Preliminary trials on the biological snail control with *Bacillus Pinottii* in Egypt

Emmanuel Dias
Instituto Oswaldo Cruz,
Rio de Janeiro, Brasil

and

Mohammed M. Dawood
Research Institute,
Cairo, Egypt

(With 10 text-figures)

Soon after starting his researches on the possibilities of the snail control by microbiological methods, DIAS (1953*a*) succeeded in isolating from *Australorbis glabratus* from Bambui, Brazil, the local intermediate host of *Schistosoma mansoni,* a spore-bearing bacillus, conventionally called BET (*bacilo de espóro terminal*) . In that paper he described 1) the procedure he had been using in attempting to increase the virulence of microorganisms against snails, through repeated passages from snail to snail, 2) the method for isolating bacteria from the ovotestes of living snails, and 3) the preliminary results of the first experiments that were carried out in small breeding places in the field. In a paper read before the V International Congresses on Tropical Medicine and Malaria, held at Istanbul in August-September 1953, DIAS (1953*b* 1954*a*) reported the encouraging results he had obtained in Bambui, by treating large breeding places with BET cultures to which powdered peptone was added.

BET bacillus proved to be an apparently new species, which has been named *Bacillus pinottii* Cruz & Dias, 1953; it is a proteolytic aerobic organism that was found to be harmless to several species of vertebrates and, under certain conditions, lethal to *A. glabratus* and other South American molluscs.

Schistosomiasis is considered to be the number one public health problem in Egypt. It is known that it occurs there since pharaonic times and that its incidence is increasing with the improvement of the irrigation system and the development of agriculture. There are there two forms of the human disease, viz., intestinal schistosomiasis due to *Schistosoma mansoni,* which is prevalent in the Delta of the Nile river, and urinary schistosomiasis, caused by *S. haematobium,* which occurs in the Delta as well as in Upper Egypt. About 60 per cent of the population is affected either by one or by both forms of the disease. Snails of several species thrive in the drains, canals, ponds, ditches and other breeding places along the Nile valley: the most important are *Bulinus*

truncatus, the intermediate host of *S. haematobium*, and *Planorbis boissyi*, the vector of *S. mansoni; Bulinus*, *Lymnaea* and others are also important from the veterinary point of view, as carriers of animal parasites, such as *S. bovis* and *Fasciola gigantica*.

An opportunity for trying the effect of *B. pinottii* on Egyptian snails would thus be most desirable, notwithstanding the fact that research work on this new line was just beginning. Such opportunity was offered last year, soon after the senior author attended the Congresses at Istanbul: he was able to spend nearly three months in Egypt and to carry on with DAWOOD the experiments that are reported in the present paper. The work has been done at the Research Institute (Director Dr. A. Halawani), from the Ministry of Health (Minister Dr. Nourredine Tarraf). Thanks to a suggestion from Prof. E. Rodenwaldt, from Heidelberg, the writers had been corresponding for some time before meeting each other in Cairo.

Acknowledgements — The writers wish to express their best thanks to Dr. Mario Pinotti, Director of the National Malaria Service from Brazil, for making possible the trip of one of them to Egypt, and to the following Egyptian authorities and technicians, for the help and facilities they have provided for the present work: Dr. Nourredine Tarraf, Minister of Public Health, Dr. A. Halawani, General Director of Endemic Diseases Control & Research, Dr. M. Gindi, from the Snail Destruction Section, Drs. Anwar and Nasr, from the Research Institute.

CULTIVATING *BACILLUS PINOTTII* ON DIFFERENT MEDIA

B. pinottii, or BET bacillus as it has been previously referred to (DIAS), originally isolated from *A. glabratus* from Bambui, Brazil, has been recently described by CRUZ & DIAS. It is a Gram variable, aerobic, motile Bacillus, bearing a terminal spore (*plectridium*). Some of its characteristics, according to CRUZ & DIAS:

Broth — Rapid grow at pH 7,0-7,2, the pH of the medium raising after some days of culture, reaching sometimes pH 9,0; no growth under pH 5,0. Abundant sediment, forming sometimes a thin pellicle. Optimum temperature 25º-37º, no growth at 50º C. *Agar slant* — Thick smooth, glistening, white-yellowish growth. Salt tolerant. *Gelatin stab* — Rapid liquefaction, infundibuliform. *Litmus milk* — No coagulation; peptonization; litmus reduced. *Cooked egg-albumin* — Peptonized, odour of putrefaction. *Blood agar* — Hemolysis in 48 hours. *Carbohydrate media* — No acid nor gas. *Indol* — Positive. *Acetyl-methyl-carbinol* — Negative. *Methyl-red test* — Negative. *Nitrates* — Not reduced. *Starch* — Not hydrolized. *Citrate medium* — No growth. *Ammonia* — Produced. *Urea* — Not decomposed. H^2S — Not produced.

