THE RELATIVE SUSCEPTIBILITIES OF BULINUS TRUNCATUS (AUDOUIN) AND SAROTHERODON MOSSAMBICUS (PETERS) TO CERTAIN MOLLUSCICIDES

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by

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ABSTRACT.

In spite of varying opinions as to the effectiveness of molluscicides in the control of schistosomiasis, the search for more effective and highly selective molluscicides should continue, so that adverse effects on non-target organisms caused by commercially available molluscicides may be reduced and the possibility of resistance guarded against.

To this end, the differences in susceptibility to Frescon and 4'-chloronicotinanilide between the schistosome-bearing snail, Bulinus truncatus and a representative tropical food fish, Sarotherodon mossambicus, were examined and discussed in relation to the rate of uptake of these compounds and their distribution among different organs.

The rate at which water is taken up by B. truncatus and S. mossambicus has also been calculated and related to the rate of uptake of molluscicides. This part of the work was an attempt to elucidate the mechanism of carriage of the chemicals into the animals being studied.

It has been demonstrated that B. truncatus and S. mossambicus concentrate Frescon and 4'-chloronicotin-anilide to high levels. For B. truncatus, both Frescon and 4'-chloronicotinanilide were concentrated in the pseudobranch, while in S. mossambicus the highest concentration of Frescon was found in the liver and that

of 4'-chloronicotinanilide in the bile.

The high tolerance of S. mossambicus to 4'-chloronicotinanilide was found to be due to the rapid metabolism of the compound in fish to more polar forms which are more easily disposed of via the bile.

Comparison of the relative susceptibilities of two field collections of B. truncatus showed that snails collected from the Frescon-treated area of the Gezira display a higher tolerance to Frescon than do snails collected from the untreated area. Frescon uptake rate was found to be lower in the less susceptible snails, and this is tentatively suggested as the basis of the observed difference in tolerance. It is additionally shown that B. truncatus infected with Schistosoma haematobium is more susceptible to Frescon than uninfected snails.

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CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

1. Schistosomiasis.

Schistosomiasis is a chronic disease caused by digenetic trematodes of the genus, Schistosoma and transmitted by fresh water pulmonate snails belonging to the family, Planorbidae and by amphibious operculates belonging to the sub-family, Hydrobiinae. There are two clinical forms of the disease, urinary schistosomiasis caused by Schistosoma haematobium (Weinland) and intestinal schistosomiasis caused by S. mansoni (Sambon) and S. japonicum (Katsurada).

The adults of S. haematobium are usually found in the veins of the pelvic plexuses. On the other hand, the adults of S. mansoni and S. japonicum commonly inhabit the mesenteric veins. The female has the capacity to lay large numbers of eggs. For example, a single S. haematobium female is able to lay up to 250 eggs per day and its life span is thought to be less than two years, but a proportion of the parasites may live for longer periods (Nelson, 1975). An infected person may harbour a large number of worms (up to 250 pairs) which, with the great mass of eggs they lay and the host reaction to such bodies, can cause considerable perturbation in associated host tissue (Cheever, 1968).

Conservative estimates place the number of individuals infected with schistosomiasis at 150 million (World Health Organisation (WHO), 1965). The true figure may eventually be shown to be considerably larger with the

more widespread use of the improved diagnostic and epidemiological techniques which have become available in recent years. The increase in the prevalence of the disease in relation to the development of water resources (McMullen et al., 1962; Sturrock, 1965; Berrie, 1970), has caused concern among health authorities and has stimulated much needed investigations into the prevalence and transmission of the disease, the development and evaluation of different control measures, and improvements in the planning of new irrigation schemes (Webbe, 1969). The public health significance of the disease may be evidenced by the recent impetus by WHO to fund research into selected priorities, in the fields of epidemiology and control, chemotherapy and immunology (WHO Scientific Working Group, 1977).

2. Control of schistosomiasis.

Webbe, 1969, stated that the objective of control of schistosomiasis is to limit the spread of infection, to reduce morbidity or to control transmission and thereby reduce the intensity and prevalence of infection. Schistosomiasis can be controlled by employing one or more of four general methods: first, mass treatment of human cases to reduce or abolish release of eggs; second, sanitation measures; third prevention of contact with water; and fourth reduction or elimination of the snails which are essential to transmission of schistosomiasis to man.

2.1. Mass chemotherapy.

The mass treatment of human cases sounds as promising a measure for control as any, but alone would not be effective under the conditions presently existing in most of the endemic areas reviewed by WHO in 1961. The lack of a suitable drug has made mass chemotherapy in most endemic areas impracticable (Jordan and Webbe, 1969).

Lucanthone hydrochloride was first used about a quarter of a century ago both in experimental animals and in humans (Archer and Dennis, 1969). It was the first effective drug that could be given orally and it required a shorter period for treatment than the classical antimonial drugs (Rosi et al., 1967). Hycanthone, the hydroxy-methyl derivative of lucanthone (Rosi et al., 1965), shows considerable promise for the treatment of S. mansoni infections, producing a considerable reduction in egg output and a high cure rate. Many physicians regard niridazole (Ambilhar) as the drug of choice for S. haematobium and providing that S. mansoni and S. japonicum infections are early and confined to the intestinal phase, moderately good results can be expected in treatment of these infections (Davis, 1966; Wolf, 1967; Webbe, 1969; Farid et al., 1970). Metrifonate (Bilarcil) is the most interesting addition to the range of schistosomicides seen in recent years. It is of mono-specific activity in being effective only against infections with S. haematobium. There is either minimal or absent activity against S. mansoni in experimental models and in infected patients (Davis and Bailey, 1969; Pleština et al., 1972). On the basis of therapeutic

efficacy, estimated population coverage, tolerance, ease of administration and cost, metrifonate was considered to be marginally superior to niridazole and more so to hycanthone in the treatment of S. haematobium (WHO, 1973). Jewsbury and Cooke (1977) reported field trial results which showed that metrifonate has a prophylactic effect, is highly effective against S. haematobium, is easy to administer and safe.

Webbe and James (1977) demonstrated that a new compound Praziquantel (Embay 8440) exhibits a high degree of activity against the 3 major schistosome species in the hamster with no apparent significant differences in efficacy against the different geographical strains of the parasite. They noted that the efficacy of the compound against S. haematobium is significantly greater than that of metrifonate. It is clear that, although many compounds are known to possess schistosomicidal activity, the approach of mass treatment has the following limitations; first, multiple examinations are necessary if all infected persons are to be found; but since this is often impracticable, a large number of cases are generally missed; second, inadequate treatment due either to failure of the patient to complete the course of treatment or the failure of the drug to cure the patient; third, reinfection is common.

2.2. Sanitation measures.

To prevent contamination of snail-infested water by schistosome eggs, it is important to provide the community with a good system of sewage disposal. But,

this is expensive to install and often requires considerable modification of human habits. Again, S. japonicum can be maintained in non-human hosts (Pesigan et al., 1958 and WHO, 1961) and S. mansoni is also zoophilic (Riggin and Berrios Duran, 1956; Vianna Martins, 1958; Nelson, 1960; Fenwick, 1969) so that provision of a sewage disposal system probably would not entirely prevent the contamination of snail-infested water by schistosome eggs.

2.3. Prevention of contact with water.

It is true that schistosomiasis could be prevented if man did not come into contact with infected water. Unfortunately, most native populations, especially children, come into contact with infected water casually or through agricultural practice, bathing and other household functions. Provision of safe water for drinking, bathing and domestic use goes a long way towards reducing human infections, but this does not help the farmers who face occupational hazards in that they are regularly exposed to schistosome cercariae. However, evidence from Brazil (Barbosa et al., 1977) and South Africa (Pitchford, 1970) suggests that provision of safe water will reduce the prevalence of schistosomiasis. Jordan et al. (1976) and Jordan (1977) confirm this suggestion from longitudinal studies in St. Lucia.

2.4. Snail control.

Various methods for snail control have been tried with varying degrees of success. These include the extermination of snails through biological, physical or

chemical control.

2.4.1. Biological control.

Although biological control has been suggested from time to time during the past 50 years (Bequaret, 1928; Mozley, 1939), the subject has not attracted serious consideration despite the extensive literature on the predators, parasites and diseases of molluscs which might furnish leads for experimental investigations. Various workers have discussed controlling schistosome-bearing molluscs through the use of leeches and rotifers (Michelson, 1957) dipteran sciomyzid larvae (Berg, 1953, 1964; Echblad, 1973; Beaver, 1974) turtles (Michelson, 1957 and Hopkins, 1973) ducks (Cawston, 1921; Humphreys, 1932; Malone, 1965; Shiff and Mowbray, 1970) and various fish (Mozely, 1951, 1953; Bowmaker, 1968; Andrade and Antunes, 1969; Barnish, 1971; Blackburn and Sutton, 1971).

One of the few cases in which biological control seems to have been effective is the successful introduction of the ampullariid snail, Marisa cornuarietis (L.) for the control of Biomphalaria glabrata (Say). The history of the utilisation of Marisa for the control of Puerto Rican B. glabrata has been amply presented and its biology has become relatively well known (Chernin et al., 1956; Oliver-Gonzalez et al., 1956; Radke et al., 1961; Bartelt, 1970; Blackburn and Sutton, 1971; Ferguson, 1972).

2.4.2. Physical control.

Physical control is a specific form of sanitation of the water channels so as to make them unsuitable for snail

life. This can be achieved by the removal of snail habitat by destroying water plants at lake and stream edges either manually or with herbicides and by modification of agricultural practice and water management techniques such as increasing velocity of water, stream straightening, canal lining and channel design (Jobin and Ippen, 1964 and McJunkin, 1970). But this is found to be expensive and becomes increasingly difficult as new irrigation schemes are set up (McMullen, 1961).

Some notable successes have been recorded, however, in the Philippines and in Japan, where control of irrigation water, proper drainage and better agricultural practices have been instituted (Okabe, 1957; Pesigan et al., 1958; Hairston and Santos, 1961; Yokogawa, 1972). The Chinese under the guidance of the Thoughts of Chairman Mao Tse-tung have scored great successes in the prevention of schistosomiasis in different counties through environmental alterations. The methods used include soil burial, snail drowning, 'anti-snail belts' and burning of waterside vegetation (Anon, 1968a, b; 1977).

2.4.3. Chemical control.

Chemical control is probably the most rapid and feasible procedure for the elimination of snails (Webbe and Jordan, 1966) and has the advantage that it controls intermediate hosts of other diseases (e.g. fascioliasis). Japanese workers (Miyagawa, 1913, 1916) were the first to use chemical compounds such as calcium cyanamide for snail control. Chandler (1920), in the United States of

America, found that certain aquatic snails were killed in 48 hours by a solution of copper sulphate as low as 1-2 ppm and suggested that the chemical might be effective against schistosome-bearing snails. Since then many countries have adopted copper sulphate as a molluscicide and it is still in use in some countries (Venezuela, Egypt and Sudan). As a result of screening tests made on a series of compounds, McMullen et al. (1948) reported that sodium pentachlorophenate (NaPCP) was molluscicidal. McMullen et al.(1951) and McMullen (1952) demonstrated that dinitro-o-cyclohexylphenol (DN-1) was highly molluscicidal.

New and more effective molluscicides have been introduced into the field during the last two decades. According to WHO (1973), two outstanding compounds, niclosamide ethanolamine salt (Bayluscide) and N-tritylmorpholine (Frescon) are currently the molluscicides of choice against aquatic snails.

Niclosamide has the advantage that, although it kills snails, snail eggs and schistosome cercariae, it is not toxic to man and has limited biocidal effect (Gönnert and Schraufstätter, 1959). It is non-corrosive, reasonably persistent but not residual, and is degraded by sunlight into harmless organic chemicals. Its disadvantages are its high cost and its lethal effect on fish and some other aquatic animals. N-tritylmorpholine has the advantages of being effective at an extremely low concentration and having limited biocidal action (Boyce et al., 1968).

3. The ideal molluscicide.

Colwill (1957) defined the ideal molluscicide as one that remains effective in water at low concentrations for a prolonged period of time; it must be non-toxic to man and warm-blooded animals and innocuous to fish and wild-life; it must not repel snails and it must be cheap, readily available, easily transported, chemically stable and preferably agreeable to handle. He goes on to point out that in practice all prospective molluscicides fall short of this ideal. Furthermore, Duncan (1969) stated that if the molluscicide is insoluble in water, it should, if possible, be formulated as an emulsion concentrate or wettable powder and that it should be non-phytotoxic as far as crops are concerned, though herbicidal action in irrigation canals could be additionally useful.

When a compound is a new biocide it is essential to investigate the possible hazards to man, domestic animals and wildlife. This is usually done by exposing laboratory animals in standardised acute and chronic tests and examining them for toxicological effects. Biochemical studies are also usually conducted with the most active compounds to determine the metabolic fate of the compound.

Wright (1959) and Muller (1965) concluded that a better understanding of the mode of action of molluscicides gained through the study of hormone and enzyme systems of snails, may uncover some peculiarity in snail metabolism or function which would lend itself to specific chemical attack. In this way it should be possible to expand our

knowledge of biochemical mechanisms and to design new compounds.

The WHO Scientific Working Group on Schistosomiasis (1977) recommended that the search for new and more effective compounds should be continued and listed a number of selected research subjects for early attention. Amongst these was included studies of mode of action, molluscan physiology and biochemistry and molecular modification and structure-activity relationships, all of which presumably being aimed at improving molluscicidal specificity if not achieving the ideal molluscicide.

4. Detoxication mechanisms.

The majority of foreign organic compounds undergo definite chemical changes in the animal resulting in the excretion of specific metabolites usually via the kidneys. There are however, some compounds which are excreted unchanged, i.e. biochemically inert compounds, although they may be pharmacologically active (Brodie and Maickel, 1962). Excretion by other channels such as the expired air, the bile and the faeces, the saliva and the skin may also occur. The type of change which occurs depends primarily upon the structure of the compound but other factors such as the species, route of administration and diet may also be involved (Williams, 1959). These changes or biotransformations are usually divided into 4 main types: oxidation, reduction, hydrolysis and synthesis. The first three are many and varied, but in general compounds of similar structure are oxidised, reduced and hydrolysed in a qualitatively similar manner. The

synthesis reactions, or conjugation processes appear to be relatively few in number and are mainly reactions involving carbohydrates and amino acids. Whether or not a given compound will undergo any of the above syntheses will depend upon its possessing a particular chemical group or "centre for conjugation". If the compound does not carry such a group it may acquire one, for example by oxidation or reduction. It has been found that some synthetic processes are confined to particular classes of animals or even species.

To maintain a state of homeostasis the body must be capable of excreting unwanted material. This may be achieved through the kidney in the case of unchanged materials which are water soluble. Lipophilic compounds do not leave the body by this route, either because they are bound to proteins or lipid in the blood and therefore cannot enter the glomerular filtrate, or having entered the kidney tubules, they are reabsorbed by the lipid membranes of the cells lining the ducts. In order to be excreted, they must therefore be altered in the direction of increased water solubility i.e. the lipophilic compounds must be modified into derivatives of greater polarity. It has been usual in the past to refer to such mechanisms as detoxication mechanisms. This is not a complete statement of the case however, as the metabolites can be more toxic than the original compound. For example, Davson (1955) found that in the body the insecticide, parathion produces the more toxic compound, paraoxan.

The conventional view holds that drugs are detoxified by enzymes of intermediary metabolism which cannot distinguish foreign compounds from their normal substrates. The non-specificity of a series of enzymes for the oxidation of foreign compounds would raise an ancillary question of how normally-occurring substrates would be protected from attack by these non-selective enzymatic scavengers. This problem appears to have been met by the microsomal enzymes (Brodie and Maickel, 1962). It is said that the oxidative enzymes, despite their low specificity have a predilection for foreign compounds and do not molest normal substrates even when these have similar chemical structure. A possible explanation is that the microsomal enzyme might be segregated by the lipid membrane of the microsome which can be penetrated only by fat-soluble substances.

Again Brodie and Maickel (1962) found that a number of varieties of fish, both from fresh and salt water, cannot oxidise drugs in vivo nor can their liver microsomes carry out in vitro the oxidative mechanisms of N- or O-dealkylation, hydration, deamination or sulphoxidation. After a series of experiments, they found that the fish do not need the help of oxidative enzymes for disposal of lipid soluble compounds for these compounds are rapidly disposed of by passive diffusion through the lipoidal gill membrane. They stated that generally, aquatic animals do not possess the same oxidative enzymes as do terrestrial animals (reptiles, birds and mammals) which have developed such mechanisms in the course of evolution to dispose of lipid-soluble, unwanted

substance ingested in food. For terrestrial animals, without these enzymes, the foreign substances would gradually accumulate to toxic levels and life would cease.

5. Toxicity of molluscicides.

A much fuller understanding of the toxicity of molluscicides might be achieved if relevant information on molluscan physiology and biochemistry were available. An understanding of several basic phenomena associated with the uptake of compounds would be helpful, for example. This information might include, first, the possible modes of entry of molluscicides from the aqueous environment into snails and secondly the relationship between chemical structure and biological activity. Thirdly, the identification of the target cells and (or) tissues which are most vulnerable to the toxic action of the molluscicides or their metabolites might be established and whether the death of the mollusc results from the effect of the chemical on such cells or tissue. Fourthly, the mode of action of the molluscicide which causes lethality could be determined. These are some of the basic data that must be looked into before a more rational approach can be taken in developing new molluscicides which possess the desirable properties of the ideal molluscicde discussed above.

5.1. The possible modes of entry of molluscicides into snails.

One of the first clues to the character of living membranes was supplied by Overton (1902). Since then considerable evidence has accumulated in support of

Overton's thesis that the cell membrane is mainly lipoid in character (Gorter and Grendel, 1925; Parpart and Dziomian, 1940; Davson and Danielli, 1952; Mayer and Maickel, 1960). Recent evidence from electron microscope pictures and X-ray diffraction patterns (Singer, 1975; Singer and Nicolson 1972; Capaldi, 1974) has revealed that all cell membranes (i.e. outer and inner) are essentially alike, being composed of protein and fatty acid substances and that lipid accounts for about half of the mass of the membranes. In inner membranes, the lipid is entirely phospholipid. Substances which are lipid-soluble might therefore be expected to pass into membranes by dissolving in them. However, much remains to be learned before there can be a good understanding of the relation between membrane structure and membrane permeability. For example, there is the question of the nature of membrane pores; their presence in the lipoid layer seems necessary to explain the passage of water and the ready diffusion of small, lipid-insoluble molecules and ions across cell membranes (Andersen and Ussing, 1957; Berliner, 1959; Paganelli and Solomon, 1957; Solomon, 1958).

There is also the question of how certain inorganic ions and lipid-insoluble molecules, such as glucose and amino acids, rapidly cross the cell boundary. Giese (1959) has discussed the factors which may influence movements of solutes through cell membranes. Amongst these he includes the effect of molecular size and ionisation. He pointed out that charged molecules enter much more readily than ions. The cell membrane is accordingly envisaged as a

mosaic of negative and positive charges with a net positive charge. An anion might, therefore, be expected to enter more rapidly than a cation. The valency of an ion will also play a part in its entry. The differences which are recordable between entry rates for various ions of the same valency could be explained by hydration of the ion which will alter the size of the various particles. The entry of such molluscicidal ions as copper may be catalysed in this way, though as will be seen later, Azevedo et al. (1958) provide an alternative explanation based on their finding that radioactive copper appears to be concentrated and absorbed through the intestinal tract.

Schanker (1962) concluded that the ways by which solutes move across membranes may be grouped into two general categories, passive transfer processes, and specialised transport processes. In the passive transfer processes, the membrane behaves as an inert solvent layer or system of aqueous channels through which the solute passes and thus many substances can move across the membrane by either simple diffusion or filtration. In specialised transport, the membranes display an active character, transporting the solute in a manner that cannot be explained by the structure or physical properties of the membrane; the substances passing through the membrane by either active transport, facilitated diffusion, exchange diffusion or pinocytosis.

Duncan (1969) reviewed the methods or routes by which the molecules might enter the body of the snail as follows:

5.1.1. Ingestion with food and water followed by transport across the gut wall. This may be the case with molluscicides formulated as very fine particles as was indicated by Hopf et al.(1963) for copper compounds of low solubility.

5.1.2. The ingestion of particulate matter by the outer surface cells of mollusc mantle is reported by Nakahara and Bevelander (1967). The mantle epithelium of the calico clam, Macroscollista maculata, was seen to ingest colloidal gold and carmine particles. Using colloidal thorium dioxide (thorotrast) and electron microscopy, these authors have also observed a similar phenomenon in two related species of bivalves, Pinctada radiata and Isognomon alatus.

