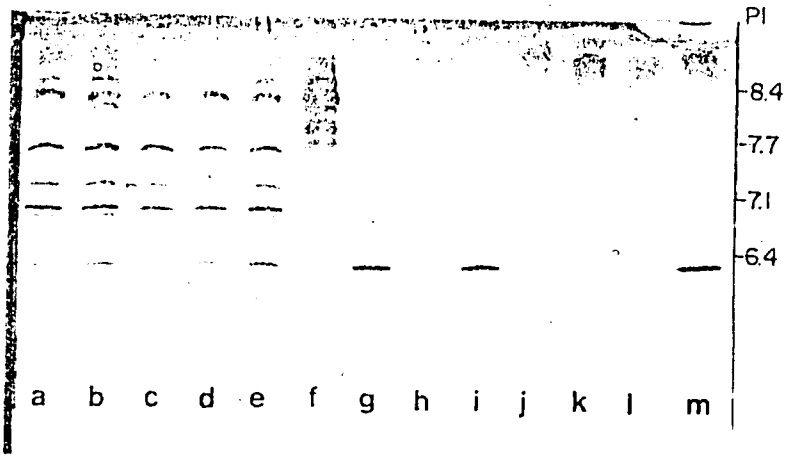

Fig. 35. Photograph of isoelectric focusing of the isoenzyme patterns of malate dehydrogenase.

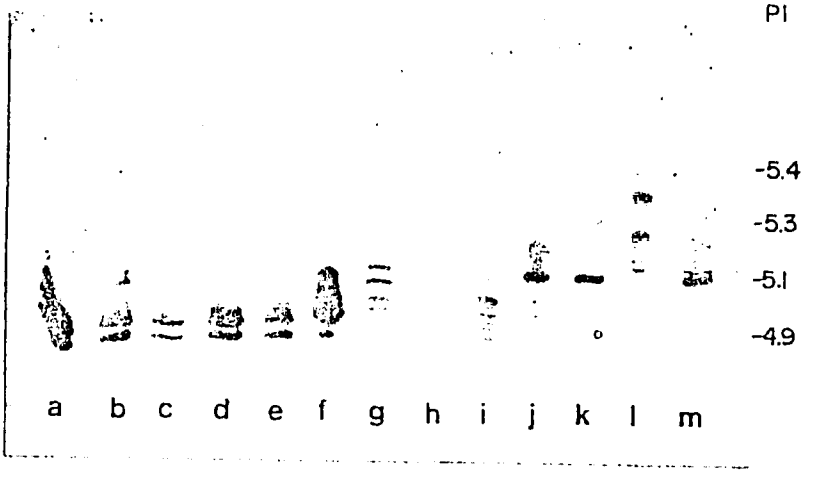

Fig. 36 . Photograph of isoelectric focusing of the isoenzyme patterns of acid phosphatase.

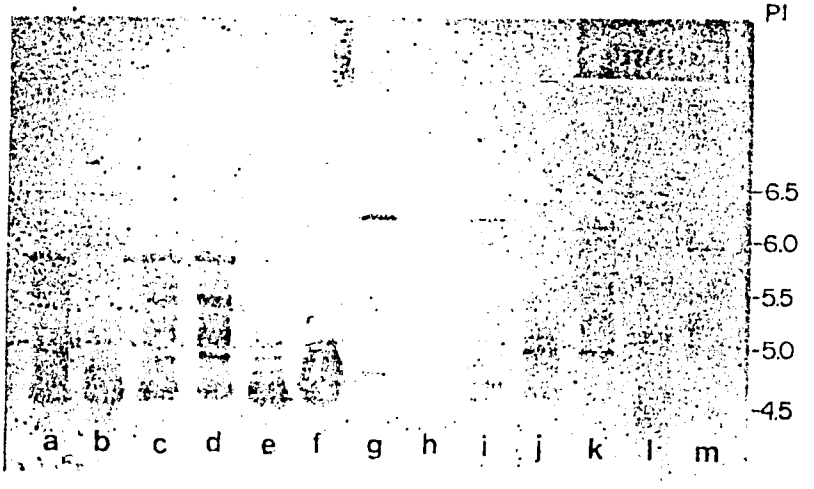

Fig. 37. Photograph of isoelectric focusing of the isoenzyme patterns of alkaline phosphatase.

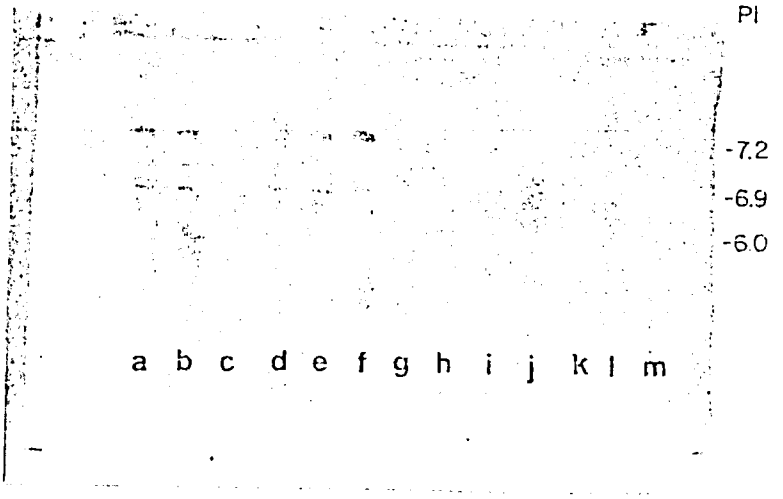

Fig. 38 Photograph of isoelectric focusing of the isoenzyme patterns of hydroxybutyrate dehydrogenase.

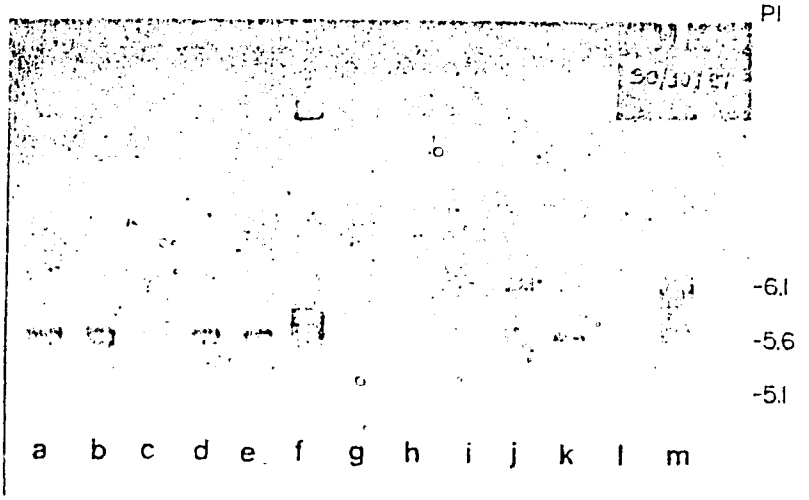

Fig. 39. Photograph of isoelectric focusing of the isoencyme patterns of hexokinase.