Bacilli morphologically similar to *B. pinottii* have been isolated from *P. boissyi* in Cairo by the writers.

For ordinary purposes for laboratory and field work *B. pinottii* may be cultivated on ordinary meat-extract broth or just in 1% peptone water. Since peptone is not commercially produced in Egypt and the imported product is very expensive in that country, we decided to try

to cultivate the *bacillus* on several cheap media. The experiments that were made in Egypt with peptone cultures were made possible since we received materials that had been shipped from Rio de Janeiro by the National Malaria Service, thanks to the Director, Dr. Mario Pinotti.

Cattle's blood — This material can be bought cheaply from the slaughter house in Cairo. It was brought to the laboratory in sterile vessels after being collected from the carotid artery of the slaughtered

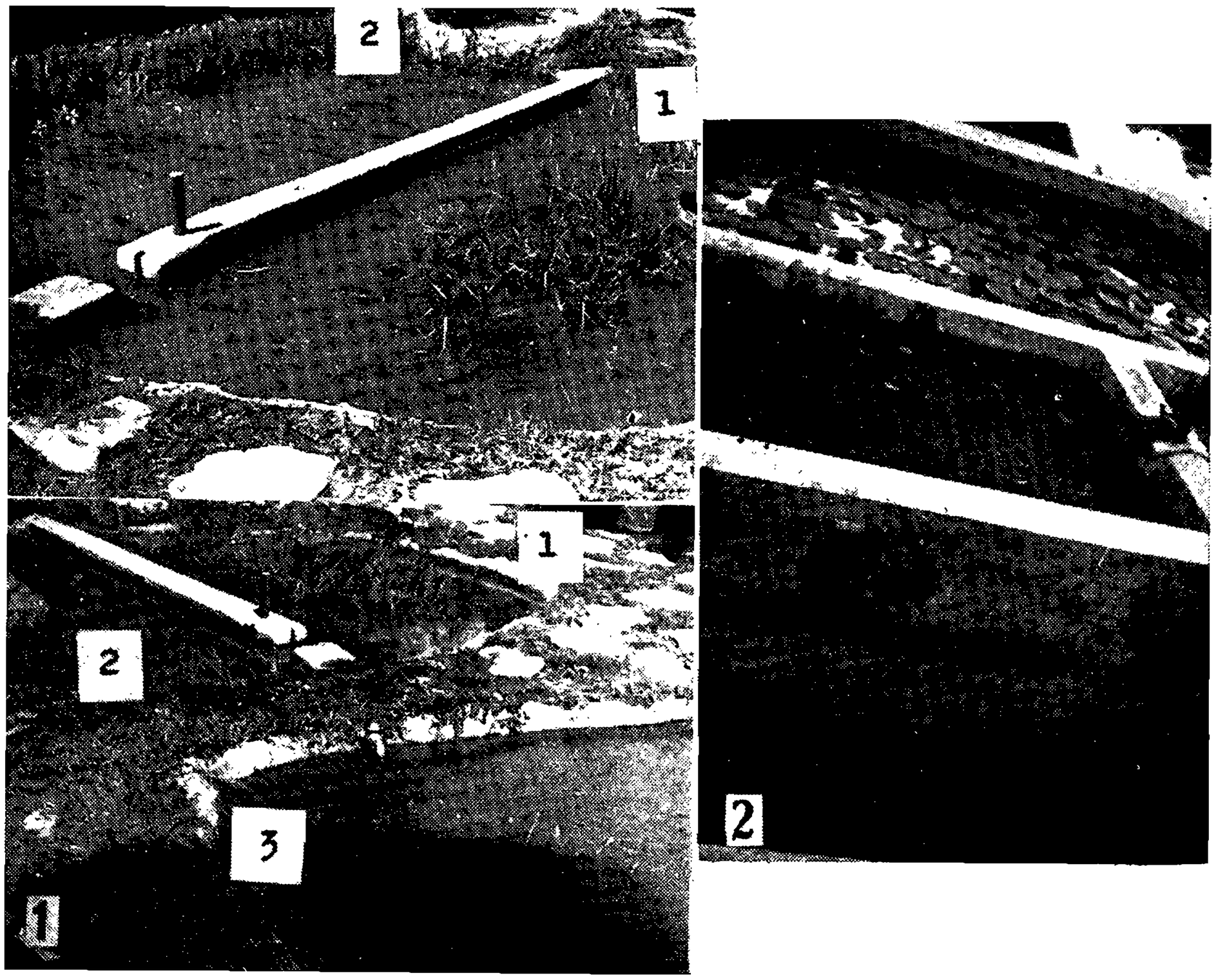

Fig. 1 — Pool at the Snail Destruction Section (experiment 5); fig. 2 — pool at the Research Institute (experiment 6).

animal. Dilutions of 1, 5, 10 and 20 per cent of whole blood in tap water were prepared in sterile vials; to these and to equivalent dilutions, to which traces of peptone were added, the bacillus was inoculated and the vials were kept at 37° for 24-48 hours. Good, pure culture of the bacillus developped in all dilutions, either with and without traces of peptone; it was more abundant after 48 hours and in the higher blood concentrations, so, for experimental purposes, concentrations from 5 to 20% or more may be used. For several reasons we tried to cultivate *B. pinottii* on cattle's boiled blood. For comparison, two flasks with 5% blood each were prepared and one of them was boiled for

several minutes. Both were inoculated with the same amount of culture and the growth appeared to be better on boiled blood. So we advise boiling the blood, a procedure that offers more than one advantage. In fact, not only less care may be exercised in collecting and transporting blood, since less contamination is expected if it is to be boiled, but also the application of the culture in the field will be better accepted by people if it is made on boiled blood, which shows a less repugnant appearance than the cultures on fresh blood.