5.1.3. Alternatively, chemicals could pass through the external membrane of the snail as molecules rather than particles. Harris (1960) has outlined the ways in which this might occur. He states that the passage of molecules or ions from a solution into a membrane when no chemical forces operate, is a consequence of the collision between the particles and the membrane which gives rise to a certain surface concentration followed by diffusion within the membrane material. Somers (1963) working on uptake of copper fungicides into fungal spores reported that the uptake of ^{64}Cu by the cells is probably an ion-exchange reaction which is followed by permeation throughout the cell. There was no evidence of an adsorption process and copper is therefore considered to be accumulated passively by the spores by unspecific reactions with cell

constituents. Cheng and Sullivan (1974) using ^{67}Cu revealed that the accumulation of copper intermingled with mucus on the surface of the foot region of B. glabrata is probably not the primary site of entry of the metal ion. On the other hand, the accumulation of copper of the surface of the rectal ridge appears to be of significance since copper in the aqueous environment is brought into contact with tissue by the water current entering the dorsal mantle chamber, and it is postulated that this area may be the site of entry.

5.2. Structure-activity relationships.

Drug action had previously been correlated with partition coefficient (Overton, 1901) but it is only in the last twelve years that serious attempts have been made to quantify relationships between physicochemical properties of compounds and the biological responses they elicit. In this way, not only the mechanisms of action might be explained but the activity of unprepared congeners may also be predicted (Dunlop, 1976). Free and Wilson (1964) suggested that biological activity is an additive property of the substituents on a molecule. Everytime a substituent appears in a molecule it is assumed that it will play a constant role either contributing to or detracting from the overall biological activity.

Dixion (1948) found that the greater the electron withdrawl from the halogen atom, X, in compounds of type $R-\overset{O}{\overset{\|}{C}}-CH_2X$, the more active the system will be as a lachrymator and thus the more reactive it will be towards

sulphydryl groupings. De Villiers and Mackenzie (1963), applying the same argument to the molluscicidal activity of the phenacylhalides, proved that phenacylbromide is more active than phenacylchloride. This is also the case with two p-iodo-phenacyl compounds and two m-nitrophenacyl derivatives. They conclude that increasing the positive nature of the halogen atom, either by going from chlorine to bromine or by the correct substitution on the phenyl ring, increases the molluscicidal activity. Similarly, Wain (1963) made a systematic investigation, of the effects on herbicidal action, of replacing the $-NO_2$ groups in DNC(2:4-dinitro-6-methylphenol) by -CN. A number of 2- and 4-hydroxybenzonitriles were subsequently synthesised and their activity as weed killers was assessed by standard methods. In general, only poor performance was shown by derivatives of salicylnitrile but a number of compounds derived from 4-hydroxybenzonitrile were shown to possess outstanding herbicidal activity and moderately good molluscicidal activity; e.g. 3¦5-diiodo-4-hydroxy-benzonitrile (ioxynil) and the 3¦5-dibromo analogue (broxynil). Dunlop (1976), examining quantitative structure-activity relationships between molluscicidal activity and the physical and chemical properties of nicotinanilides, revealed a positive correlation between molluscicidal activity and partitioning.

5.3. Distribution of molluscicides among organs.

Little work has been done on this line of investigation, which could be an important one in providing clues as to the mode of action of molluscicides. Application of

radiotracer methods can tell whether compounds accumulate in any particular tissue or cells and the rate of accumulation in the various organs might also be related to molluscicidal effect.

Azevedo et al.(1958), using radioactive sodium orthophosphate, showed that the radioactivity of the soft parts of the snail was x4 that of the shell. Using solutions containing ^{32}P, ^{131}I and ^{64}Cu they succeeded in showing by means of autoradiographs of Helisoma duryi tissue, that the radioactive compounds were absorbed by the intestinal tract and became diffused through the various organs, with a tendancy to accumulate in the hepatopancreas. Duhm et al.(1963), using ^{14}C-niclosamide, found that storage of activity could not be established in any organ of the rat, not even following repeated application. Less than 0.5% of the applied activity could be traced in the animal 48 hours after the final application. Of this 0.5%, the greater part was found in the gastrointestinal tract. Somers (1963) and Martin (1969) used radioactive copper to show that fungal conidia may concentrate copper by a factor of up to 100-fold over that in the immediate environment.

Beynon (1971), using Rasbora spp., related the build up of ^{14}C-Frescon (up to x50 the actual concentration in the water in 4 hours) to the filtration of the solid particles of the chemical from the water by the gills of the fish. Matthiessen (1977), using Sarotherodon mossambicus, found that after 55 hours exposure to a sub-lethal solution of Frescon, approximately 40% of the Frescon was found in

the bone, 16% in the muscles, 16% in the gut and 10% in the gill. He suggested that the relatively high concentration found in the bile, liver and gut indicates that liver is involved in Frescon excretion.

5.4. Action of molluscicides.

The process leading to the production of energy i.e. to the synthesis of adenosine-5'-triphosphate (ATP), starts in the cytoplasm with the biotransformation of glucose to pyruvate. Energy production and biosynthesis are also required for growth. Lower molecular weight compounds such as amino acids and purines are produced in the cytoplasm; protein synthesis takes place in the ribosomes; DNA in the nucleus and RNA partly in the nucleus and the remainder in the cytoplasm. Kaars Sijpesteijn (1970) summarised the process which leads to the production of ATP into four main stages; first, alteration of glucose to pyruvate, second, oxidation of pyruvate to carbon dioxide via the tricarboxylic acid cycle (TCA cycle), third, hydrogen or electron transfer via flavin enzymes, cytochromes and cytochrome oxidase to form water using up oxygen and fourth, oxidative phosphorylation to form the energy rich compound, ATP from adenosine-5'-diphosphate (ADP). These four processes are coupled in such a way that inhibition of any of them leads to inhibition of respiration as well as inhibition of ATP production.

Brand et al. (1949) surveyed 72 compounds for their inhibitory effect on the oxygen consumption of

Australorbis glabratus (= B. glabrata). They could distinguish 4 types of reaction: no effect; transitory inhibition; slow inhibition, rapid and lasting inhibition. But, due to the sharp initial retraction of snails into their shells and the implications of this retraction on oxygen uptake, the mode of action of the compounds could not be ascertained with certainty. There were some indications however, that enzyme inhibition is involved in the latter two reaction types listed above. The most effective inhibitor reported by them was α-nitrostilbene which, at a concentration of 10 ppm, reduced oxygen consumption by more than 99%.

Wain (1963), investigating the mode of action of the 3¦5-dihalogeno-4-hydroxybenzonitriles as herbicides found that these compounds were strongly active in uncoupling oxidative phosphorylation. Since this property is shown also by 2¦4-dinitro-6-methyphenol and pentachlorophenol, both of which are well known molluscicides, he examined them for molluscicidal activity and found that ioxynil had a LC_{50} of about 0.5 ppm and broxynil about 2.0 ppm when tested, as their sodium salts, against B. glabrata.

5.5. Mode of action of some molluscicides in current use.

5.5.1. Sodium pentachlorophenate (NaPCP).

Weinbach (1954) suggested that the molluscicidal activity of pentachlorophenol (PCP) is due to the interference with the oxidative phosphorylation process. He indicated that it is a powerful uncoupler of oxidative phosphorylation in both snail and rat tissues. Weinbach and Nolan (1956) found that PCP, unlike 2,4-dinitrophenol (DNP), inhibits

the ATP-ase activity in a variety of mitochondrial preparations including 'soluble systems'. Ishak et al. (1970) revealed that the activity of NaPCP is due to its uncoupling action when used in low concentrations (3 ppm) but at higher concentrations (above 3 ppm) this could be attributed to the inhibition of the glycolytic pathway.

5.5.2. Copper compounds.

Hopf et al.(1963) tested various copper compounds of low solubility and found the activity increased with decreasing pH. It was concluded that toxic action was due to cupric ion (Cu^{2+}). Martin (1969) also suggested that some copper compounds probably act via the cupric ion which means that cuprous oxide must be oxidised before becoming effective; the mechanism, enzymatic or otherwise, by which this is done is unknown however.

Ishak et al.(1970) found that the oxidation of various substrates by homogenates of B. glabrata was inhibited by concentrations of copper sulphate at micromolar levels but that neither pyruvate nor α-ketoglutarate were involved. Kaars Sijpesteijn (1970), in a review of the mode of action of agricultural fungicides, suggests that copper ions might be fungitoxic through interference with pyruvate dehydrogenase. Although copper sulphate has been shown to inhibit oxygen uptake by some fungal spores at concentrations that just prevent germination (McCallen et al., 1954) there do not appear to be any studies on whether pyruvate or α-ketoglutarate accumulates in fungi treated with inorganic copper. Corbett (1974) concluded

that the inhibition of dihydrolipoyl dehydrogenase, and hence the pyruvate (and possibly α-ketoglutarate) dehydrogenase system, provides a possible explanation of the effect of cupric ions on fungi, but that other mechanisms may operate as well.

If copper does work by inhibiting keto-acid oxidation, one would expect copper salts to be generally biocidal. However, possibly due to lack of uptake by higher plants and insects, inorganic copper salts are not widely used to control pests other than fungi, though copper sulphate has been used as a selective herbicide and algicide (Martin and Worthing, 1972) besides its use as a molluscicide.

5.5.3. Organotin compounds.

Trisubstituted-tin compounds, whose activity and mode of action has been reviewed by Kaars Sijpesteijn et al. (1969) are thought to work by inhibiting oxidative phosphorylation. This is mainly based on the evidence by Aldridge and Street (1971) that these or related compounds are active inhibitors of oxidative phosphorylation in isolated mammalian mitochondria. There is evidence that triethyltin inhibits ATP synthesis by binding to a component of the energy conservation mechanism, and by interfering with the exchange of hydroxide ions and anions across the mitochondrial membrane (Rose and Aldridge,1972).

5.5.4. Niclosamide.

Gönnert and Schraufstätter (1959) showed that the concentrations of niclosamide which are lethal to snails

strongly inhibit oxygen uptake by the whole snail, while lower concentrations were found to stimulate respiration by up to 40%. Ishak et al.(1970) explained the molluscicidal activity of the molluscicide on the basis of its powerful inhibitory action on the oxidative processes of the snail. They showed that it stimulates respiration at very low concentrations (0.000033 ppm) and that at a concentration of 0.3 ppm it inhibits succinate oxidation by 45%, glutamate oxidation by 70% and reduced tetramethylparaphenylene diamine (TMPD) oxidation by 15%. Later,Ishak and Mohamed (1975) confirmed the inhibition of the respiratory rate by niclosamide and they were able to demonstrate that sub-lethal concentrations markedly reduced the respiration rate and that the rate of oxygen consumption decreased at increasing concentrations of the chemical.

5.5.5. Frescon.

Beynon (1971) stated that Frescon breaks down readily to form triphenylcarbinol (TPC) and morpholine in water, soil and plants and that these compounds are known to be of a low order acute and sub-acute toxicity to mammals. Both Frescon and TPC are lipophilic but they will not be stored in animal fat since mammals convert them to hydroxylated derivatives which are readily excreted.

Brown and Hubble (1969) associated the toxicity of Frescon with the N-tritylmorpholine molecule rather than with the hydrolysis products, TPC and morpholine. Beynon (1971) found that the metabolism of Frescon itself in rats and dogs was similar to that of a mixture of TPC and

morpholine and he suggested that it is probable that Frescon is hydrolysed in the gut prior to adsorption. The morpholine is excreted largely unchanged in urine but TPC is metabolised mainly by hydroxylation in the para-position followed by conjugation with glucuronic acid. These conjugates are hydrophilic compounds unlike Frescon which is lipophilic. Moreover, Griffiths (1968) found that Frescon is more toxic to rats when administered intravenously than orally and this may be due to gastric acids speeding up the hydrolysis rate. He also showed that the mammalian liver can hydrolyse Frescon and that traces of hydroxycarbinols could be detected as metabolites both free and conjugated.

Using ^{14}C-Frescon, Beynon (1971) suggested that its metabolism in fish would probably occur with longer exposure times, as he was able to identify only unchanged Frescon in fish exposed to treated water for 30 minutes. Similarly, using thin layer chromatography (TLC) on ether/ethanol extracts of fish exposed to ^{14}C-Frescon for 30 minutes, Griffiths (1968) showed that the material in the fish was unchanged, since the radioactive peak co-chromatographed with unlabelled Frescon. He also reported that the rate of removal of Frescon or its metabolites from the fish body appears to be slower than in mammals, possibly due to the inability of the fish to convert Frescon and TPC to more hydrophilic compounds and this may be due to the absence of drug-metabolising enzymes in fish as has been reported by Maickel (1960) and Brodie and Maickel (1962).

It seems that Frescon does not directly affect respiration like many other molluscicides e.g. niclosamide and pentachlorophenol by interfering with oxidative phosphorylation and it appears more likely that it acts on the nervous system of the snail (Shell Chemicals, 1974). It was added that Frescon appears to act on the synapses, either by increasing the amount of transmitter-substance released from the presynaptic terminals, or by potentiating the action of the transmitter at the post-synaptic cell membrane. It is not yet known whether the actual concentrations of Frescon required to produce these effects on the isolated nervous system can account for the molluscicidal activity in Frescon. Moreton and Gardner (1976) have suggested the nervous system as a possible site for Frescon action in freshwater snails. They showed that Frescon modifies the action of synaptic networks so as to cause intermittent massive discharges affecting the entire nervous system. These "Frescon bursts" were shown to occur after a minimum of 10-20 minutes in nerve cells from the visceral or right parietal ganglion of the isolated central nervous system of Lymnaea stagnalis exposed to 10 ppm of molluscicide. Banna (1977) working on both Archachatina and Bulinus was able to demonstrate these "Frescon bursts" in some neurons together with the inhibition of normal activity in others. Accordingly he suggested that the chemical may also be affecting the interneurone pools.

6. Present work.

It is evident from the above survey that a large amount

of information exists on the different aspects of the mechanisms of action of molluscicides. However this information is diffuse, lacking the coordination in direction and continuity which would help in achieving a better understanding of the susceptibility of molluscs, aquatic biota or domestic animals to molluscicides.

With the above points in mind, the present study was made in order to examine the difference in susceptibility between the schistosome-bearing snail, Bulinus truncatus and a representative tropical food fish, Sarotherodon mossambicus to two molluscicides; Frescon, which is known to be toxic to both snails and fish at more or less the same concentrations and 4'-chloronicotinanilide, a candidate molluscicide which is relatively non-toxic to fish (Matthiessen, unpublished).

The ratio of uptake and loss of the two compounds were studied in order to find out whether these rates influence molluscicidal activity and also whether they are the cause of the difference in susceptibility between the two species. It is also essential to study their metabolism in order to determine the nature of their metabolites and whether they are excreted or retained in the body.

The relative susceptibilities to Frescon of various groups of B. truncatus collected from the Gezira were also examined and the implications of these results on future application of Frescon in the Gezira are discussed.

CHAPTER 2

MATERIALS AND METHODS

MATERIALS AND METHODS

1. Molluscicides.

Strictly speaking N-tritylmorpholine or N-triphenylmethylmorpholine is the active ingredient of the commercial emulsifiable concentrate formulation, Frescon. The names are often used interchangeably, however, as they are in this text.

1.1. N-Tritylmorpholine (Frescon).

N-Tritylmorpholine was kindly supplied as a 16.5% w/v emulsifiable concentrate (Frescon FX 28) by Shell Research Ltd., Sittingbourne, Kent; sample FC 5730.

1.2. Radioactively-labelled N-tritylmorpholine (^{3}H-Frescon).

Radioactively-labelled N-tritylmorpholine, (N-triphenylmethyl [G-^{3}H] morpholine), with universal tritium label in the morpholino ring was also supplied by Shell Research Ltd. (Specific Activity = 21.85 μCi/mg on 1st June, 1974).

1.3. 4'-Chloronicotinanilide.

This compound was prepared by slowly adding nicotinyl chloride-hydrochloride to 4-nitroaniline in a minimum of dry pyridine and refluxing for 90 minutes. The precipitate gave a satisfactory elemental analysis (C,H,N ± 0.3%) (Dunlop, 1976).

1.4. 4'-Chloronicotin[ar-^{3}H]anilide (^{3}H-4'-chloronicotinanilide)

This was synthesised by an acid catalysed exchange reaction between tritiated water and the substituted aniline, followed by acylation with nicotinylchloride. The chemical

had chemical and radiochemical purities > 99% (Specific Activity = 47.0 μCi/mg on 21st August, 1974)(Dunlop,1976).

2. Other radioactive compounds.

2.1. n-[1,2(n)-^{3}H]Hexadecane.

Tritium labelled n-hexadecane (Specific Activity = 2.27 μCi/gm on 1st December, 1973) was obtained from The Radiochemical Centre, Amersham, Buckinghamshire.

2.2. Tritiated water ($^{3}H_2O$).

This was from stock from The Radiochemical Centre, (Specific Activity = 11.2 mCi/ml on 15th March, 1977).

3. Scintillation materials.

Insta-gel and Dimilume-30 (universal liquid scintillation scintillants) and Soluene-350 (tissue solubiliser) were obtained from Packard Instrument Ltd., Caversham, Berks.

4. Chromatography materials.

Silica gel GF(250 microns) precoated analytical thin layer chromatography plates were obtained from Anachem, Luton, Bedfordshire.

5. Reagents and solvents.

All reagents and solvents were of analytical grade and were obtained from British Drug Houses Ltd.,Poole, Dorset.

6. Animals.

6.1. Bulinus truncatus (Audouin).

These were collected from two different locations; from Hebeika canal in the Frescon-treated area of the Gezira

Scheme, Sudan and from Abu Gueli canal in the non-treated area of the same scheme.

6.2. Biomphalaria glabrata (Say).

This is a Brazilian strain reared under standard conditions (Duncan and Brown, unpublished). This snail replaced Bulinus truncatus in one experiment on the distribution of radioactive material among organs. This was considered acceptable since they are of the same family though it is also recognised that species differences may occur.

6.3. Physa acuta (Muller).

The strain originated from the Wonji-Shoa Sugar Plantation, Ethiopia and has been bred in London at the C.O.P.R. for 8 years.

6.4. Sarotherodon mossambicus (Peters).

This is a laboratory bred strain (= Tilapia mossambica (Peters)) originally obtained from the Malaysian Agricultural Research and Development Institute, Malacca.

6.5. Schistosoma haematobium (Weinland) miracidia.

These were hatched from eggs collected from the urine of infected schoolboys in Kereiba Village, Gezira, Sudan.

7. Rearing methods.

7.1. Bulinus truncatus.

The two collections were bred separately in identical perspex aquaria containing 9 litres of an aerated, artificial hard water (AHW : 0.104 gm CaCl and 0.26 gm $MgSO_4.7H_2O$ per litre of distilled water (Hopf and Muller, 1962)) at 26°C and under fluorescent light on a

12 hr/12 hr diurnal cycle. To rear the snails for experimental use, about 200 adults were transferred to a new tank for about 24 hours and then removed again, leaving behind egg masses whose age was therefore known within 1 day. The eggs began to hatch after 8 days and the hatchlings were fed on small additions of ground TetraMin fish food (Tetra Werke, West Germany). When the snails were 10 days old, 100 snails were moved to a new tank and from then on were fed on dried lettuce twice a week with frequent additions of TetraMin fish food. This method arranged that all snails in any one aquarium were of the same age, presumably in the same physiological condition and more or less of the same size and weight. The relationship between the shell height and body weight was examined. The shell height was measured at the greatest distance between the basal margin and the uppermost point of the shell, the apex, taken parallel to the axis (Mandahl-Barth, 1962). It is evident from Fig. 1 and Appendix 1 that a curvilinear relationship represented by the biparametric equation $y = ax^b$ exists (where a = 0.16 and b = 3.08) and that a straight line relationship seems best satisfied by plotting logarithms of the two parameters (Fig. 1a).

7.2. Biomphalaria glabrata.

These were cultured at 26°C and under fluorescent light on a 12 hr/12 hr diurnal cycle. About 100 snails were put into 8 litres of AHW when they were 3-4 days old, fed on boiled lettuce and provided with under-gravel and airlift filtration through charcoal (Duncan and Brown, unpublished).

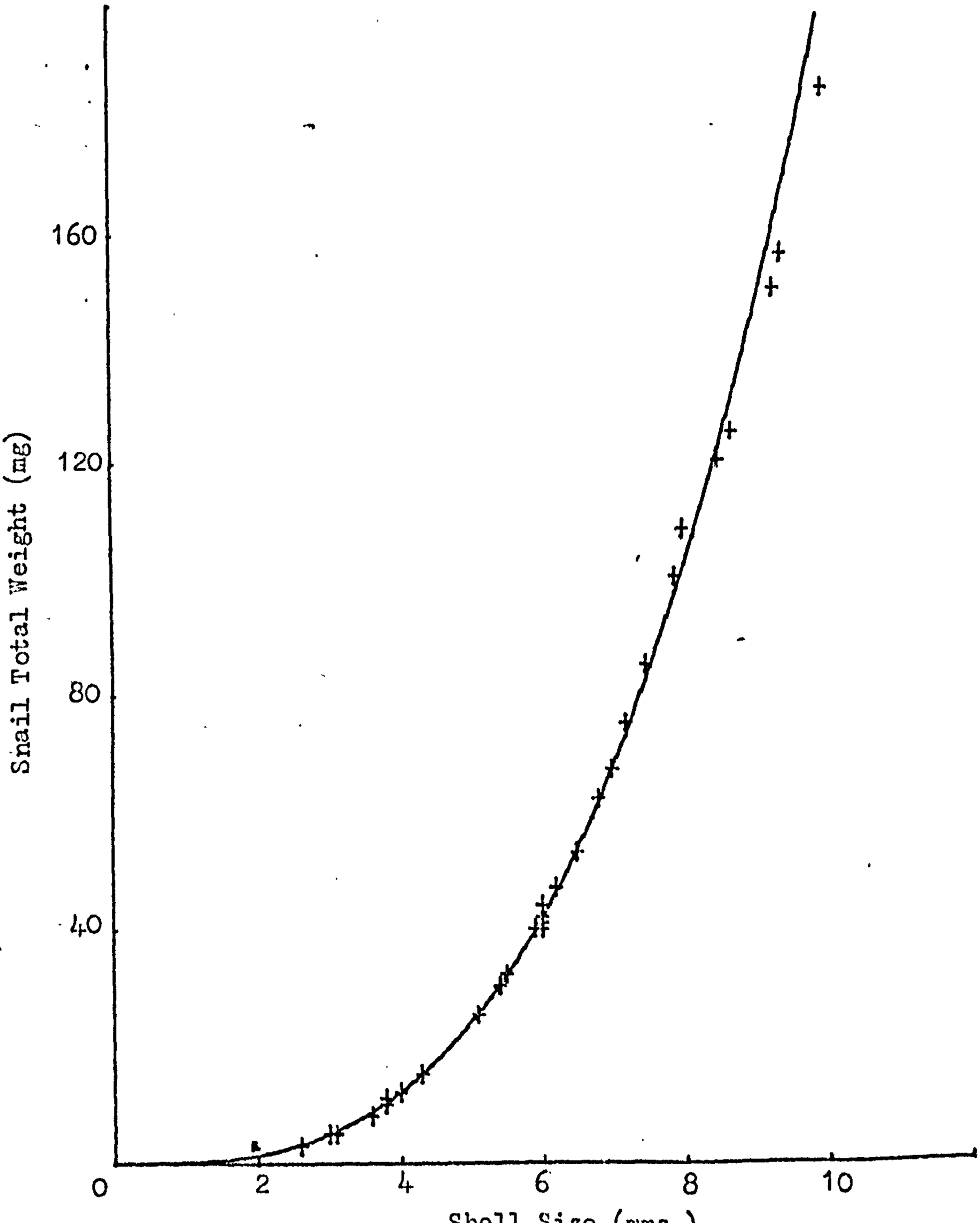

Figure 1. RELATIONSHIP BETWEEN SHELL SIZE AND SNAIL TOTAL WEIGHT OF BULINUS TRUNCATUS.

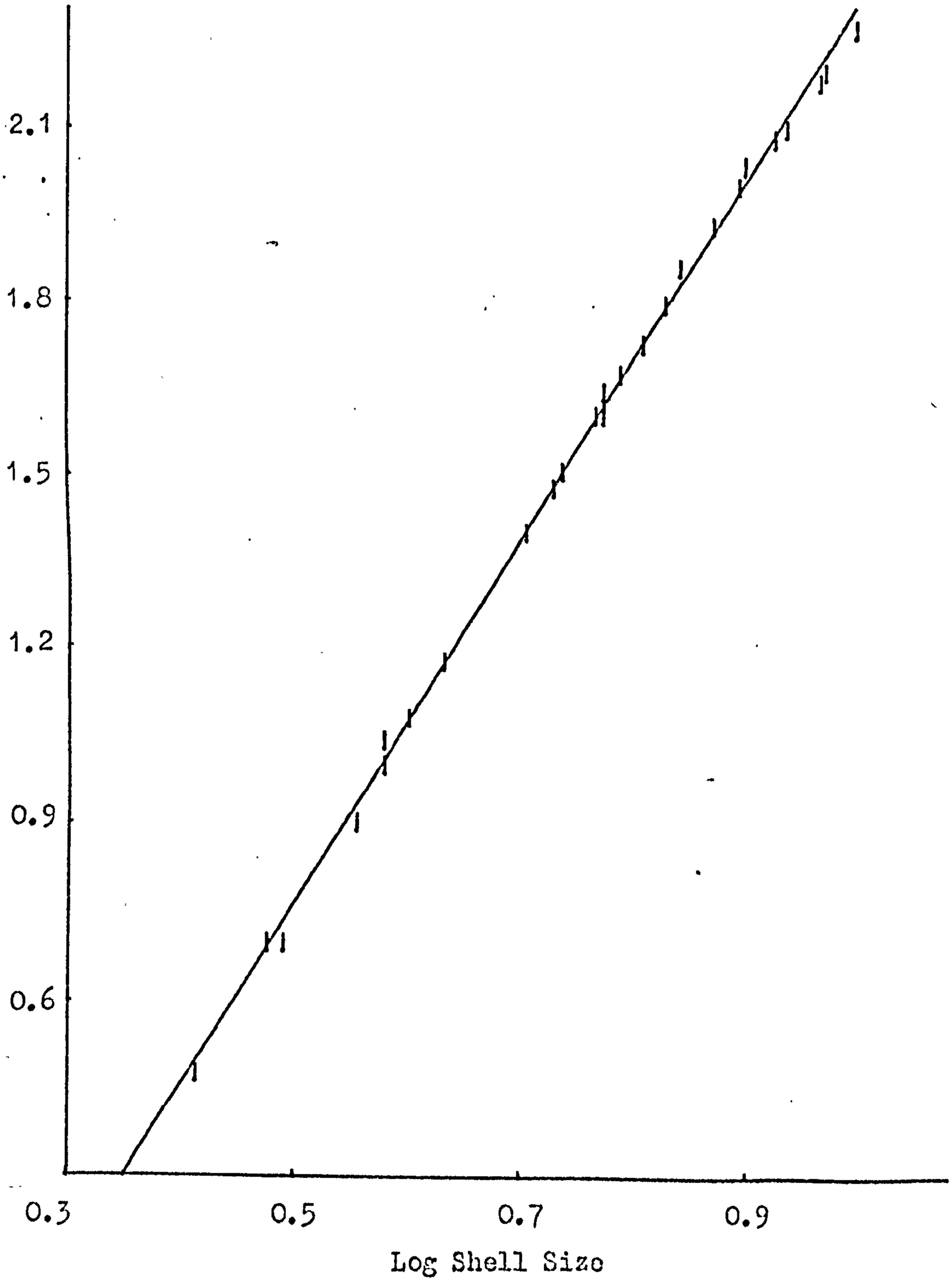

Figure 1a. RELATIONSHIP BETWEEN SHELL SIZE AND SNAIL TOTAL WEIGHT OF BULINUS TRUNCATUS.

7.3. Sarotherodon mossambicus.

These were reared and bred under standardised laboratory conditions (Matthiessen, unpublished). A maximum of 10 adults was kept in a 30 litre glass aquarium through which pre-heated (27-28°C), dechlorinated and air saturated London tapwater (total hardness = 291 mg/l as $CaCO_3$, pH = 7.9-8.0) was pumped at a rate of 6 litres per hour. The tanks were illuminated by fluorescent light on a 12 hr/12 hr diurnal cycle. The fish were fed on Main Stream Trout Diet, Standard 2 expanded, pellets (BP Nutrition Ltd., Witham, Essex). For the first four weeks, after the fry have been removed from the female, they are kept in 20 litre perspex tanks at a density of about 5 fish per litre. The fry were fed on ground Trout Diet twice a day. All the fish used were the first generation descendants of the original stock.

8. Flow-cell apparatus.

In order to expose snails to radioactive molluscicides under constant conditions of molluscicide concentration, oxygen tension and temperature, the flow-cell apparatus described by Duncan et al.(1977) was used.

The apparatus (Fig. 2) consisted of a cylindrical glass cell 5 cm high x 2.5 cm diameter. A glass tube with a bell-shaped end was clamped so as to stand 1 mm away from the bottom and sides of the cell. Another glass tube whose end had been turned through 90°, was held within the first tube by a polythene collar. The exposure cell was held level with the tops of the two brown-glass, Winchester bottles. A peristaltic pump was used to drive air in to

Figure 2. Single Cell Unit With Inflow And Outflow Connections.

displace water from one of the bottles and so into the cell. The air line passed almost to the bottom of the Winchester thus helping to keep the water aerated. After passing downwards under the bell, the water was evacuated through the stainless steel tubing by means of a second peristaltic pump set to work slightly faster than the first. A three-way tap was employed to redirect the air flow to a second Winchester bottle which contained the molluscicide solution so that the molluscicide was passed into the cell. The exposure cell was replicated 10 times and the entire apparatus (Fig. 2a) was contained in a 135 litre water bath which was maintained at constant temperature by means of 6 thermostatically controlled heaters distributed evenly on the bottom of the bath, and 2 electric mixers, one on each side of the bath, to distribute the heat evenly.

9. Measurement of radioactivity by the Liquid Scintillation Counting method.

9.1. Introduction.

Liquid scintillation counting is a method of assessing the radioactivity of beta-emitting isotopes (e.g. ^{3}H, ^{14}C, ^{32}P). The samples are dissolved in a solvent containing a solute that fluoresces or scintillates when excited by beta-particle energy. Solubiliser may be necessary in order to dissolve some samples and the whole mixture is termed a 'cocktail'. Since the radioactive sample is in intimate contact with the cocktail constituents, the efficiency of the energy transfer is maximised. The beta particles excite the solvent molecules which in turn transfer the energy to the scintillator resulting in the

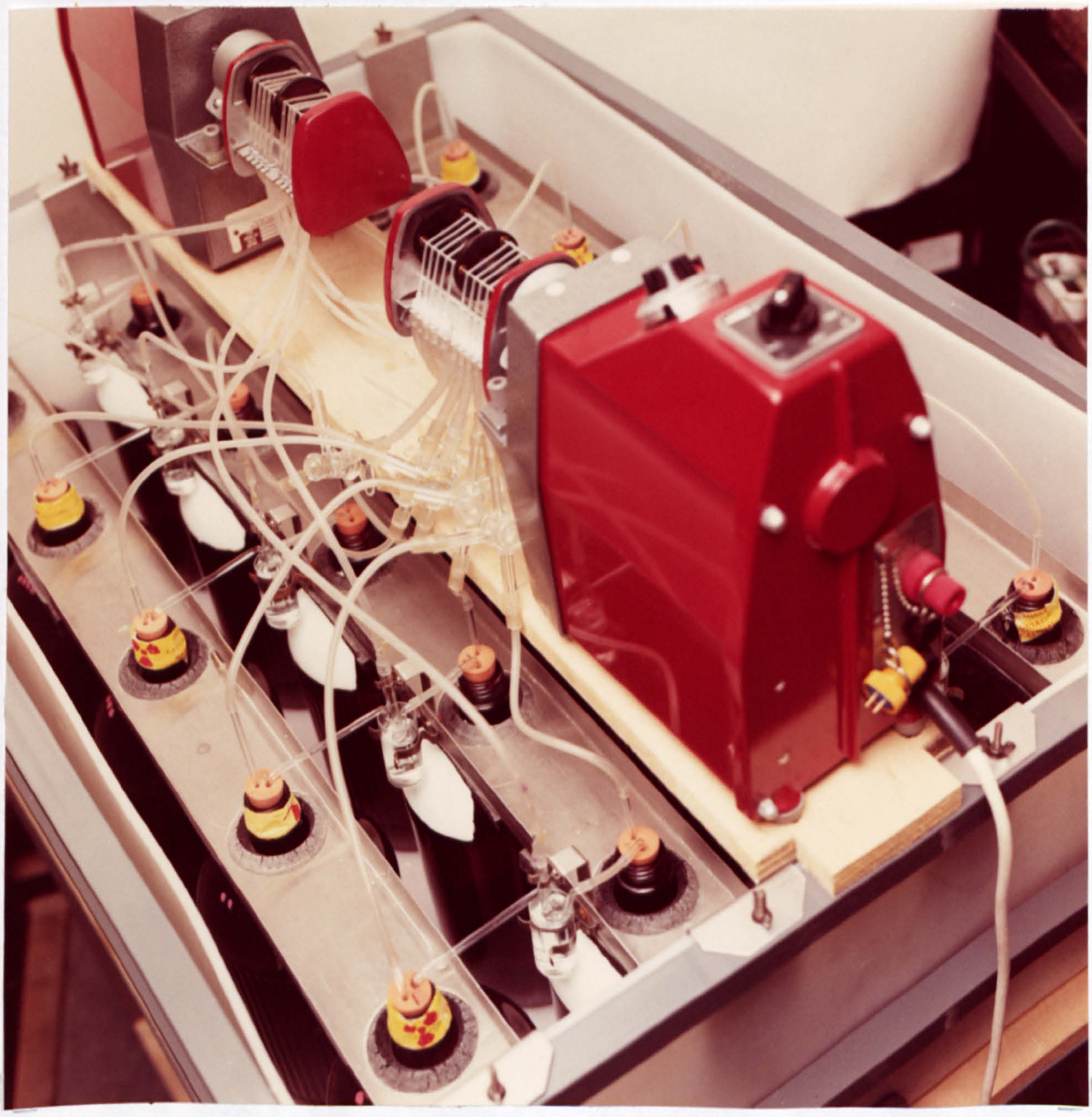

Figure 2a. Replicated Cell Arrangment Housed In Water Bath.

production of photons of light which pass through the wall of the vial containing the sample to be detected and converted to electrical pulses by photomultipliers. The pulses produced are proportional to particle energy and are sorted into channels by analyser units incorporating pulse height discriminator circuits.

With experimental samples, a number of undesirable effects occur. These include interference with the sample-solvent-scintillator energy transfer mechanism by chemical substances (e.g. dissolved oxygen and solubilisation chemicals) or the colour of the solution absorbing emitted light. The result of these effects is to cause a reduction in the detector photomultiplier pulse height voltage, resulting in a shift of the pulse height spectrum as observed by the system analyser circuits. These effects are termed chemical and colour quenching respectively.

9.2. Sample preparation for Liquid Scintillation Counting.

9.2.1. Combustion.

After exposure to labelled compounds, whole adult *B. truncatus* and fry *S. mossambicus* were removed from the solution, blotted dry, weighed and measured, then wrapped in a small piece of tissue and folded into a Packard 'Combustocone' and immediately burnt in a Packard model 305 Oxidiser set to wash the condenser with distilled water (1 ml) and add Instagel scintillant (14 ml) to a scintillation vial.

9.2.2. Solubilisation.

Weighed portions of various tissues of S. mossambicus and B. glabrata were placed in counting vials and Soluene-350 (1 ml) was introduced into each vial which was kept at 28°C for 24 hours. To ease the solubilisation the vials were frequently agitated. Dimilume (14 ml) was then placed in each vial and the samples were left to stand over night. Dimilume is a commercial scintillant which also suppresses chemiluminescence.

9.3. Liquid scintillation spectrometer.

The samples were counted at 13.5 ± 1°C in a Packard model 2425, liquid scintillation spectrometer using the automatic external standard ratio method to correct for quenching.

9.4. Quench calibration.

The model 2425, liquid scintillation spectrometer incorporates an external gamma-ray emitting source (^{266}Radium) in a shielded compartment which is automatically positioned close to the vial for quench calibration. The gamma source interacts with the solvent and scintillator and gives rise to a spectrum of photon energies very similar to the ^{3}H beta spectrum. The emission spectrum due to the external source is affected by quenching agents in a similar way to that of the sample beta source and this fact is employed in developing an automatic quench correction for experimental samples. Calibration curves of counting efficiency versus external standard ratio (ESR) are generated as described below.

9.4.1. Combusted sample preparations.

Different volumes of distilled water (0.25, 0.50, 0.75, 1.00, 1.25, 1.50 and 1.75 ml) were pipetted into separate vials and Insta-gel (14 ml) was added to each vial. An internal standard or 'spike' of 50 µl of ^{3}H-n-hexadecane (100% efficiency = 174010 disintegrations per minute (dpm)) was then introduced using a microsyringe. (GKN Sharlow Meterology Ltd. The samples were then counted in the spectrometer. From the counts per minute (cpm) obtained for each vial the % efficiencies were calculated

$$\% \text{ Efficiency} = \frac{\text{cpm} \times 100}{174010}$$

The calculated % efficiencies were then plotted against the external standard ratio obtained for each vial (Appendix 2). The result as shown in Fig. 3 was a linear-relationship represented by the equation

$$y = 0.0644 + 0.0150x$$

9.4.2. Solubilised B. glabrata.

Eight snails were crushed in 8 ml of Soluene-350 and kept at 28°C for 24 hours. Different volumes (0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 ml) of the solubilised sample were pipetted into separate vials and 14 ml of Dimilume were added. A spike of 50 µl ^{3}H-n-hexadecane (100% efficiency = 179445 dpm) was introduced using a microsyringe. Single snails were also crushed and solubilised in 1 ml of Soluene-350 and treated in the same manner. The samples were then counted in the spectrometer. From the cpm obtained for each vial the % efficiencies were calculated and these were plotted against the ESR obtained for each vial (Appendix 2a). The result as shown in Fig. 3a is a

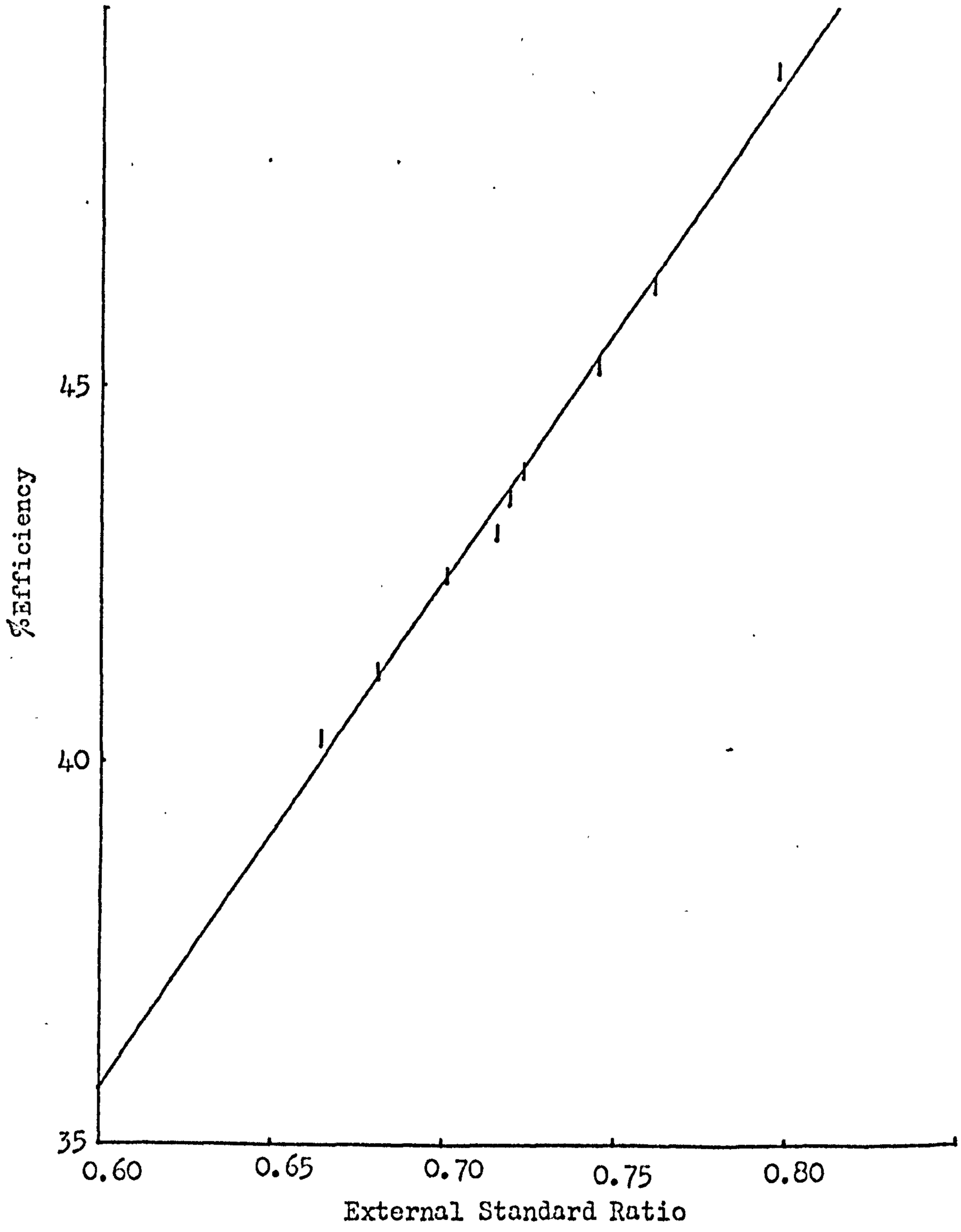

Figure 3. TRITIUM QUENCH CALIBRATION CURVE FOR COMBUSTED SAMPLE PREPARATIONS.