Yeast — Used yeast (*Saccharomyces cerevisiae*) may be obtained freely from beer factories, or at low cost (100 kg of liquid or pressed yeast cost respectively 1 and 3 Egyptian pounds). Dried yeast contains 60-70% proteins. Ten per cent liquid yeast in tap water was tried as a culture medium for BET bacillus but it was found that, although some growth developped, it was inferior to the one that takes place on peptone or blood cultures. Both living and dead yeast were tried. Growth is improved by the addition of peptone.

Laboratory-made peptone — We prepared it in the usual way (1 litre tap water, 300 g cleaned, minced stomach, 100 cc hydrochloric acid, etc.) (cf. Hook & Fabian, 1943) using stomachs from pig, sheep and cattle. Good cultures were obtained in media prepared with such peptones.

ANIMAL EXPERIMENTS

Although previous experiments, carried out in Brazil, had shown that *B. pinottii* was harmless to dogs and guinea pigs, either by inoculation, *per os* or by rubbing cultures on the skin, some more experiments were performed in Cairo:

One cock was given 5cc of peptone culture by mouth for two consecutive days. One dog (11 kg) took 100 cc by mouth twice a day for two days and then once a day for two more consecutive days. The animals did not show any ill effects during an observation period of two months. Blood was taken from the dog's vein one week after it started to be fed with cultures and was inoculated into culture media, which remained sterile.

Twelve gerbils were given by mouth 1 cc culture once a day for three consecutive days. A pregnant female died on the third day, but post mortem examination did not show any significant changes and typical bacilli were not found in smears from different organs. Another gerbil died on the 7th day, but cultures from heart blood were negative for *B. pinottii*. The nine other animals remained alive and healthy for over two months.

Culture was rubbed on the shaved and scratched skin of a guinea pig's abdomen, and was applied to the wounded skin of a gerbil: no further lesions developped and the wounded skin of the animals healed quickly. Pure culture was dropped in the eye of a dog and a gerbil, and no harm was noticed.

Mortality of fish and other vertebrates and invertebrates (excluding molluscs) has never been observed in Brazil in waters treated with *B. pinottii* cultures and small amounts of peptone.