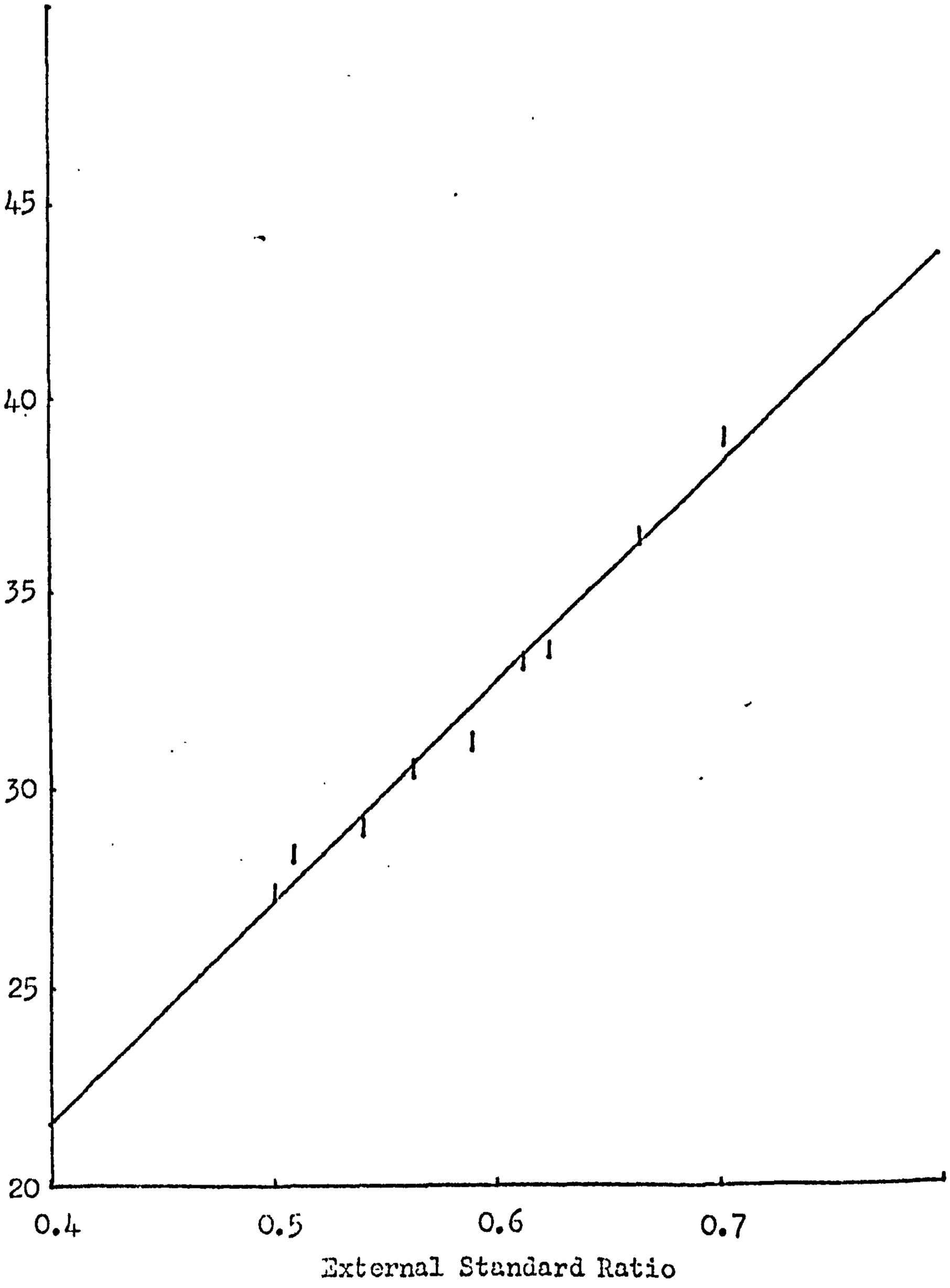

Figure 3a. TRITIUM QUENCH CALIBRATION CURVE FOR SOLUBILISED B. GLABRATA.

linear relationship represented by the equation

$$y = 2.57 + 59.21x$$

9.4.3. Solubilised S. mossambicus.

S. mossambicus was dissected and between 0.1 and 0.6 gm of various tissues were placed in counting vials. Six ml of Soluene-350 were introduced into each vial and the tissues were left to solubilise at 28°C for 24 hours. Varying amounts (0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 ml) of solubilised tissue were pipetted into separate vials. Dimilume (14 ml) was placed in each vial followed by 50 µl of ^{3}H-n-hexadecane (% efficiency = 182004 dpm) using a microsyringe. The samples were left overnight to reduce chemiluminescence resulting from the interaction between the solubilised tissue and the scintillant (Peter Matthiessen, personal communication) and were then counted in the spectrometer. From the cpm obtained for each vial the % efficiencies were calculated.

It is evident from Fig. 3b that a linear correlation is best obtained when the logarithm of the calculated % efficiencies were plotted against ESR obtained for each vial (Appendix 2b). From the relationship the % efficiency can be calculated by the equation

$$\% \text{ efficiency} = \text{antilog } (0.734)(\text{ESR}) + 1.072$$

10. Recording of snail activity.

10.1. Time-lapse cinematography is one of a number of techniques which can be used to record animal activity. Single snails were held in 4.5 mm petri dishes partially submerged in a water bath at 27°C, while groups of 6 snails were held

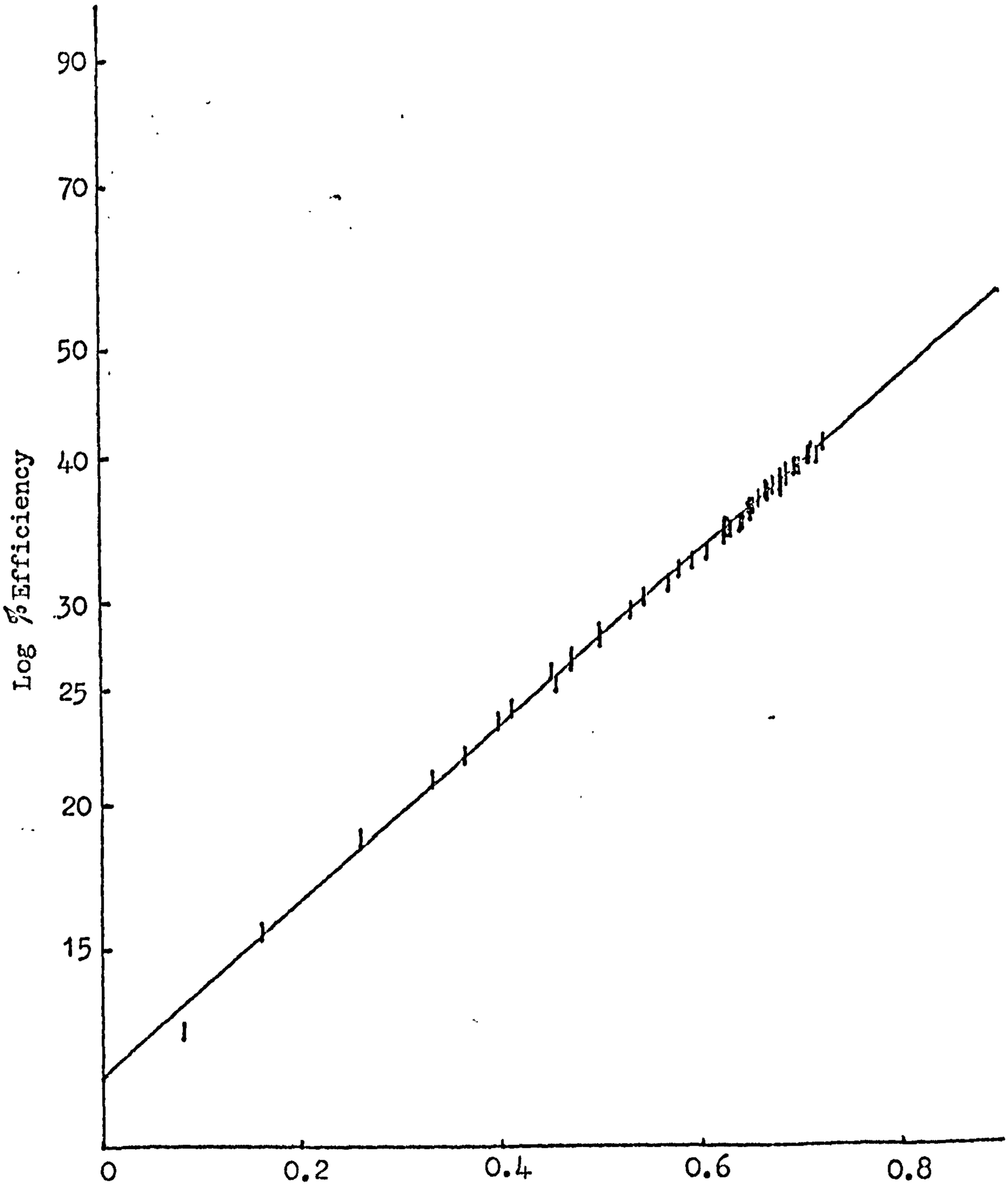

Figure 3b. TRITIUM QUENCH CALIBRATION CURVE FOR SOLUBILISED TISSUE OF S. MOSSAMBICUS.

in glass vials (similar to those used in the flow-cell apparatus) through which water was made to flow by means of a peristaltic pump (Watson-Marlow Ltd.). In order to differentiate individual snails in each cell, their shells were painted different colours with a non-poisonous enamel (Humbrol, Hull)(Fig. 4).

Direct illumination was provided by a 100w bulb placed 1 metre from the water bath when a monochrome (Ilford Mark V) 16 mm. motion picture safety negative was used and by 2 photoflood lamps (Philips, Photolita No. 1) placed 2 metres from the cell when Ektachrome (Kodak, commercial film 7252) film was used. The heat generated by the lamps was absorbed in a 3 litre round-bottom flask filled with tap water and placed midway between the lamps and the cell.

Photographs were taken using a H16 Reflex Bolex camera fitted with an electric motor drive (Paillard-Wild motor MBF-A). The camera was triggered at regular intervals of 30 seconds by an electronic timing device (Paillard-Wild variometre control unit MBF-B).

The films were developed by Filmatic Laboratories Ltd., London. A remote controlled motion analysis projector was used for the analysis of the snail's activity. Each frame was projected onto a piece of paper, at right angles to the projected image, and the position of each snail was recorded. From the position of each snail on consecutive frames the distance moved by each snail in 30 seconds could be measured.

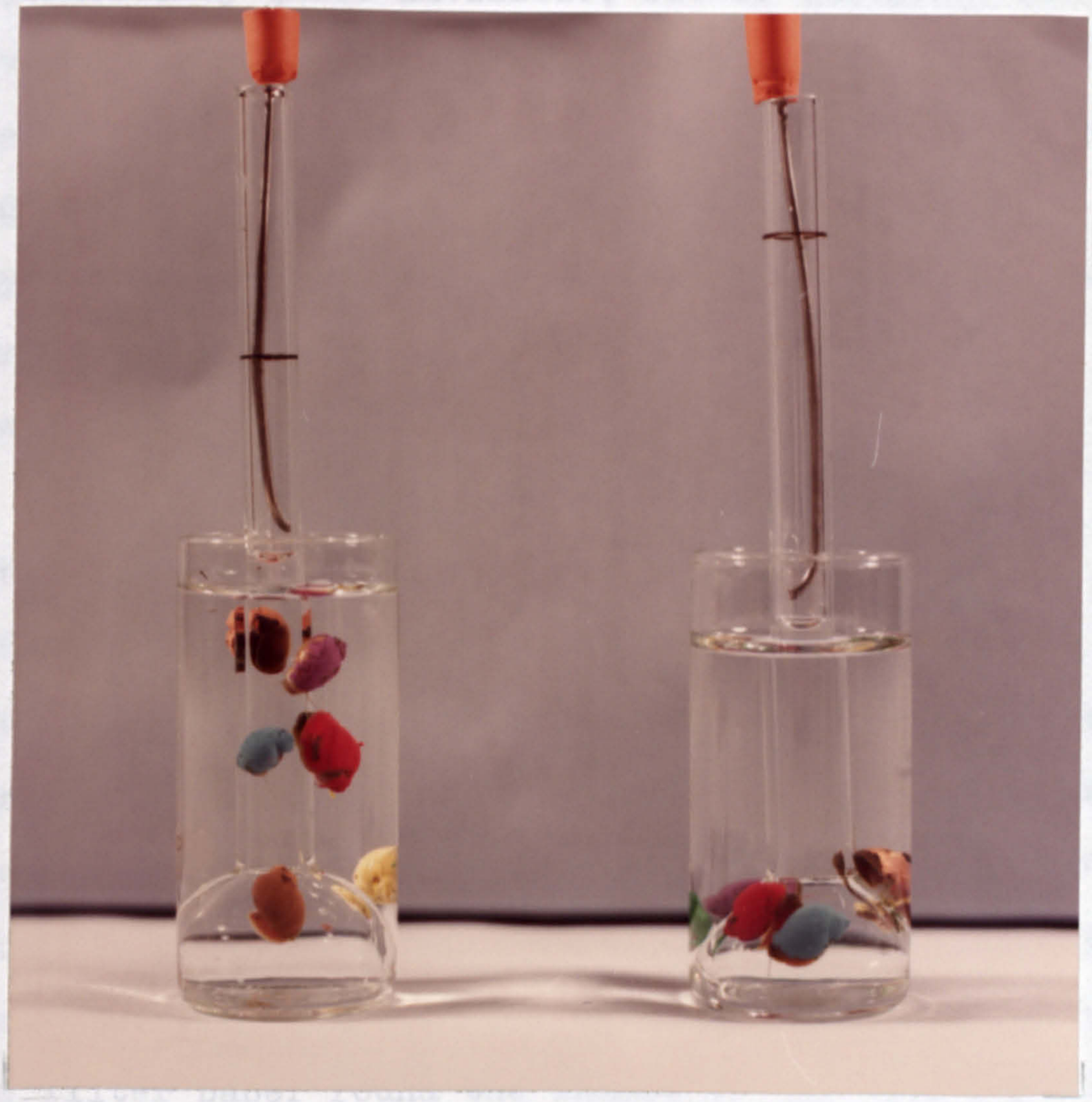

Figure 4. Colour-coded B.truncatus In The Flow Cell.

10.2. Recording snail trails.

The method of Ullyot (1936), for recording the direction of the movement of the flatworm, Dendrocoelum lacteum, was used with slight modifications. The bottom of the aquarium was covered by a sheet of clean glass on which the snail crawled, leaving a mucus trail. This mucus track was normally quite invisible, but if the glass plate was put into a suspension of fine mud, the fine particles became entangled in the mucus, so that after gentle agitation of the plate in the clean water the path traced by the snail stood out as a brown line. The plate was then dried and photoprints were made from it to give a permanent record (Fig. 5).

11. Thin layer chromatography.

The plates were activated before use by heating to 150°C for 4 hours. Using a spotting jig, test materials and markers were spotted onto the plates 1.5 cm from the bottom by means of a 10 µl micropipette. The plates were run, in ascending mode, in closed tanks. The atmosphere was equilibriated with the solvent by using a large area of filter paper round the inside of the tank and dipping into the solvent. The solvent mixtures used routinely for N-tritylmorpholine and its hydrolysis products were 10% v/v diethylether in hexane or ethylacetate/diisopropyl ether (1:1, v/v) and for 4'-chloronicotinanilide and its hydrolysis products, 20% v/v ethanol in benzene or dioxan/acetone (1:1, v/v). Ultra-violet light was used to locate individual spots after the plates had been run.

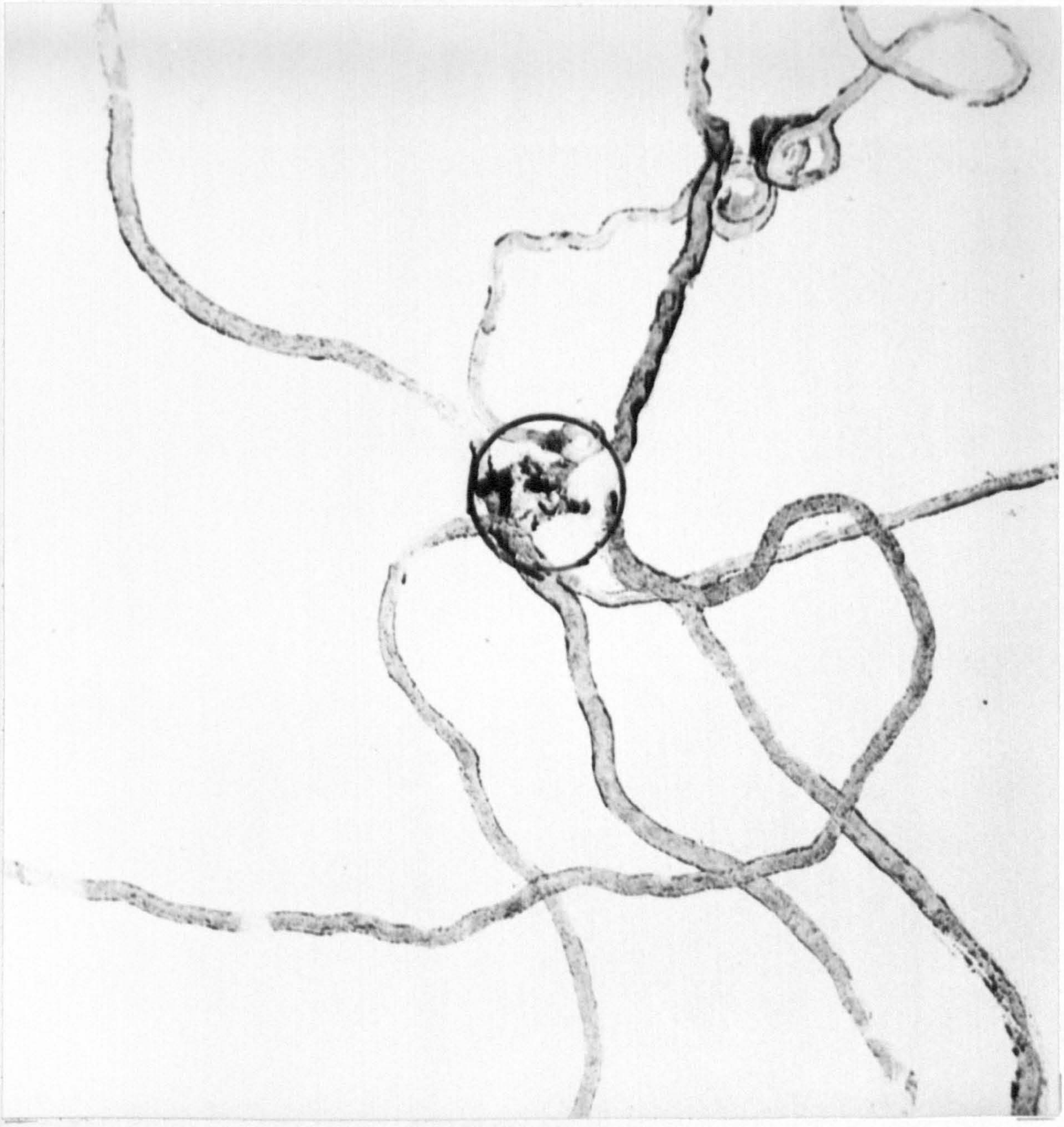

Figure 5. Trails Of B.truncatus.

12. Infection of snails with S. haematobium.

B. truncatus was exposed to miracidia hatched from S. haematobium eggs. The sample was rapidly washed, in urine flasks, several times with tap water until it was a clear suspension. The suspension was transferred from the urine flasks into several petri dishes. The dishes were left under artificial light for 1 hour at room temperature (20-25°C) to stimulate hatching. The hatched miracidia were picked up with the aid of a Pasteur pipette under a binocular microscope.

Snails (4.0-5.0 mm shell height) were exposed separately to 5-8 miracidia in a 2 cm depth of water for 4 hours at room temperature. The snails were returned to the rearing tanks and 4 weeks later, they were examined for infection. They were placed separately in glass tubes (5 cm high x 2.5 cm diameter) containing 2 cm depth of water (AHW) and exposed to artificial light for 6 hours at room temperature (McClelland, 1967) to stimulate shedding of cercariae. The positive snails were grouped together and transferred to new tanks. The rest were tested every other day for about a month to see if they were infected.

CHAPTER 3

PRESENT WORK (PART I)

Comparative Toxicity and Uptake of N-tritylmorpholine (Frescon) and 4'-chloronicotinanilide by *Bulinus truncatus* and *Sarotherodon mossambicus*.

Comparative Toxicity and Uptake of N-Tritylmorpholine (Frescon) and 4'-Chloronicotinanilide by B. truncatus and S. mossambicus.

1. Introduction.

Susceptibility to molluscicides is known to vary from species to species of snail (Shiff and Ward, 1966). It is of interest therefore, to know whether this is due to different rates of uptake of molluscicides, their distribution into various tissues, detoxication mechanisms or simply a function of size or body weight. N-Tritylmorpholine is a molluscicide which is used to control schistosome-bearing snails (Boyce et al., 1966). It is frequently applied to snail-infested water bodies as a 16.5% w/v emulsifiable concentrate (Frescon, FX 28). It has the advantage of being extremely effective at low concentrations. The applied dosage however, depends on the pH of the water since it is highly susceptible to hydrolysis, to triphenylcarbinol and morpholine at acidic pH (Beynon et al., 1967). The acute toxicity of Frescon to snails and fish (Tables 1 and 2) is variable and depends on the species as well as physical factors such as temperature and pH of the treated water.

4'-Chloronicotinanilide is one of a number of chemicals developed by Dow Chemicals, U.S.A., as candidate molluscicides (Ehrenford, 1969). It has been shown that these compounds have low water solubility and low oral toxicity to mice; and that they are non-toxic to fish (de Souza and Paulini, 1969). The LC_{50} of 4'-chloro-nicotinanilide against B. glabrata after exposure for

Table 1. Toxicity of Frescon to snails on 24 hrs exposure.

Species	Reference	Water pH and temp.	Toxicity (ppm)
Biomphalaria glabrata	Boyce et al. (1966)	pH 7.8, 21°C	LC_{90}=0.042 LC_{50}=0.025
Biomphalaria pfeifferi	Boyce et al. (1966)	pH 8.0, 22°C	LC_{90}=0.023 LC_{50}=0.014
Biom. sudanica tanganyicensis	Webbe and Sturrock (1964)	pH 7.5-7.8, 22°C-24°C	LC_{90}=0.078 LC_{50}=0.044
Bulinus(Physopsis) nasutus productus	Webbe and Sturrock (1964)	pH 7.5-7.8, 22°C-24°C	LC_{50}=0.090
Bulinus(Physopsis globosus	Boyce et al. (1966)	pH 8.0, 22°C	LC_{90}=0.085 LC_{50}=0.050
Bulinus truncatus	Boyce et al. (1966)	pH 7.8, 21°C	LC_{90}=0.100 LC_{50}=0.053

24 hours, was found by de Souza and Paulini (1969) to be 0.14 ppm and by Dunlop (1976) to be 0.37 ppm. Matthiessen (unpublished) found that x200 the dosage required to give an LC_{50} for B. glabrata is necessary to obtain a similar LC_{50} for S. mossambicus.

The display of apparent selectivity is of great importance because no other molluscicide shows such differential toxicity between snails and fish, and it was therefore chosen for study in view of this unique property.

2. Results.

2.1. The susceptibility of B. truncatus and S. mossambicus to Frescon and 4'-chloronicotinanilide.

Adult B. truncatus and fry of S. mossambicus were tested

Table 2. Toxicity of Frescon to fish.

Species	Reference	Water pH & temp.	Toxicity (ppm)
Carassius auratus	Deschiens & Floch (1968)	pH 7.0	LC_{100}=0.50 (24 hrs)
Carassius auratus	Shiff et al. (1967)	pH 7.8, 23°C	LC_{50} =0.07 (24 hrs)
Lebistes reticulatus	Shiff et al. (1967)	pH 7.8, 23°C	LC_{50} =0.25 (24 hrs)
Cyprinus carpio	Willomitzer & Lucky(1970)	pH 6.6, 21°C	LC_{100}=1.00 (10 hrs)
Rasbora heteromorpha	Shiff et al. (1967)	pH 7.8, 23°C	LC_{50} =0.03 (24 hrs)
Sarotherodon mossambicus (Tilapia mossambica)	Shiff et al. (1967)	pH 8.1, 20°C	LC_{50} =0.11 (10 day)

under identical conditions against Frescon and 4'-chloro-nicotinanilide. In all instances, dosage was calculated as active ingredient.

The snails (6.5 mm shell height) and fry (17 days old) were of the same body weight (40 ± 1 mg). They were exposed in 200 ml of molluscicide solution prepared in aerated, dechlorinated tap water, pH 8.1, at 25°C. Ten animals were exposed singly at each concentration so that dead or dying animals could not affect the response of others. After an exposure period of 24 hours, the animals were left to recover for 48 hours in fresh tap water.

Death was presumed to have occurred in snails when

prodding with a seeker elicited no response, while in fish it was assessed either by their floating on the surface or sinking to the bottom with complete inactivity. Controls were established for each test although it is noteworthy that in fact no deaths ever occurred in controls for fish or snails.

The dose-mortality data from each test (Appendix 3) were analysed using log-probit plot computer program (Scoppa, 1972). The results are shown in Figure 6; the lethal concentration values and data describing the line fit are given in Table 3.

Table 3. A comparison of the dose-mortality data for Frescon and 4'-chloronicotinanilide to Bulinus truncatus and Sarotherodon mossambicus after 24 hour exposure and 48 hour recovery period.

	Frescon		4'-Cl-nicotinanilide	
	Bulinus truncatus	Sarotherodon mossambicus	Bulinus truncatus	Sarotherodon mossambicus
LC_{50} (ppm)	0.034	0.019	0.30	32.00
LC_{90} (ppm)	0.060	0.021	0.44	33.00
Degrees of freedom	1,6	1,4	1,5	1,3
F test	88.00	11.88	169.95	23.33

It is found that the susceptibility of the two species to Frescon and 4'-chloronicotinanilide differs significantly ($p = 0.05$), the susceptibility of the two species to Frescon being of the same order while that of S. mossambicus to 4'-chloronicotinanilide is x100 < that of B. truncatus.

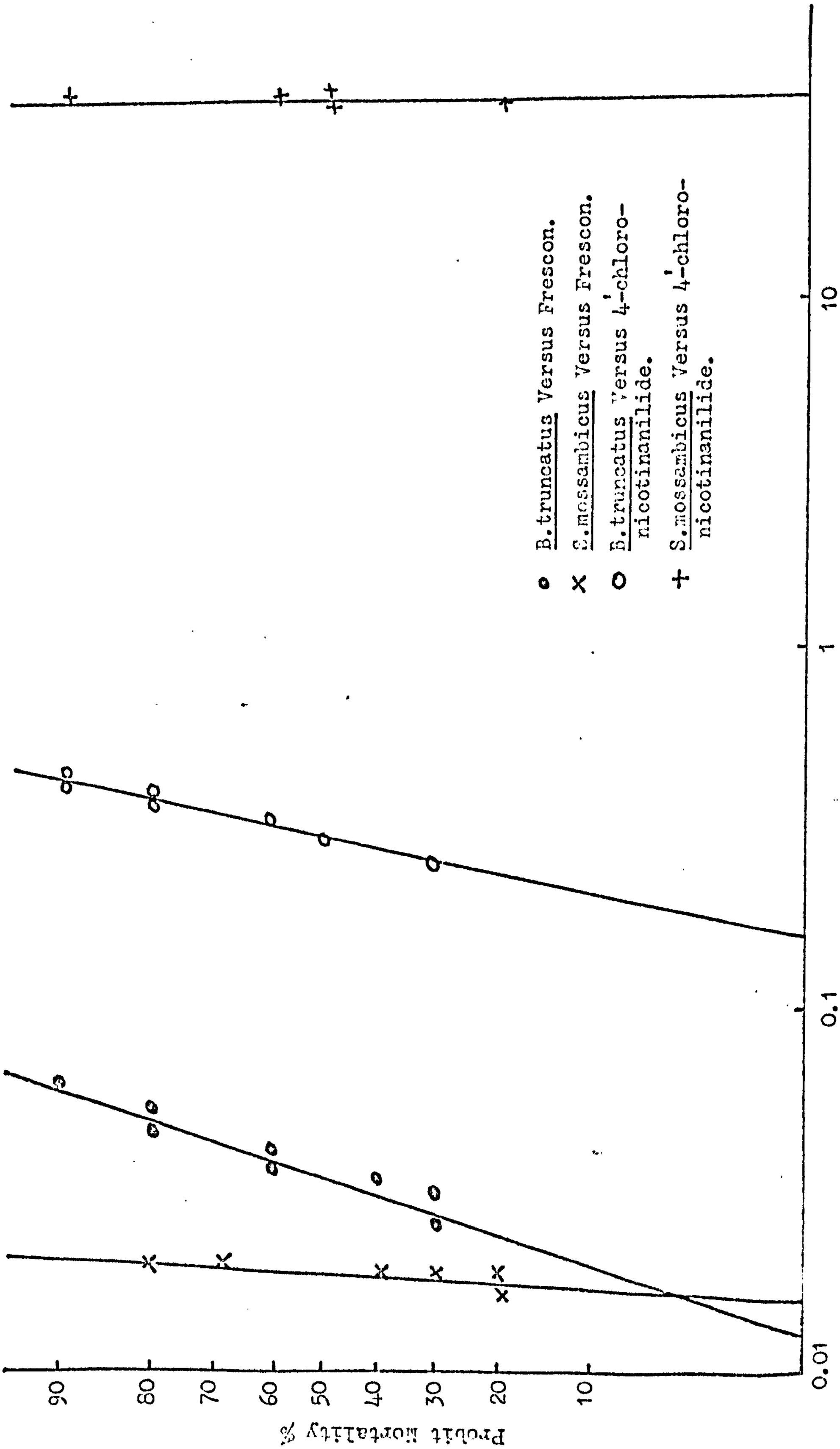

Figure 6. SUSCEPTIBILITY OF B.TRUNCATUS AND S.MOSSAMBICUS TO FRESCON AND 4'-CHLORONICOTINANILIDE.

2.2. The acclimatisation period in relation to the activity of snails.

Yager and Harry (1964) reported that the uptake of metals by B. glabrata may be in part a function of the activity of the snails. Duncan et al. (1977) showed that the uptake of 4'-chloronicotinanilide by B. glabrata is influenced by the activity of the snails and that activity decreased with time after a snail was placed in a new environment. Thus, they allowed for an acclimatisation period of 2 hours prior to measuring the rate of uptake. It was considered desirable to investigate this in more depth before proceeding to the present studies on rates of uptake. To establish the length of the acclimatisation period required for snails, before exposure to molluscicides, the activity of B. glabrata and B. truncatus was studied under different conditions by recording the snail's mucus trails and by time-lapse cinematography.

2.2.1. Snail trails.

It is apparent from the recording of snail's trails (Fig. 5) using a modification of the method due to Ullyot (1936) that the snails do not follow trails laid by other individuals of the same species. This may be seen by the trails crossing one another instead of the original trail being followed.

2.2.2. Time-lapse cinematography.

The movement of single, and groups of, B. truncatus and B. glabrata was recorded in 4.5 mm diameter petri-dishes containing static water and in cylindrical, glass tubes

arranged with water inflow and outflow as in the flow cell apparatus, in which the water was kept flowing at the rate of 1 ml per minute by means of a peristaltic pump. In subsequent experiments, the water flow was increased to 10 ml per minute for 10 minute intervals after 2 and 3 hours from the start of the transfer of the snails to the cylindrical tubes. This replicates the change-over to molluscicide exposure as used in the flow cell apparatus. The distance moved by each snail every 20 minutes was summed (Appendix 4a and b) and the results are illustrated graphically in Fig. 7a and b.

It is apparent that increased activity is demonstrable at the start of the transfer to the new environment in the two species, B. glabrata being more active than B. truncatus. This activity decreases to an almost constant level after approximately 2 hours in the two species. This was found to be independant of the number of snails in the container and the shape of the container. The snails on reaching the constant activity level were found to be undisturbed by changing the speed of pumping water over them.

It is concluded therefore, that a 2 hour acclimatisation period is necessary in rate of uptake experiments and that the system for quick change-over to molluscicide exposure media in the flow cell apparatus will not influence rate of uptake measurements.

2.3. Uptake and loss of Frescon and 4'-chloronicotinanilide.

The rates of uptake and loss of Frescon and

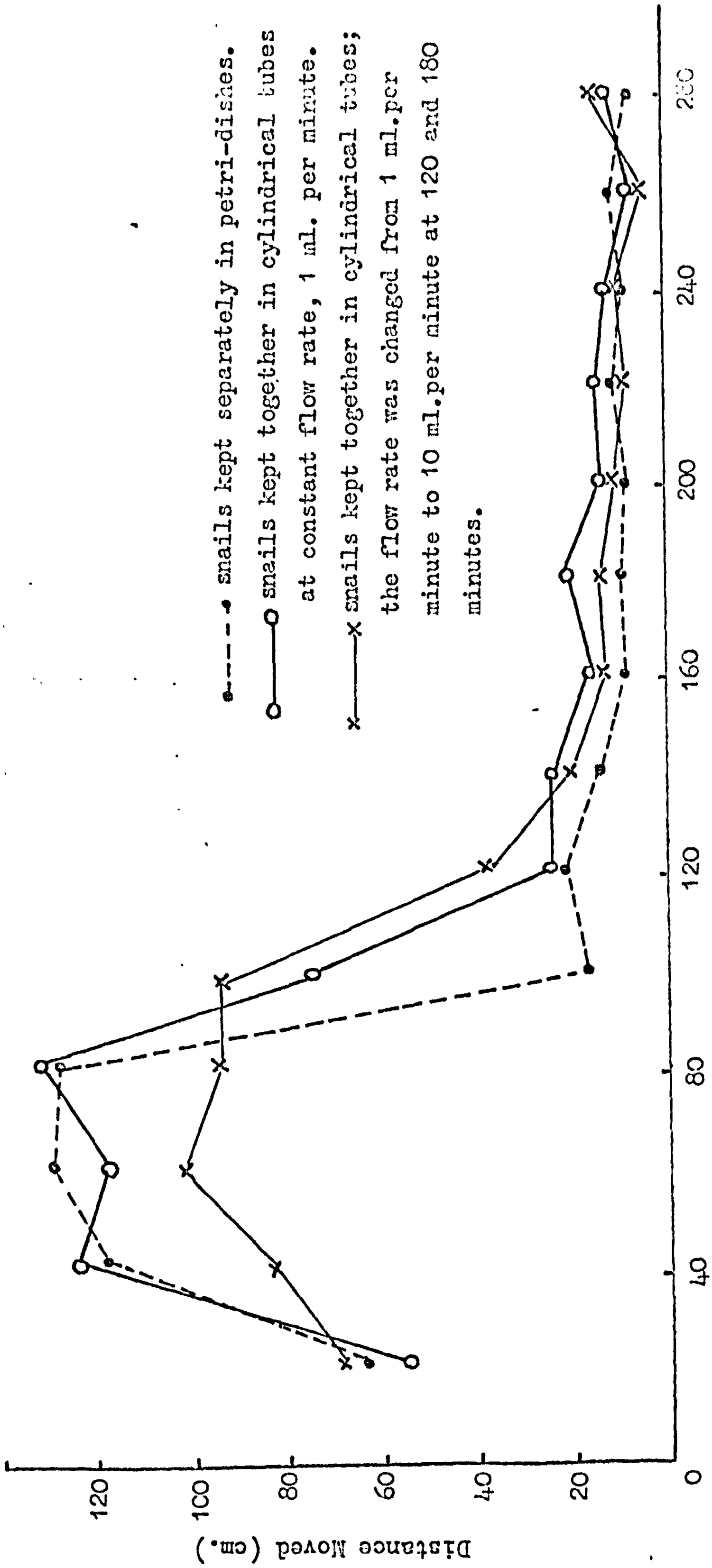

Figure 7a. ACTIVITY OF BIOMPHALARIA GLABRATA.

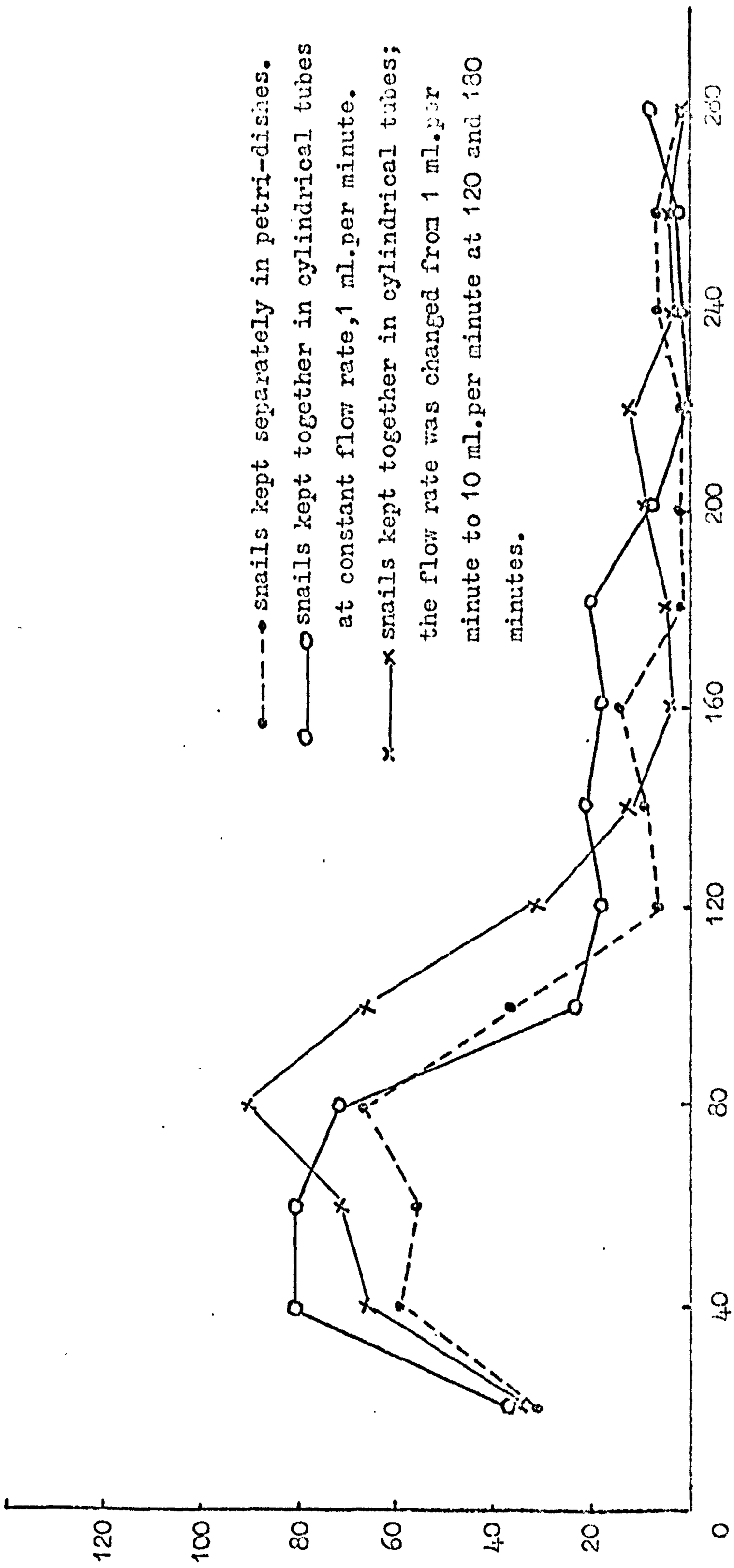

Figure 7b. ACTIVITY OF BULINUS TRUNCATUS.

4'-chloronicotinanilide by R. truncatus and S. mossambicus were studied in order to find out whether these rates influence molluscicidal activity and also whether they are the cause of the difference in susceptibility between the species.

^{3}H-Frescon and ^{3}H-4'-chloronicotinanilide of the same Specific Activity (18.7 µCi/mg) were formulated in dimethylsulphoxide (DMSO) and dispersed in aerated, dechlorinated tap water, pH 8.1, to give 0.0152 µM solutions (0.005 ppm Frescon and 0.0037 ppm 4'-chloronicotinanilide). This concentration was judged from the bioassay results to be sub-lethal to snails and fry at least for the exposure periods which were to be used.

Groups of 7 snails of shell height 2-9 mm were exposed to the labelled molluscicide solution in the flow cell apparatus described in the previous chapter. Fry weighing 40 $\pm$ 1 mg were exposed in 100 ml beakers through which the solutions were kept flowing by means of a peristaltic pump. A steady loss of 21% of Frescon after 3 hours was found to be due to adsorption onto the polythene tubing of the pump (Appendix 5). The amount adsorbed has been calculated and allowed for. All the exposures were made at 25.5°C and an acclimatisation period of 2 hours was allowed before exposure to molluscicidal solutions. In loss experiments, the initial exposure to molluscicide solutions was 4 hours.

The rates at which the molluscicides are taken up and lost has been measured over different periods of time.

The results are given in Appendices 6-13 and are shown graphically in Figs. 8 and 9. In the case of snails, the amount of chemical per mg of body weight was calculated from the amount of chemical present in each group of snails in a cell divided by the total weight of the snails in that cell. For fish, the mean of the chemical present in approximately 6 fry was divided by the mean weight of fry i.e. by 40 mg. It can be seen from Fig. 8 that the uptake rates of Frescon and 4'-chloronicotinanilide by B. truncatus and S. mossambicus are all non-linear relationships which can be represented by the general biparametric equation $y = a + bx$. At the same time, it is evident that the rates of loss are also of a non-linear relationship of the form $y = \frac{1}{(a + bx)}$. The coefficients describing each curve are given in Fig. 9. These curves were chosen by computer-program (Vostry, 1972) to give the closest fit from a range of 15 possible equations; they are not necessarily the best fit. The uptake rate of 4'-chloronicotinanilide is lower than that of Frescon for both species, the uptake by S. mossambicus being the lowest of all. The rates of loss of the chemical are of the same order. Fifty per cent of the Frescon absorbed by B. truncatus and S. mossambicus was eliminated by 32 and 23 hours respectively. Similarly, 50% of the 4'-chloronicotinanilide absorbed by B. truncatus and S. mossambicus was eliminated by 23 and 30 hours respectively.