Fig. 3 — Transportation of cultures in drums for the drain near the Pyramids (experiment 7); fig. 4 — preparing a partition of a drain with a mud and stone wall (experiment 7).

People working with the bacillus in the field for a long time, taking no special precautions, did not show any ill effects. Peptone used to be dissolved into cultures with bare hands. The writers and their helpers have been doing so and besides they have taken incidentally small amounts of culture into the mouth, but no harm was ever noticed.

EXPERIMENTS ON SNAILS

a) In the laboratory and in artificial pools

1 — A thick fluid (prepared some weeks previously by mixing powdered peptone with a culture of *B. pinotii* 1:1) was added to water containing snails in a vial, at a concentration of 1:50. After 24 hours, 73,3% of *P. boissyi* and 93,3% of *B. truncatus* were dead. After 3 days all snails were dead, while in the control vials a good number of them were living.

2 — Some *Bulinus* and *Planorbis* remained for 24 hours in water containing bacilli and peptone. After that, the dead and living ones were transferred to another jar containing just water, where they all died in three days. The water became turbid and presented the characteristic odour, while in the control it remained more or less clear. For several days the turbid water of the experiment jar was examined and found to be swarming with typical bacilli and spores.

3 — A paste of peptone and culture (as in 1) was diluted from 1:10 to 1:10 million in several glasses containing living *Planorbis* and *Bulinus*. At the end of 7 days all snails were dead, except in the last dilution, in which 50% of *P. boissyi* were living; in the controls the snails remained alive. Dead snails were not removed from the glasses. Microscopic examination showed that BET bacilli were present in the different dilutions, and were more abundant in the higher concentrations. No typical bacilli were seen in the water of the control vial.

This last experiment has been repeated in two series of dilutions of only pure culture and of only peptone, but the killing of snails was delayed. This suggests that peptone activates the bacillus in attacking the molluscs, as it had been previously shown (Dias, 1953). A similar experiment was made with water containing grinded snails (that had been killed by culture plus peptone), but again the killing of the snails was slower than in the equivalent dilutions of a mixture of peptone and pure culture.

Incidentally, no effects of cultures were noticed on mosquito larvae in these experiments.

4 — One per cent dilutions of *B. pinottii* in cultures (1) on 5% boiled blood, (2) 5% fresh blood and (3) in water from snails that were killed by culture and peptone, were tried on snails in similar glasses, one with just water serving as control. While in this one the molluscs remained alive after 5 days exposure, the other results were as follows:

Mortality of snails, per centage

Days	*Boiled blood*	*Fresh blood*	*Water from snails*
1	10	0	20
2	20	20	40
3	50	40	40
4	80	70	90
5	100	90	100

5 — Pool at the Snail Destruction Section.

This artificial cement pool, 900 litres capacity, was divided in two communicating parts (fig. 1). Part 1, with about 500 litres water, was

Fig. 5 — Culture spraying (experiment 7); fig. 6 — transportation of culture in drums for experiment 8; fig. 7 — spreading cultures (experiment 8).

treated on September 17 with 3,700 cc of a thick fluid prepared some weeks earlier by dissolving peptone into *B. pinottii* culture. 48 hours

later a good growth was apparent in both parts of the pool. The characteristic odour, which was intense, subsided on the 3rd day and disappeared on the 4th day, when some dead and apparently diseased snails were seen; fish were alive and remained so during all the experiment's duration. In the first days after the treatment the water's volume increased and some water passed to a nearby pool (3, fig. 1). It was noticed that the number of dead snails increased with time in parts 1 and 2 of the pool. On September 29 dead *Planorbis* and *Lanistes* were again seen there, and dead *Bulinus* and *Ampullaria* were seen in 3 (one dead *Ampullaria* was then taken from there and put in an aquarium containing several living *Lanistes* and other snails, which died in some 12 days). On September 30 (13 days), microscopic examination of the water from parts 1 and 2 (which had turned green) showed a large number of green protozoa. On October 5 (18 days) 86 dead (51,2%) *P. boissyi* were counted in part 1. Although the experiment was intended to show the propagation of the bacilli and their effect upon snails, a count was made on the 23rd day but the molluscs from (1), (2) and (3) were mixed up before counting; the following numbers and percentages of dead snails were obtained on October 10: *Planorbis* 178 (22,5%), *Melania* 37 (68,5%), *Vivipara* 22 (30,1%), *Lanistes* 13 (24,6%), *Bulinus* 16 (64,0%), *Physa* 12 (37,5%), *Cleopatra* 8 (88,8%) and only one living *Lymnaea* was found on that day.