2.4. The rate of water uptake by B. truncatus and S. mossambicus

The rate of water uptake by B. truncatus and S. mossambicus was studied in order to find out whether

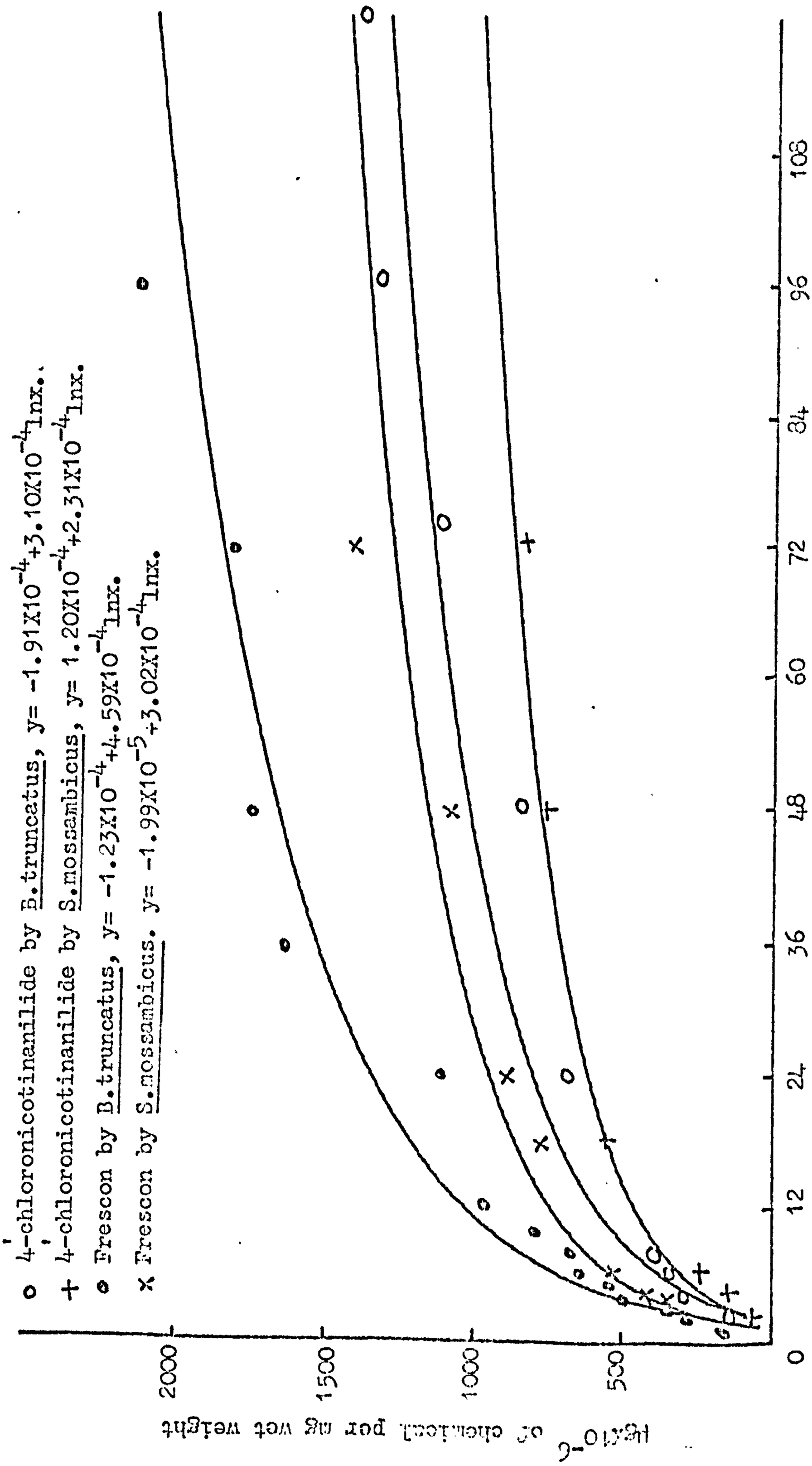

Figure 8. UPTAKE OF N-TRITYLMORPHOLIDE AND 4'-CHLORONICOTINANILIDE BY B. TRUNCATUS AND S. MOSSAMBICUS.

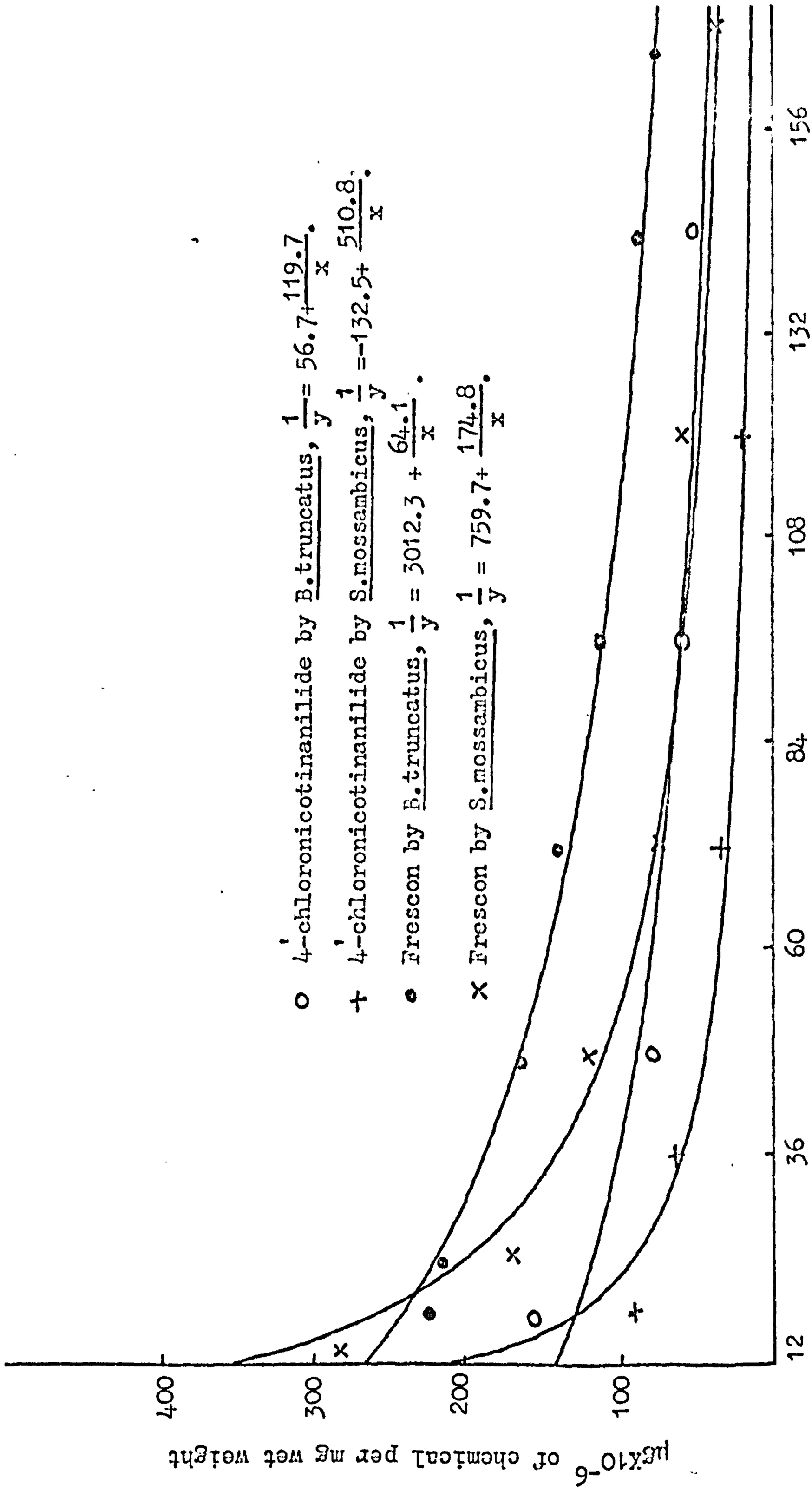

Figure 9. LOSS OF N-TRITYLMORPHOLINE AND 4'-CHLORONICOTINANILIDE BY B. TRUNCATUS AND S. MOSSAMBICUS.

water influx is responsible for the carriage of chemicals into the animals and whether this might be a basis for selective action between compounds.

Weighed, adult B. truncatus and fry B. mosambicus were held singly in 50 ml beakers containing 25 ml of aerated, dechlorinated tap water. The beakers were themselves held in the surface of a water bath running at 25.5°C. After an acclimatisation period of 2 hours the water in the beakers was removed by suction by means of a peristaltic pump and immediately replaced with tritiated water (Specific Activity = 57600 cpm/ml). Temperature changes were avoided by keeping the tritiated water in the same bath. The snails and fry were allowed to remain in the radioactive solution for a known time before being removed and oxidised as described previously. The results are shown in Appendices 14 and 15 and in Fig. 10. Each point on the graph was determined from at least 3 replicates. The results indicate that both snails and fry take up water very rapidly and that equilibrium is established in both animals after approximately 6 minutes. The graph continues to rise slightly after this which is probably due to the final establishment of equilibrium between influx and efflux. The amount of water taken up per unit time is measured either by calculating the amount of water taken up in the first second using the equation describing the curve or by drawing a tangent to the curve at zero time i.e. before equilibrium begins to develop. Using the first method, it is found that 1.0 mg of snail tissue takes up 225 µg of water in 1 minute

$$= 0.225\ \mu l\ min^{-1}$$

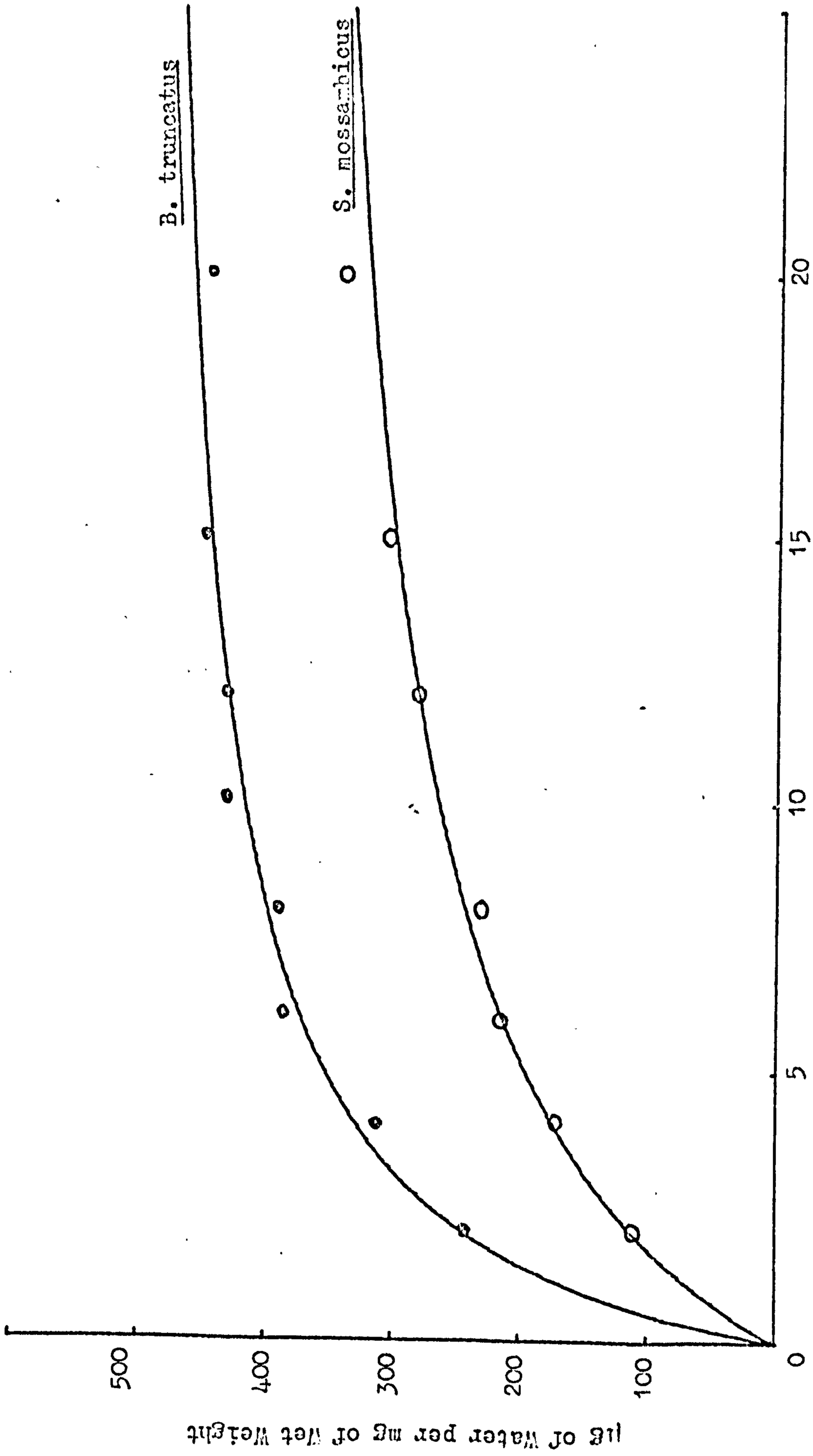

Figure 10. RATE OF WATER UPTAKE BY B. TRUNCATUS AND S. MOSSAMBICUS.

and 1.0 mg of fry tissue takes up 74 µg of water in 1 minute

$$= 0.074 \ \mu l \ min^{-1}$$

using the tangent method, it was found that 1.0 mg of snail tissue takes up 210 µg of water in 1 minute

$$= 0.210 \ \mu l \ min^{-1}$$

while 1.0 mg of fry tissue takes up 70 µg of water in 1 minute

$$= 0.070 \ \mu l \ min^{-1}$$

2.5. Distribution of Frescon and 4'-chloronicotinanilide within the tissues and organs of B. glabrata and S. mossambicus.

Other work presented in this study has shown that snails and fish absorb Frescon and 4'-chloronicotinanilide from water and concentrate it to relatively high levels but it is not known how these concentrations are internally distributed and whether their distribution bears any relation to the susceptibility of the species. It would also be of interest to relate the distribution of these molluscicides to possible modes of action and routes of excretion.

In this experiment, B. glabrata was used instead of B. truncatus. The former species being greater in size would obviously be expected to produce larger amounts of tissue and would therefore be easier to manipulate in this type of experiment. Adult B. glabrata (shell diameter 25 mm) and juvenile S. mossambicus (mean weight = 1.30 gm) were exposed for 4 hours to 0.02 ppm solutions of ^{3}H-Frescon (Specific Activity = 20.9 µCi/mg) and

^{3}H-4'-chloronicotinanilide (Specific Activity = 52.8 μCi/mg). At the end of the exposure, the snails were killed by immersion in hot water (75°C) for 20 seconds and the snail body was then removed from the shell using forceps; the fish were killed by a blow on the head. Both animals were dissected immediately. Most tissues and organs were used completely, while some were merely sampled. Due to limitations, such as the amount of tissue which can be dissolved by tissue solubilisers and the volumes which can be incorporated into the scintillation cocktail, it would be impracticable to solubilise whole fish tissues for example, skin, muscle and bone. Each sample was immediately weighed and placed in a counting vial for solubilisation and counting as described in the previous chapter.

The amount of chemical per mg of tissue was calculated from the mean of three samples and from this, the concentration factor was calculated as;

$$\frac{\text{amount of chemical per mg wet weight of tissue}}{\text{amount of chemical per mg of solution}}$$

The results are summarised in Table 4 and show that the highest concentration of Frescon in B. glabrata was found in the pseudobranch with intermediate levels present in mantle collar, head and foot regions. In S. mossambicus, the highest concentration was recorded in the liver with moderate concentrations in the gut, spleen, heart, kidney and brain. For 4'-chloronicotinanilide, again the highest concentration was found in the pseudobranch of B. glabrata with intermediate concentrations in kidney, mantle and mantle collar. In S. mossambicus, 4'-chloronicotinanilide

was concentrated in the gall bladder with moderate concentrations in the kidney, liver and spleen.

Table 4. Distribution of Frescon and 4'-chloronicotinanilide in B. glabrata and S. mossambicus after exposure for 4 hours to 0.02 ppm solutions. The results are shown as concentration factors: µg/mg wet weight.
µg/mg solution

Biomphalaria glabrata			Sarotherodon mossambicus		
Tissues	Frescon	4'-chloronicotinanilide	Tissues	Frescon	4'-chloronicotinanilide
shell	2	15	skin	107	44
foot	170	118	muscle	47	26
head	147	128	eye	82	30
mantle	41	150	brain	147	28
mantle collar	122	158	spinal cord	104	31
pseudobranch	433	515	backbone	132	32
kidney	44	153	gill	166	55
heart	39	74	kidney	149	150
liver	33	24	heart	168	78
gut & content	40	30	liver	1322	135
albumen gland	30	29	spleen	200	130
ovotestis	44	22	gall bladder	47	1058
genitalia	35	95	gut & content	244	70

2.6. Metabolism of Frescon and 4'-chloronicotinanilide by *B. glabrata* and *S. mossambicus*.

B. glabrata (shell diameter > 25 mm) and *S. mossambicus* (mean wt = 11.0 gm) were exposed for 4 hours to 0.02 ppm solutions of Frescon and 4'-chloronicotinanilide. At the end of the exposure, the animals were killed and dissected immediately. The gall bladder and the liver from each fish were taken separately, while the pseudobranchs from 10 snails were collected together. The tissues were then weighed, homogenised in chloroform (1mg/20ml) and allowed to extract for 24 hours at room temperature. The supernatant was then reduced to approximately 1 ml in a rotary evaporator at 30°C. The extract, standard solutions of N-tritylmorpholine and its hydrolysis products (triphenylcarbinol and morpholine) and 4'-chloronicotinanilide and its hydrolysis products (4-chloroaniline and nicotinic acid) were chromatographed using the solvent systems described in the previous chapter.

Qualitative analysis of the chromatograms of all the chloroform extracts of both the gall bladder and liver of *S. mossambicus* and the pseudobranch of *B. glabrata* indicated the presence of unchanged parent compound, except in the extract of the gall bladder of fish exposed to 4'-chloronicotinanilide where spots similar to those recorded for 4-chloroaniline and nicotinic acid were produced.

3. Discussion.

Many countries where schistosomiasis is endemic cannot afford to meet the financial requirements for the control of the disease due to their limited health budgets and, in some cases, due to the allocation of resources to other more pressing disease problems. It seems that because of the lack of an assured market for molluscicides, the necessary research by industry on either improving the available molluscicide formulations or looking for new chemicals has been neglected. However, in the last 20 years, considerable effort has been made in screening different compounds for molluscicidal activity. Any candidate molluscicide must at least equal the standard set by those commercially available.

In the laboratory, 4'-chloronicotinanilide was found to be less toxic than Frescon. Its 24 hour LC_{50} of 0.30 ppm against B. truncatus compares unfavourably with an LC_{50} of 0.034 ppm for Frescon. Against B. glabrata, the LC_{50} was found by Dunlop et al. (in press) to be 0.37 ppm compared to 0.044 ppm for Frescon (Webbe and Sturrock, 1964). However, the compounds must not be compared on this factor alone. The standard requirements for an ideal molluscicide described by a number of authors (Colwill, 1957; Wright, 1959; Ritchie, 1973; Duncan, 1974) include many other criteria which are of relevance. Specificity of the compound is a high ranking criterion among these requirements. Since fish and snails have, to some extent, the same habitat, it is important from both the economic and public health point of view that fish should not be affected by molluscicidal applications. But, the

currently used molluscicides are known to be toxic to fish to a greater or lesser degree.

The present study has shown that Frescon is more toxic to S. mossambicus than B. truncatus while 4'-chloronicotinanilide was shown to be non-toxic to S. mossambicus at concentrations about x100 those required to kill B. truncatus. The differences in susceptibility between snails and fish to both Frescon and 4'-chloronicotinanilide were thought to be influenced by the amount of chemical penetrating each species.

Since it has been suggested that uptake of chemicals by snails is in part a function of the snail activity (Yager and Harry, 1964; Duncan et al., 1977), the understanding of the behaviour of schistosome-bearing snails on exposure to molluscicides is of importance in studies of rates of chemical uptake.

Wells and Buckley (1972) have reported that the pulmonate, Physa acuta and the prosobranchs, Monodata lineata and Littorina saxitalis follow trails laid by the individual and also respond to the trails left by others of the same species. The trails apparently fade in 30 minutes. The limited experiments undertaken here, using Ullyott's modified technique for recording the movement of snails, show that both B. truncatus and B. glabrata do not follow trails laid by other snails, even though these were fresh trails less than 15 minutes old.

The finding that a significantly higher activity is

demonstrable during the first 2 hours following the transfer of the snails to a new environment indicates that the snails are probably adapting after transfer to the new environment. Further work needs to be done in order to ascertain whether this adaptation is associated with differences between, for example, the quality or temperature of the water in which the snails were reared and that of the cell.