30 days after the treatment the following result was obtained in snails collected from (1):

Snail	*Total no.*	*Dead*	*Dead* %
Planorbis	1 005	837	83,3
Physa	137	135	98,5
Vivipara	101	89	88,1
Bulinus	67	53	79,1
Lanistes	78	45	57,7
Cleopatra	20	20	100,0
Lymnaea	14	14	100,0
Ampullaria	12	0	0,0

Planorbis collected from the three parts on the 30th day showed a global mortality of 77,5% and on the 40th day, the last available result, a global mortality of 82,5%.

6 — Pools at the Research Institute

High concentrations of cultures were used in these experiments, in order to show that quick results could be obtained, so that the procedures could eventually be tried in the field under similar conditions. Trials were carried out in cement pools (fig. 2) with a thin layer of water and numerous molluscs. The following *B. pinottii* cultures were used: (1) in 1% peptone, (2) in 2% peptone and (3) in 10% boiled blood. One

similar pool was kept as control. Unfortunately these experiments were partly spoiled because not all the pools contained all the molluscan species that were used.

Results after 5 days exposure were as follows:

SPECIES	1% PEPTONE		2% PEPTONE		10% BLOOD		CONTROL	
	Nr. exam.	% dead	Nr. exam.	% dead	Nr. exam.	% dead	Nr. exam.	% dead
Cleopatra	240	62,5	973	98,7	235	76,6	149	14,1
Physa	150	66,6	175	100	42	95,2	=	=
Vivipara	120	75,0	287	99,7	69	95,7	44	0
Planorbis	55	90,9	=	=	=	=	=	=
Bulinus	48	83,3	5	100	=	=	=	=
Lymnaea	45	88,9	7	100	1	100	9	0
Lanistes	35	28,6	36	100	66	54,5	44	0
Melania	30	83,5	56	89,3	15	66,6	30	13,3
	723	69.8	1 539	98,7	428	77,8	276	8,7

b) Experiments in the field

7 — A part of a drain near the Pyramids (figs. 3, 4, 5), nearly 40 m long, 1,5 wide and 0,5 m deep was separated from the rest of the drain by a mud wall. After most of the water had been removed, it was heavily sprayed with *B. pinottii* cultures to which 5 per cent powdered peptone was added (November 3, 1953). Vegetation was only grass and there were many snails from different species. Next day the water volume had increased and dead snails as well as small dead fish could be seen there. On November 5 the drain section was full of water and was starting running over the mud wall; a number of snails was then collected, washed in fresh water in the laboratory and counted next day, with following results:

Species	*No. collected*	*No. dead*	*Per cent mortality*
Vivipara	338	338	100
Lymnaea	111	111	100
Physa	104	99	95
Melania	34	34	100
Bulinus	36	36	100
Lanistes	32	32	100
Cleopatra	11	10	90
	666	660	99

Two other small sections of the same drain were similarly treated, one with culture in 20% boiled blood and the other with water from a drum that contained a large number of dead snails; although some dead snails were found 48 hours later, results proved to be far interior to the above ones.

Fig. 8 — Examining of a drain section 24 hours after treatment (experiment 8); fig. 9 — spraying a small drain with *B. pinottii* culture and peptone.

8 — In a similar partition of another drain (near Imbaba) a preliminary experiment had been performed, with large amounts of blood and peptone cultures, but without the water volume being reduced (nearly 30 cubic meters); since during some weeks only a small snail

mortality was noticed, another treatment was performed there (figs. 6, 7), with nearly 150 liters of peptone culture plus 5% powdered peptone, after most of the water had been taken off (November 25, 1953). Next

Fig. 10 — Spraying a small drain with *B. pinottii* culture and peptone; fig. 11 — workers from the Snail destruction Section with nets.

day, however, the water had come back (fig. 8) and it looked as if a good growth occurred *in loco*, in spite of the temperature being low; dead snails were seen.

Results after one week were as follows:

Snail	*Total*	*Dead*	%
Physa	150	148	98,7
Planorbis	35	34	97,1
Lymnaea	4	4	100

9 — A small drain containing a small amount of water, grass and many *Physa* was closed from the distal end and sprayed with 24 liters culture plus 10% peptone (figs. 9, 10). *Planorbis* did not exist there, but some specimens were introduced there from a nearby canal. Next morning snails were collected and examined after being washed in fresh water for two days: 52 out of 94 *Physa* (55,3%) and 20 out of 22 *Planorbis* (90,0%) were dead. After the only counting of dead and living snails from this drain, the experiment was spoiled by the owner of the drain, who opened and cleaned it next day.

10 — (Luxor, Nov. 21, 1953) — A small collection of water, containing many *Bulinus*, was separated from the main body by a mud wall and divided into two parts. To one of them, 2 liters of a thick paste (prepared on August 8) made of peptone and BET culture. Ten days later 36% of the snails from the treated part were found to be dead and 13 days after the treatment the mortality was 79%, while in the control part there were no dead *Bulinus*. On this day fresh snails of same species were added to the previously treated water and 70,6% of these were dead a week later.

DISCUSSION AND CONCLUSIONS

Although research work on the biological snail control has just started, from the results described in the present paper and in others it may be seen that this line of investigation seems to be a very promising one. As it is rightly stated by McMULLEN (1952), "Observations in the laboratory and the field by various individuals have indicated that snail colonies sometimes disappear but reasons for this are not understood. The effect of bacteria, fungi and viruses on snails and their environment has received little attention and it is possible that something of value would be discovered in such investigations". During his observations upon the rearing of *A. glabratus* in the laboratory, STANDEN (1951) incidentally noticed that "Inclusion of meat extracts, 'Marmite' or other similar substances, is not advisable, because they diffuse out into the aquaria and soon set up undesirable bacterial activity", and COWPER (1946) has found that in certain localities "tre tap-water contains a fungus (*Catenaria*) which attacks and destroys mollusc (*P. guadeloupensis*) egg-nests".

Bacillus pinottii was apparently the first useful microbe to be isolated from snails, in Brazil. It appears to be a saprophytic bacterium that under certain conditions may develop some degree of virulence.

Morphologically similar bacilli from snails have been encountered in Venezuela (personal communication from J. F. TORREALBA) and now in Egypt.

The search for other useful microbes is being carried out and should be encouraged everywhere, and opportunities should not be missed for trying to isolate pathogenic organisms from snails, that could possibly be responsible for naturally occurring epizootics (which, by the way, the writers never happened to observe).

It is important to look not only for new, useful microbes but also for cheap peptones or other cheap nutrient substances that might be used as culture media. Quite recently, DIAS (1954 b) has published a paper in which are described new biochemical procedures for snail control, based on the effect of mollasses and other materials on the snail's environment, with or without the addition of special microorganisms (yeasts, bacteria). He also succeeded in quickly eradicating molluscan colonies by introducing into the water spontaneously fermented mollasses plus pure mollasses or pure mollasses alone (unpublished data).

In the present paper it has again been shown that *B. pinottii* is harmless to vertebrates (guinea pig, dog, chick, gerbil) and that it is also probably harmless to man. As to its effects on Egyptian snails, it has been shown that under certain conditions it is lethal for *Bulinus*, *Planorbis* and other molluscs. Field experiments have clearly demonstrated the quick lethal effect of cultures plus peptone in drains containing comparatively small amounts of water, although these amounts have increased soon after the experiments have started. Substitution of peptone cultures by blood cultures did not prove to be as effective in the field. Since peptone is rather expensive in Egypt, this method of control, as it has been used in the field trials here described, probably could not be extensively adopted there. On the other hand, we feel sure (from the results already obtained in Brazil and from what is known about the conditions of breeding places in Egypt) that similar good results may be easily obtained by means of one of the much less expensive biochemical methods recently developed, especially the ones in which mollasses are used.

Biological control measures are probably easier to be taken in Egypt against *P. boissyi* than against *B. truncatus*. In fact, according to HELMY (1953), *P. boissyi* has a limited, irregular distribution in the Nile Valley; its chance of life is only offered if the snail finds its way into stagnant water, which is the only place in which it can settle and breed. The Nile River is apparently free from the snail, so destruction of a colony means irreparable extinction of a focus. It is thus clear that the essential characters of a *P. boissyi* focus in Egypt are that the water should be (a) sufficiently stagnant and (b) sufficiently permanent".