The studies described here with Frescon and 4'-chloro-nicotinanilide indicate that these compounds are rapidly taken up from water by B. truncatus and S. mossambicus and assimilated into various tissues. It is obvious from the longer-term exposure that the amount of Frescon taken up was higher than that achieved with 4'-chloronicotin-anilide; the amounts taken up being greater in B. truncatus than in S. mossambicus. Comparison between these rates of uptake and the relative susceptibilities of B. truncatus and S. mossambicus to Frescon and 4'-chloronicotinanilide indicate that some connection between rate of uptake and potency of molluscicides might be possible. It is apparent that S. mossambicus which is highly tolerant to 4'-chloronicotinanilide takes up this compound less rapidly than Frescon. On the other hand, both B. truncatus and S. mossambicus, which are highly susceptible to Frescon, take this compound up more rapidly than 4'-chloronicotinanilide. The plateau in the amount of chemical taken up, which developed after approximately 24 hours (Fig. 8), should not be taken as a steady level of chemical, it is more likely to be due to simultaneous removal of chemical from the tissue rather than a true

steady state due to saturation of tissue. Fifty per cent of the chemicals taken up were found to be eliminated after 23-32 hours of recovery.

The rapid uptake of molluscicides by both snails and fish has been mentioned by other authors. Azevedo et al. (1957) and Cheng and Sullivan (1974), using radioactive copper sulphate, demonstrated the rapid uptake of this compound from the environment by planorbid snails and verified its diffusion into different tissues and organs. Duhm et al. (1963), studying the accumulation of ^{14}C-niclosamide by B. glabrata found that within 24 hours up to 0.2% active ingredient was absorbed from a 1 ppm solution. Boynon (1971), using Rasbora spp., found that after 4 hours, the fish concentrate Frescon up to x50 the concentration in water. Similarly, Matthiessen (1977) reported that S.mossambicus concentrates Frescon up to x1300 from a 0.005 ppm solution. Glickman et al.(1977) also reported the rapid uptake of pentachlorophenol by the rainbow trout (Salmo gairdnerii). Dunlop (1976), Duncan et al., (1977) and Duncan and Brown (unpublished) were able to measures rates of uptake of Frescon and nicotinanilide and some substituted nicotinanilides by B. glabrata. They hinted that the rate of uptake of molluscicides might be directly dependant on the rate of water influx.

The rate of water uptake by B. truncatus and S. mossambicus was, therefore, studied in an attempt to relate water and molluscicide uptake. The experiments show that both B. truncatus ans S. mossambicus are freely permeable to water and that the flow of water through the animals when

they are in their normal environment is very fast. Van Aardt (1968), using Lymnaea stagnalis, found that tritiated water equilibriates in the snail within 80 minutes after exposure while Potts et al.(1967) estimated the biological half-life for the uptake of tritiated water by S. mossambicus (given as Tilapia mossambica) to be well below 1 hour. The high influx rates of water observed for B. truncatus and S. mossambicus undoubtedly are related to their existence in water and the relative unprotectedness of the body surface for water permeation. In contrast, the biological half-life for tritiated water for the rat under normal feeding and housing conditions is 3-4 days (Herzer and Haberich, 1966). The flow of water into B. truncatus is greater than that into S. mossambicus. However, the equilibrium is established in both at the same time. Values of 0.224 µl min^{-1} and 0.074 µl min^{-1} per mg of tissue were calculated for B. truncatus and S. mossambicus respectively. Using weight as a basis for comparison, the value calculated above for B. truncatus is remarkably close to the one derived from the value of 17.74 µl min^{-1} calculated by Duncan (1969) for an 8.5 mm B. glabrata. An 8.5 mm B. glabrata weighs 84.0 mg which therefore gives an uptake rate of 0.210 µl min^{-1} mg^{-1}. The calculated rates of influx of water have been used to find a value for the amount of molluscicide expected to enter after a certain time. Such a calculation rests on the assumption that the molluscicide enters with the water in the same concentration as it exists in the medium. It was hoped that such calculations would give values similar to those obtained from the uptake of Frescon and 4'-chloronicotin-

anilide by B. truncatus and B. mossambicus. However, the values calculated, i.e. the amount of chemical expected to enter the animals, were found to be less than the values obtained for uptake (Appendix 16). This indicates that the rate of uptake of the compounds was faster than the rate of water influx.

The mechanism involved in the diffusion of the compounds through the animal membranes might be a specialised transport process in which the membrane plays an active part, transporting the solute in a manner that cannot be explained by the structure or physical properties of the membrane (Schanker, 1962). Specialised transport processes, for example, pinocytosis, are known to occur in snails. Nakahara and Bevelander (1967) reported the ingestion of particulate matter by the outer surface cells of mollusc mantle. The mantle epithelium of the calico clam, Macrocallista maculata, was seen to ingest colloidal gold and carmine particles. These authors have also observed a similar phenomenon in 2 related species of bivalves, Pinctada radiata and Isognomon alatus. But, pinocytosis appears to operate too slowly to account for the rapid uptake of these compounds. Harris (1960) stated that the passage of molecules or ions from a solution into a membrane, when no chemical forces operate, is a consequence of collision between the particle and the membrane which gives rise to certain concentrations followed by diffusion within the membrane and across it to redissolve in the aqueous phase on the other side. Lieb and Stein (1969) explained the diffusion of non-

electolyte molecules through membranes by the concept of porous behaviour i.e. the membrane behaving as homogenous polymer networks.

The present experiments have demonstrated high levels of Frescon and 4'-chloronicotinanilide in the pseudobranch of B. glabrata, the accumulation in other organs being relatively slight by comparison. The presence of large concentrations of molluscicide in the pseudobranch may be indicative of uptake and/or elimination. However, the TLC analysis of a chloroform extract of snails exposed to Frescon and 4'-chloronicotinanilide shows that the pseudobranch contained unchanged N-tritylmorpholine and 4'-chloronicotinanilide respectively. This would imply that this organ is an uptake rather than an elimination one since metabolism to form more hydrophilic compounds might be expected in a tissue responsible for excretion.

The gastropod epithelium functions not only for protection but also in respiration (Hess, 1964). The uptake of compounds through the epithelium has been reported on many occasions. Zylstra (1972) demonstrated the uptake of colloidal ferritin by the dorsal head epidermis and inner mantle epithelium of Lymnaea stagnalis. Furthermore, Sullivan and Cheng (1974), believe that the rectal ridge greatly increases the area of the mantle epithelium-water interface and postulated that the cilia-mediated water flow pattern, made possible by its contiguity with the dorsal ridge, strongly suggests that the rectal ridge is involved in the uptake and elimination

of substances by the snail.

The distribution of Frescon and 4'-chloronicotinanilide within the body of snails and fish does not show any relation with the fat content despite the fact that both molluscicides are lipophilic in character. This seems to be in agreement with the findings of Matthiessen (1977) who exposed S. mossambicus to Frescon. The general dependance of distribution of pesticides on fat content was however confirmed by Matthiessen et al. (1976) when S. mossambicus was exposed to the organophosphate insecticide, Abate. Anderson and Fenderson (1970) and Ernst et al.(1976) have shown that DDT residues in fish are broadly dependant on fat content, with high concentrations in adipose tissue and brain.

The high concentration of Frescon found in the fish liver indicates that the compound is possibly metabolised and excreted via this organ. Statham and Lech (1975) reported that Bayluscide is metabolised by fish liver, and Griffiths (1968) has shown that mammals also metabolise Frescon in the liver and excrete its metabolites via the bile and urine. However, the presence of a low concentration of Frescon in the bile and the fact that most of the Frescon found in the liver after 24 hours exposure consisted of unchanged N-tritylmorpholine does not support the hypothesis of metabolism and excretion of this compound via this route. Brodie and Maickel, (1962) suggested that aquatic animals do not have the capability of detoxifying drugs and that

lipid-soluble compounds are rapidly disposed of by passive diffusion through membranes into the water. Consequently, it is suggested that the liver may be the site of action of the molluscicide which causes fish mortality by interfering with the normal function of the organ. That is to say, Frescon accumulates in the liver until a threshold of toxicity level is attained. This 'target organ' hypothesis has been suggested by Sullivan and Cheng (1974, 1975). In an autoradiographic study of the uptake of ^{67}Cu in B. glabrata they concluded that the principal site of copper action is located in the rectal ridge. Moreton and Gardner (1976), experimenting with Lymnaea stagnalis, reported the nervous system as a possible site for Frescon action in fresh water snails. However, the validity of such suggestions remains to be seen; the studies needed to further investigate these points were beyond the scope of the present study.

The high concentration of 4'-chloronicotinanilide reported in the fish gall bladder suggests that this compound is most probably excreted via the bile after being hydrolysed into 4'-chloroaniline and nicotinic acid as shown by the TLC analysis of chloroform extracts of the bile of S. mossambicus exposed to 4'-chloronicotinanilide. The metabolism and excretion of molluscicides in the bile has been previously reported in mammals (Griffiths, 1968) and in fish (Statham and Lech, 1975). A number of insect pests such as the tobacco wireworm (Conoderus vespertinus), differential

grasshopper (Melanoplus differentialis) and the cigarette beetle (Lasioderma serricorne), have a high capacity to degrade pesticides e.g. nicotine to non-toxic metabolites such as continine (Self et al., 1964). Hollingworth (1976) states that it is quite rare for any foreign compound (xenobiotic) to enter the body and not be converted, at least in part, to metabolites that are usually more polar than the parent compound which can then be rapidly excreted from the body. He adds that differences in metabolic rate between organisms represents probably the most important force behind selective toxicity. Therefore, the capability of fish to withstand high concentrations of 4'-chloronicotinanilide and its analogues may be related to the rapid breakdown of the compound in fish to more polar forms which are easily disposed of via the bile.

CHAPTER 4

PRESENT WORK (PART II)

Comparison of the Relative Susceptibilities of Various Groups of *Bulinus truncatus* to Frescon.

Comparison of the Relative Susceptibilities of Various Groups of Bulinus truncatus to Frescon.

1. Introduction.

The Gezira lies between the Blue and White Niles to the South of Khartoum. Its consists of an area of 1 million hectares of irrigated land with a further million hectares potentially irrigable. It is supplied from the Blue Nile via three main types of distribution canals, main, major and minor canals. The function of both the main and major canals is to transport the water rather than to irrigate the land. The minor canals, positioned at right angles to the major canals, provide a reservoir for irrigation of the field via field channels known as Abu eshreens and Abu sittas. It was realised from the outset of the scheme in 1925, that schistosomiasis could become a major public health hazard and copper sulphate was therefore introduced as a molluscicide in 1956 (El-Hager, 1958). The disease has spread nevertheless and large numbers of snails can be found in the minor canals (Warley, 1976). In 1969, a comparison between copper sulphate and the then new molluscicide, N-tritylmorpholine or Frescon showed that the latter was more effective and easier to apply (Amin, 1972). As a result of this study, copper sulphate was gradually replaced by Frescon. The regimen of Frescon application has since evolved through the use of drip-feed dispensers backed up by knapsack spraying of the tail ends of minor canals to the use of aerial spraying in which applications are made 5 times a year in early September, mid-November, late January, mid-March and early June. However, the 'blanket' applications

of Frescon have had limited success in the Gezira and have proved to be uneconomic (Fenwick, personal communication). During 1977, a pilot study indicated a strong correlation between human habitation, well defined water contact sites and the presence of infected snails. Infected snails were often found in the Abu eshreens near villages and dwelling sites. Following this study, 'focal' control was thought to be an alternative strategy which will be economically viable and feasible.

The complete elimination of snails by focal mollusciciding is unlikely to be achieved in many of the defined transmission habitats and a proportion of the snail population may therefore receive only a reduced concentration of the molluscicide. A study of the susceptibility of infected snails to the molluscicide was therefore thought to be essential for the evaluation of a focal control strategy.

As the phenomenon of pesticide resistance has become more prevalent with the exposure of pest populations to widespread chemical treatments, the possibility of development of resistance in snail hosts of schistosomiasis has become an intriguing question for those concerned with the application of molluscicides (WHO, 1976). A pest population is described as resistant when it has lost its original susceptibility to a toxic chemical to the point that it can no longer be controlled with that chemical (Brown, 1969). With these points in mind, it was decided to investigate whether any level of Frescon-resistance has developed in the snail populations of the treated areas of

the Gezira and to provide base-line information with which to examine the situation at any given time in the future.

2. Results.

2.1. The susceptibility of B. truncatus to Frescon; data for snails either infected or uninfected with S. haematobium.

Two groups of B. truncatus, either infected or uninfected with S. haematobium, were bioassayed at the same time and under identical conditions in order to determine their relative susceptibilities to Frescon e.c. The snails used were of the same size (shell height = 6.5 ± 0.05 mm). They were exposed singly at 25°C in molluscicide solution (200 ml) prepared in aerated, dechlorinated tap water (pH 8.10), 10 snails at each concentration. After an exposure period of 24 hours, the snails were left to recover for 48 hours in fresh, aerated tap water.

The dose-mortality data from each test (Appendix 17) were analysed using a log-probit computer program. The results are shown in Fig. 11; lethal concentration values and data describing the line fit are given in Table 6. It is apparent from the results that infected snails are more susceptible to Frescon than uninfected ones. Comparison of the dose-mortality data for slope and position showed a significant difference in position at the 5% level (F = 32.81).

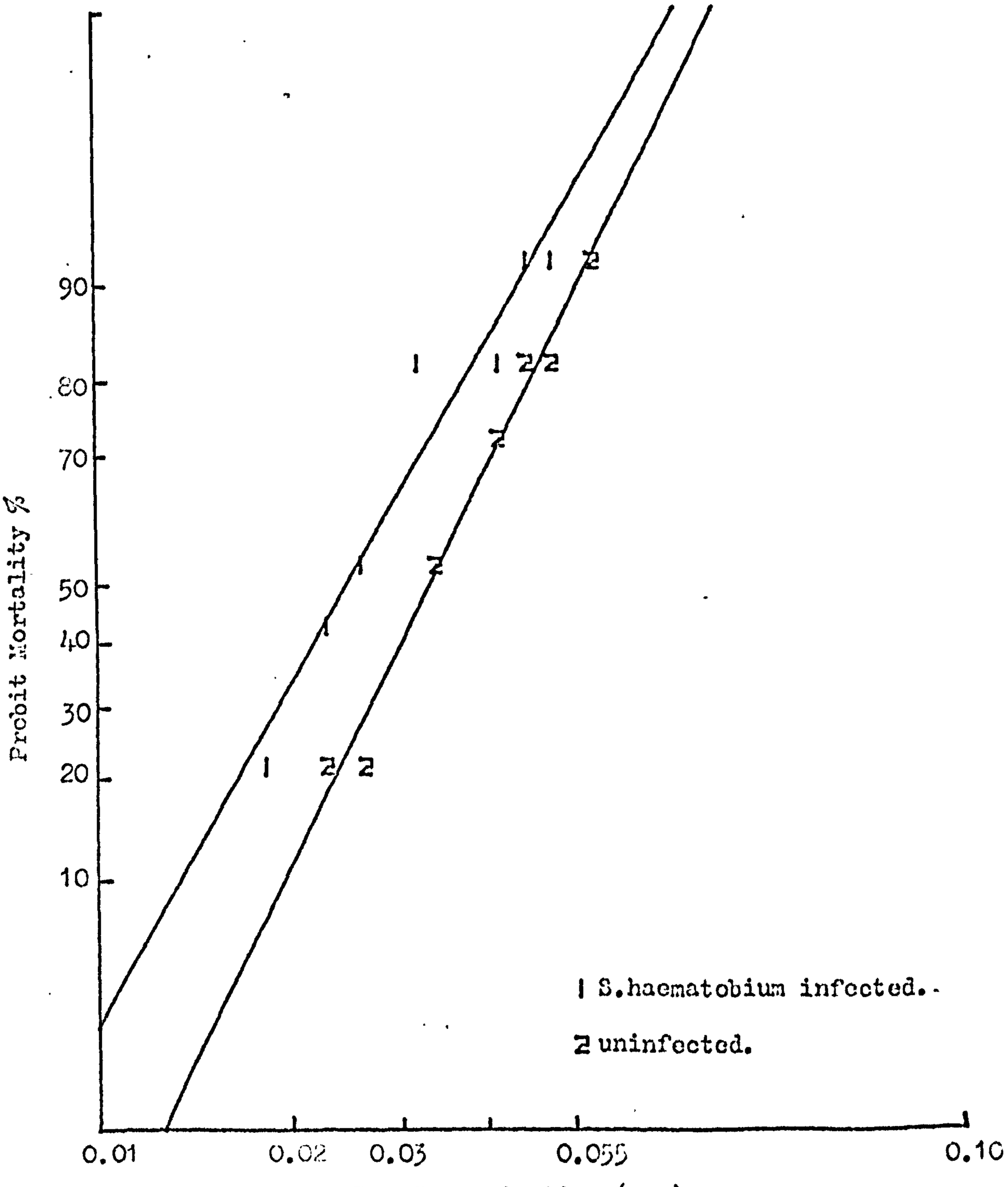

Figure 11. SUSCEPTIBILITY OF B.TRUNCATUS TO FRESCON.

Table 5. A comparison of the dose-mortality data of Frescon against infected and uninfected B. truncatus after 24 hour exposure and 48 hour recovery period.

	Infected B. truncatus	Uninfected B. truncatus
LC_{50}	0.025	0.032
LC_{90}	0.044	0.055
Degrees of freedom	1,5	1,5
F test	77.35	256.19

2.2. The rate of uptake of Frescon by B. truncatus either infected or uninfected with S.haematobium.

The rate of uptake of Frescon by two groups of B. truncatus either infected or uninfected with S. haematobium was studied using ^{3}H-Frescon (Specific Activity = 20.36 μCi/mg). Batches of 6 snails (shell height 2-9 mm) were exposed to labelled molluscicide solution (0.005 ppm) in the flow cell apparatus described previously, 5 cells for each of the two groups. An acclimatisation period (2 hours) was allowed prior to exposure. Two batches at a time, one from each group, were removed from the flow cell apparatus after known exposure times.

The results are given in Appendix 18 and are shown graphically in Fig. 12. Each point on the graph represents the total amount of chemical (μg) taken up by a batch of 6 snails in a cell divided by the total wet

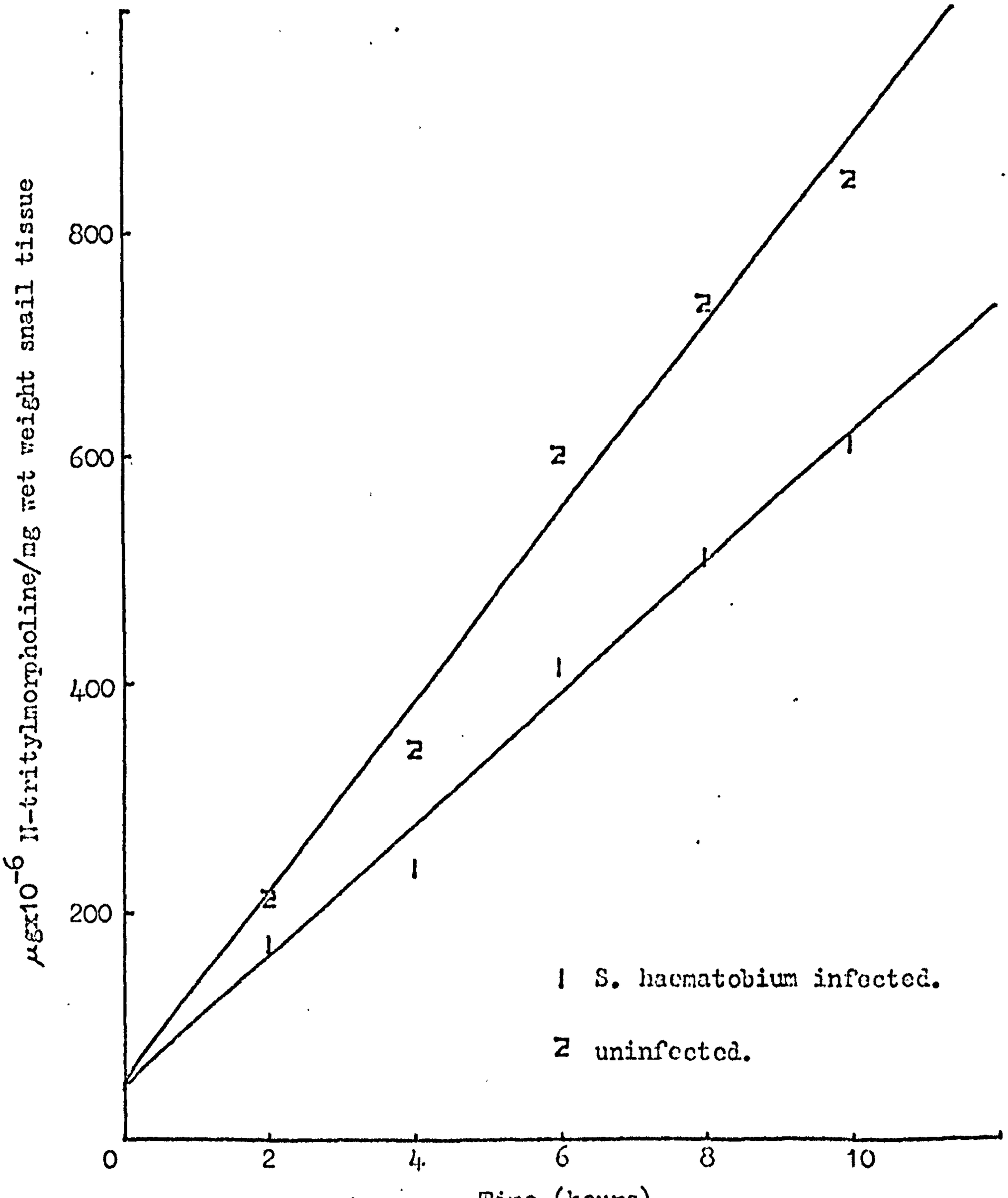

Figure 12. UPTAKE OF N-TRITYLMORPHOLINE BY B. TRUNCATUS.

weight (mg) of the snails in the cell. It is apparent from the results that uninfected snails take up Frescon more rapidly than infected ones. Comparison of the rates of uptake indicated a significant difference at the 5% level ($F = 20.77$).