As to *Bulinus*, it is known that it occurs both in Lower and in Upper Egypt and that it is common in the source basin of the Nile. "Surveys of the banks of the Nile showed the snails in some back-water baylets containing vegetation. From March to July, when the water level of

the river is at its lowest, residual pools are found on either side of the bed. These are full of aquatic weeds and serve as favorable breeding places for *Bulinus* and in many instances have been responsible for local bilharzia infection". (ABDEL AZIM, 1948). "As to the bays which lie along the banks of the river... in Egypt they do not become suitable for *Bulinus* breeding before April, that is to say six to seven months after the end of the flood" (HELMY, 1953).

Biological treatments of breeding places (preferably with mollasses) should apparently be best carried out during occasions when the water is at its lowest, and it is known that desiccation of the irrigation channels is easy to obtain; for this, profit should be taken from the usual irrigation operations: "In Egypt water is under control in that irrigation channels are constantly submitted to such operations as I) The winter stoppage, when the water is shut off in the agricultural districts for the period of forty days... II) The summer rotations, when the canals are given a full supply every alternate week only, so as to avoid water--logging of the lands during the Nile flood. This repeated desiccation inflicts serious losses on the snail fauna, even to its entire destruction... (HELMY, 1953).

Since "The Egyptian species, *Bulinus truncatus* and *Planorbis boissyi* are nonoperculate and entirely aquatic. Their successful destruction would cut the bilharziasis problem at its root and it is the method of choice for the control of the disease" and since "From 1942 onwards, the Bilharzia Snail Destruction Section has been making complete snail surveys... The surveys are still going on and will ultimately cover the whole country, giving a complete picture of the distribution and intensity of infestation with these snails" (ABDEL AZIM), conditions for trying and using new effective weapons for controlling these pests appear to be especially favorable in this country. As Dr. ABDEL AZIM put it, "The prospects for effective control in Egypt are favorable, for, unlike other countries with a bilharzia problem, the area infested with snails is sharply defined. The water supply is under the control of the Irrigation Department, which makes possible a large measure of control over the breeding places".

DISCUSSÃO E CONCLUSÕES

Embora tenham apenas começado as pesquisas sôbre as possibilidades do contrôle biológico dos caramujos, pode ver-se, pelos resultados descritos neste e em outros trabalhos, que esta linha de investigação parece de fato ser muito promissora. Como bem disse MCMULLEN (1952), "Observations in the laboratory and the field by various individuals have indicated that snail colonies sometimes disappear but reasons for this are not understood. The effect of bacteria, fungi and viruses on snails

and their environment has received little attention and it is possible that something of value would be discovered in such investigation".

No decurso de suas observações sôbre a criação de *A. glabratus* no laboratório, notou incidentemente STANDEN (1951) que "Inclusion of meat extracts, 'Marmite' or other similar substances, is not advisable, because they diffuse out into the aquaria and soon set up undesirable bacterial activity". E COWPER (1946) observou que em certas localidades "the tap-water contains a fungus (*Catenaria*) which attacks and destroys mollusc (*P. guadeloupensis*) egg-nests".

O *Bacillus pinottii* foi aparentemente o primeiro micróbio útil a ser isolado de caramujos, no Brasil, parecendo ser êle uma bactéria saprófita que em certas circunstâncias pode se tornar virulenta. Bacilos que se lhe assemelham morfològicamente foram encontrados em caramujos na Venezuela (comunicação pessoal de J. F. TORREALBA) e agora no Egito. Deve ser incrementada a pesquisa de outros micróbios úteis por tôda a parte, e não se deve perder oportunidade para tentar isolar microorganismos patogênicos de moluscos, que serão possìvelmente responsáveis por epizootias naturais dêstes animais (que, aliás, nunca foi dado aos presentes autores observar).

É importante procurar, não sòmente novos micróbios úteis, mas também conseguir a obtenção de peptonas baratas ou outras substâncias nutritivas de baixo custo, que possam servir como meio de cultura. Recentemente, DIAS (1954b), publicou um trabalho no qual descreve novos processos bioquímicos para contrôle de caramujos, baseados na ação do melaço e de outros materiais sôbre o meio aquático, com ou sem a adição de microorganismos especiais (levêdos, bactérias): também conseguiu erradicar ràpidamente colônias de moluscos por meio de tratamento em que empregou o melaço fermentado espontaneamente e acrescido de melaço puro, ou de sòmente melaço puro (dados não publicados).

No presente trabalho tornou a ser demonstrado que o *B. pinottii* é inócuo para os vertebrados (cobaia, cão, galinha, *"girbil"*) e que também é, provàvelmente, inócuo para o homem. Quanto a ação em relação aos caramujos egípcios, foi demonstrado que em certas condições êle é letal para *Bulinus, Planorbis* e outros moluscos. Experiências de campo mostraram claramente o rápido efeito letal de culturas com peptonas em drenos contendo volume d'água comparativamente pequeno, embora êsse volume aumentasse logo depois de começadas as experiências. A substituição das culturas em peptona por culturas em sangue de boi não se mostrou tão eficaz, no campo. Desde que a peptona importada é um produto caro, no Egito, êste método, tal como foi empregado nas experiências aqui descritas, provàvelmente não poderia ser extensivamente adotado no país. Por outro lado, parece-nos certo (pelos resultados já obtidos no Brasil e pelo que se sabe das condições dos focos do Egito) que resultados semelhantes e igualmente bons podem ser fàcilmente obtidos por meio de um dos processos bioquímicos muito mais baratos recentemente criados, especialmente aqueles em que o melaço é empregado.

Provàvelmente as medidas de contrôle biológico serão mais fàcilmente tomadas no Egito em relação ao *P. boissyi* do que ao *B. truncatus*. Com efeito, segundo HELMY (1953), "*P. boissyi* has a limited, irregular distribution in the Nile Valley; its chance of life is only offered if the snail finds its way into stagnant water, which is the only place in which it can settle and breed. The Nile River is apparently free from the snail, so destruction of a colony means irreparable extinction of a focus. It is thus clear that the essential characters of a *P. boissyi* focus in Egypt is that the water should be (a) sufficiently stagnant and (b) sufficiently permanent".

Com relação ao *Bulinus*, sabe-se que êle ocorre tanto no Baixo quanto no Alto Egito e que êle é comum na própria Bacia do Nilo. "Surveys of the banks of the Nile showed the snails in some back-water baylets containing vegetation. From March to July, when the water level of the river is at its lowest, residual pools are found on either side of the bed. These are full of aquatic weeds and serve as favorable breeding places for *Bulinus* and in many instances have been responsible for local bilharzia infection" (ABDEL AZIM, 1948). "As to the bays which lie along the banks of the river... in Egypt they do not become suitable for *Bulinus* breeding before April, that is to say six to seven months after the end of the flood" (HELMY, 1953).

O tratamento biológico dos criadouros (de preferência com melaço) deve aparentemente ser executado de modo mais eficaz nas ocasiões em que a água está reduzida ao mínimo, e é sabido que a dessecação dos canais de irrigação é fácil de se obter; com êste fim, devem aproveitar-se as usuais operações de irrigação: "In Egypt water is under control in that irrigation channels are constantly submitted to such operations as I) The winter stoppage, when the water is shut off in the agricultural districts for the period of forty days... II) The summer rotations, when the canals are given a full supply every alternate week only, so as to avoid water-logging of the lands during the Nile flood. This repeated desiccation inflicts serious losses on the snail fauna, even to its entire destruction... (HELMY, 1953).

Desde que "The Egyptian species, *Bulinus truncatus* and *Planorbis boissyi* are nonoperculate and entirely aquatic. Their sucessful destruction would cut the bilharziasis problem at its root and it is the method of choice for the control of the disease" e que "From 1942 onwards, the Bilharzia Snail Destruction Section has been making complete snail surveys... The surveys are still going on and will ultimately cover the whole country, giving a complete picture of the distribution and intensity of infestation with these snails" (ABDEL AZIM), as condições para experimentar e empregar novas armas para o contrôle dos caramujos parecem ser especialmente favoráveis nesse país. Como disse o dr. ABDEL AZIM "The prospects for effective control in Egypt are favorable, for unlike other countries with a bilharzia problem, the area infested with snails is sharply defined. The water supply is under the control of the Irrigation Department, which makes possible a large measure of control over the breeding places".

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