2.3. Susceptibility of B. truncatus to Frescon; data for snails collected from two different locations of the Gezira scheme.

Two groups of B. truncatus were collected during January, 1976; one group from Hebeika canal in the Northern Group of the scheme and the other from the Abu Gueli canal near Hassaheisa. The two canals are 50 km apart though they both receive water from the main canal. Hebeika canal is in an area treated with Frescon for 6 consecutive years; Abu Gueli has never been treated.

Two experiments were conducted, the first in June, 1976 with snails from the two groups and the second in September, 1976 with 2 batches of snails from the untreated area group and 1 batch from the treated area group. On each occasion, the snails from the two groups were bioassayed at the same time, under identical conditions. The snails used were of the same size (shell height 6.5 $\pm$ 0.05 mm) and of the same age. They were exposed singly at 25°C for 24 hours in molluscicide solution (200 ml) prepared in aerated, dechlorinated tap water (pH 8.10); 10 snails at each concentration.

After the exposure, the snails were left to recover for 48 hours in fresh tap water. The mortality data from all these tests (Appendix 19) were analysed by a log-probit plot as before. The results are illustrated in Figs. 13a and 13b from which it may be seen that the tolerance of the snails from the treated area was slightly higher than that of the snails from the untreated area on each of the two occasions. Lethal concentration values are given in Table 7.

Table 7. Lethal concentration values for Frescon against the two snail groups described in the text.

Snail groups	First experiment LC_{50}(ppm)	LC_{95}(ppm)	Second experiment LC_{50}(ppm)	LC_{95}(ppm)
Frescon-treated area	0.036	0.060	0.046	0.071
Untreated area	0.030	0.064	0.036	0.070
Untreated area	-	-	0.034	0.068

Comparison of the dose-mortality data for slope and position showed a significant difference in position, at the 5% level, between the treated and untreated area groups in both experiments ($F = 5.5$ and 20.1 respectively) and that there was no significant difference between the two untreated area groups in the second experiment ($F = 0.6$).

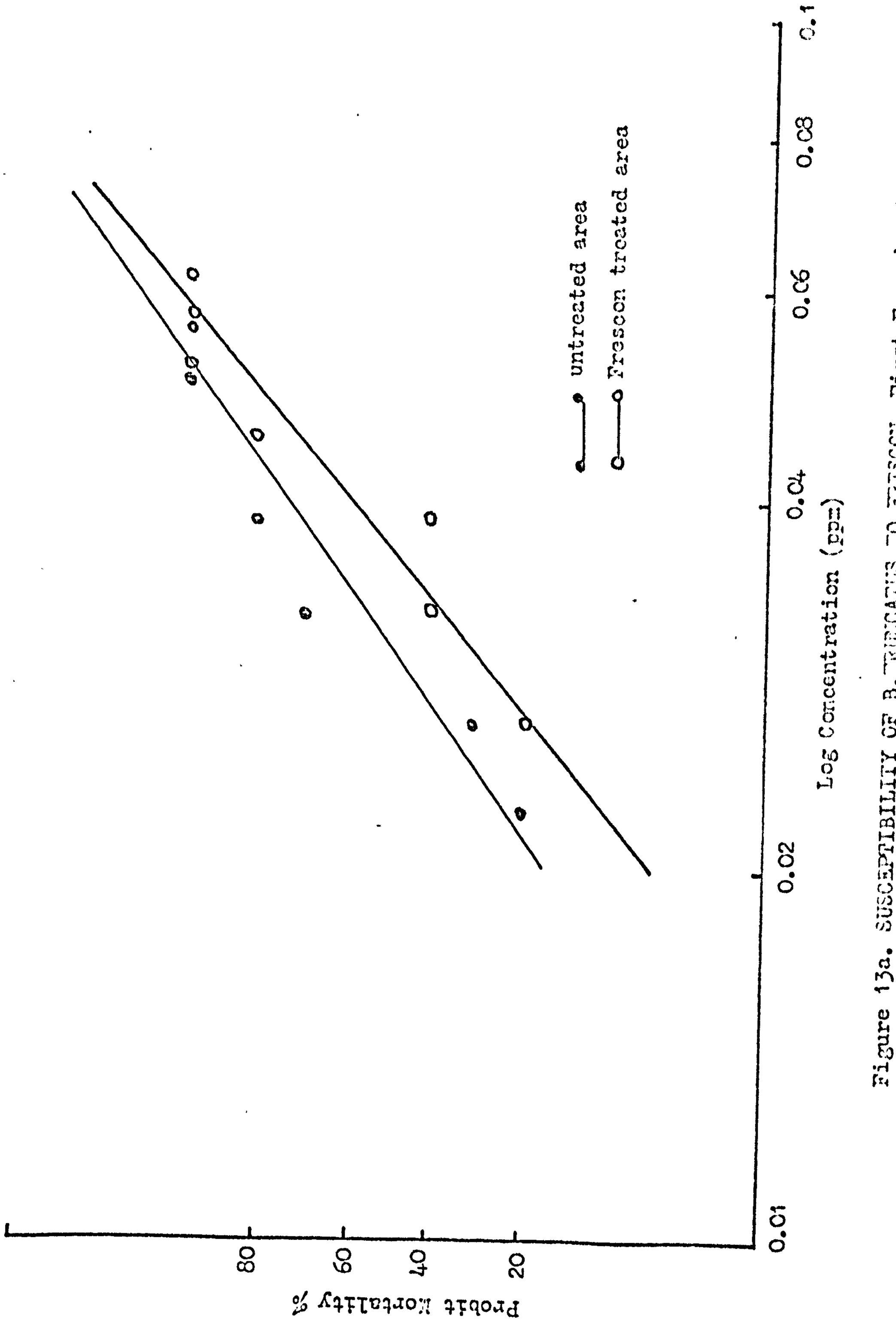

Figure 13a. SUSCEPTIBILITY OF B. TRUNCATUS TO FRESCON, First Experiment.

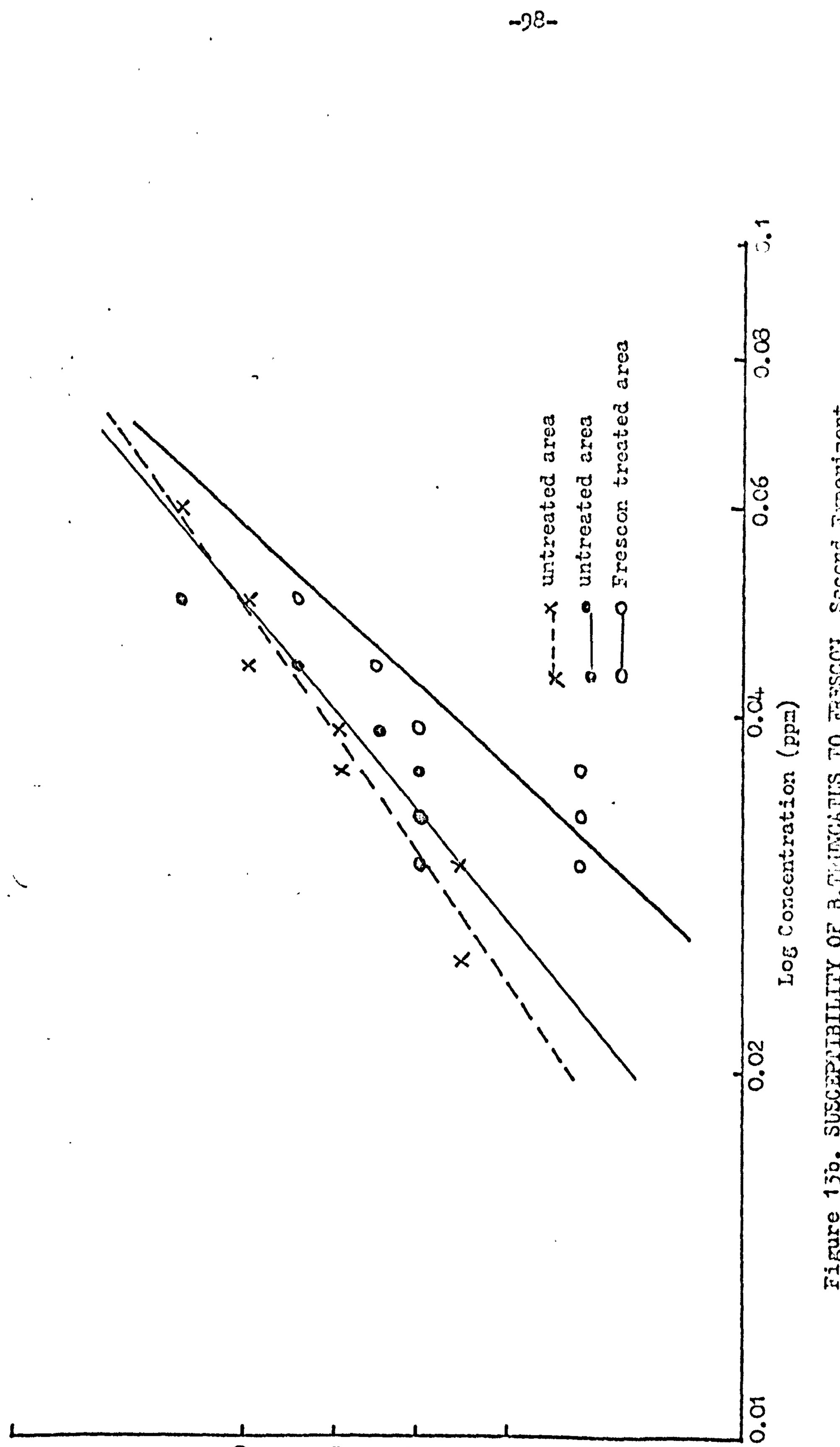

Figure 15. SUSCEPTIBILITY OF B.TRUNCATUS TO FRESCON, Second Experiment.

2.4. The rate of uptake of Frescon by the two field collections of B. truncatus.

This experiment was also repeated twice in June and August, 1976 with exposure periods of 5 and 12 hours respectively. On each occasion, batches of snails from the treated and untreated areas were exposed in the flow cell apparatus, to 0.005 ppm of N-tritylmorpholine (Specific Activity = 21.16 μCi/mg) in aerated, dechlorinated tap water.

Results are given in Appendix 20 and are shown graphically in Figure 14. Each point on the graph represents the total amount of chemical (μg) taken up by each group of snails in a cell divided by the total wet weight (mg) of the snails in the group. It is clear that the rate of uptake of Frescon in the two groups is different.

Comparison of the uptake rates of Frescon for the two groups indicates a significant difference in position at the 1% level (F = 189.5 in the first experiment and 65.3 for the second).

3. Discussion.

Comparison of the susceptibility to Frescon of two groups of B. truncatus, which were either infected or uninfected with S. haematobium, showed that the former are more susceptible than the latter. The difference was significant and therefore, it might be assumed that the infection is the cause of this difference in susceptibility. However, comparisons of the rates of uptake of Frescon indicate that infected snails take up the molluscicide less rapidly than uninfected ones. The slow uptake of Frescon

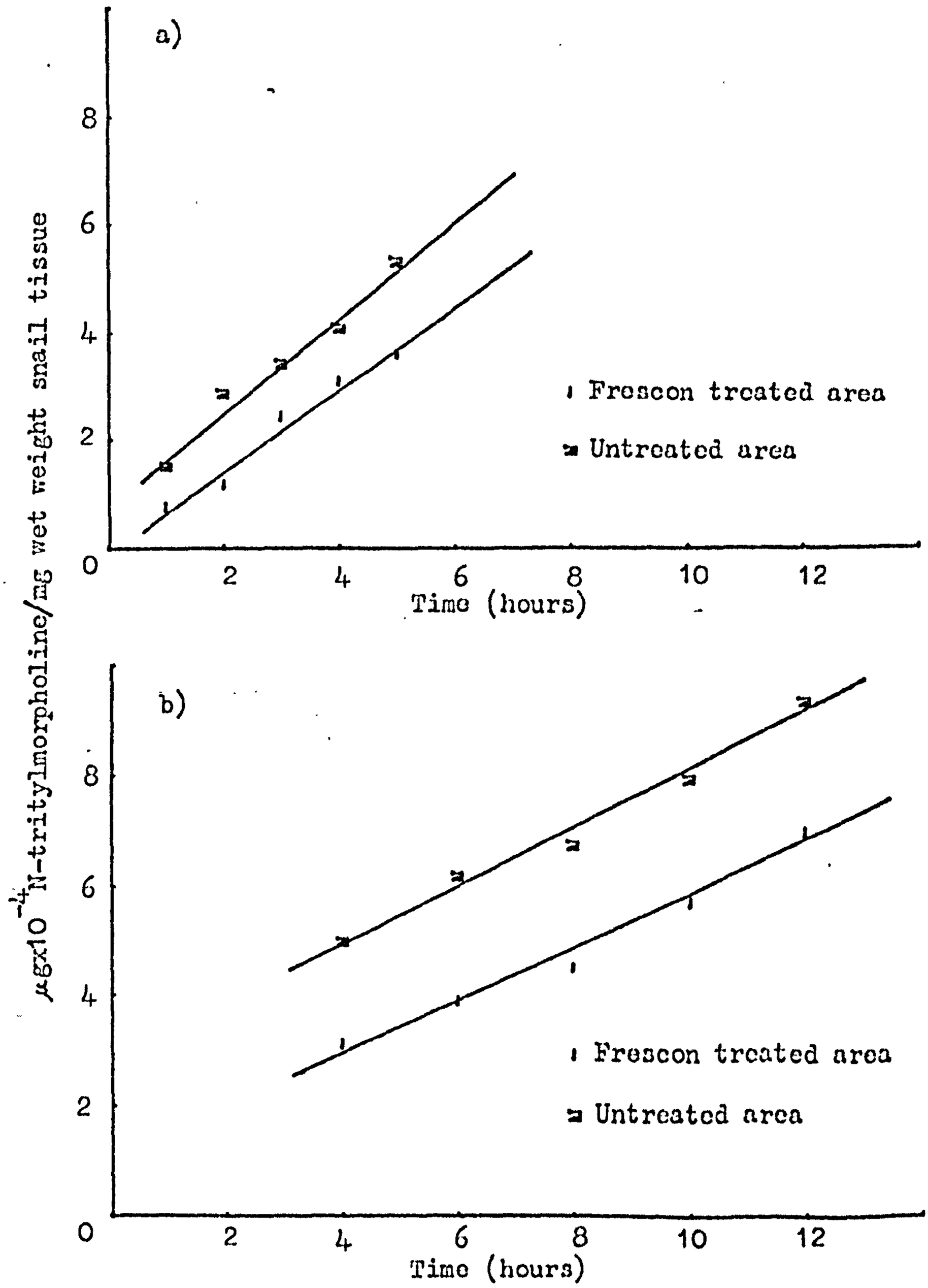

Figure 14. UPTAKE OF N-TRITYLMORPHOLINE BY B. TRUNCATUS
a) 5 hours exposure b) 12 hours exposure.

by infected snails may be due to the damage caused by the escaping cercariae in the loose, vascular connective tissue characteristic of the molluscan pseudobranch (Pan, 1965) which has been here identified as a possible site of Frescon uptake.

The deleterious effect of schistosome parasites of man on their snail hosts has been mentioned by various workers. Brumpt (1941) observed that B. glabrata infected with S. mansoni was apparently less resistant to dessication. Paulini and Pellegrino (1956a, b) showed that B. glabrata infected with S. mansoni was more susceptible to sodium pentachlorophenate and copper sulphate than uninfected snails. Hira and Webbe (1972) demonstrated the combined effect of a molluscicide and infection on the survival rate of snails.

It is apparent therefore, that the application of molluscicides to potential transmission foci may eliminate or greatly reduce the numbers of infected snails in greater proportion than those uninfected snails even when a complete kill is not achieved overall.

From the data obtained here, it is also evident that the group of snails from the Frescon-treated area is more tolerant to Frescon than that from the non-treated area. However, this is hardly enough to mark the former group resistant and the dosage applied in the Gezira would be adequate to control both groups judging from the presently reported dosage-mortality results. It might be

said, of course, that the difference uncovered between the two groups was always present and not due to selection pressure at all. Certainly, differences in susceptibility can occur between geographical strains (Olivier et al., 1962). The groups dealt with here however, were collected from confluent bodies of water and are therefore judged to have come from the same population.

The overlap in the bioassay results of the two experiments could possibly be due to seasonal variation. It has been pointed out by Tsuda (1953) and Yasuraoka et al. (1966) that the susceptibility of Oncomelania to molluscicides varies with the season of the year. This is not thought to be a factor operating in the present studies with laboratory bred animals, unless the seasonal rhythm is a very strong inherent one. It seems much more likely that the variance is inherent in the bioassay technique.

Two different and opposing views on development of resistance by snails to molluscicides once prevailed in the Far East. One group of workers argued against this as a possibility (Walton et al., 1958; Gancarz, 1958; Komiya et al., 1961; Yasuraoka and Hosaka, 1971) and another group claimed its existance (Okabe et al., 1956; Ota and Sato, 1956). The differences were finally thought to be due to variations in bioassay technique in the laboratories (Yasuraoka, 1972). More recently, however, some preliminary results have been reported, purporting to show Bayluscide resistance in B. truncatus from Iran (Jelnes, 1977).

The difference in the rate of uptake between the two groups in this study is, on the other hand, more marked suggesting that any difference in tolerance is due to differential penetration rates and that the snails with prior exposure to Frescon may have the capability to slow down the rate of penetration of the molluscicide and thus tolerate the chemical. The concept of decreased penetration has been suggested as one of several methods by which an insect becomes resistant to a toxicant (Lovell, 1963) and many resistant strains of insects have been shown to absorb insecticides at a slower rate than susceptible strains (Oppenoorth, 1958; Dinamarca et al., 1962; Sanchez and Sherman, 1966; Plapp and Hoyer, 1968; Szeicz et al., 1973).

Since the aquisition of resistance in an animal population is initially often an insidious process, there is a possibility that we are witnessing the early stages of such an event and that a strain of high tolerance, reaching the level of resistance, might develop with the protracted use of Frescon in the Gezira. It is suggested therefore, that this or other situations could be monitored from now on using the same methods outlined here. Two factors might be expected to mitigate against the development of resistance in irrigation schemes; the influx of untreated snails from the intake which will continually contribute to the gene pool and secondly, the use of focal chemical treatment of transmission sites, rather than blanket applications. Additionally, in the particular context of the Gezira, Bayluscide is being

actively considered as a replacement for Frescon on the grounds of efficiency if not specifically as a guard against incipient resistance (Amin, personal communication).

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS.

Although a large body of information exists on the biochemistry and particularly on the physiology of molluscs, little has been achieved towards a better understanding of the modes of action of molluscicides. It is well known that most pesticides, at their lowest inhibiting concentrations, act rather specifically only on one vital function, localised at one particular site. Hence, one can speak of pesticides which are primarily inhibitors of energy production whereas others are inhibitors of synthesis and still others act on the structure of the cell. All the work that has been done in the field of molluscicides seems to indicate that many of these compounds are primarily inhibitors of energy production.

It must be realised that few, if any, of the compounds used as molluscicides act selectively on molluscs; many have broad spectrum activity including toxicity to other animals or phytotoxic properties. This is not surprising since at the biochemical level, processes are often basically very similar in different organisms. It is only possible to find a more selective molluscicide among compounds which act on processes which are more or less specific for molluscs e.g. shell formation.

From the results of the present study, it seems possible that the differences in susceptibility to molluscicides between species are, to some extent, influenced by the

amont of chemical taken up by the species. The mechanisms involved in the uptake of compounds are thought to be a specialised transport process, similar but faster than pinocytosis, in which the membrane plays a major part, transporting the solute in a manner that cannot be explained by the structure or physical properties of the membrane. However, cellular components involved in the processes need to be identified by further studies, using for example electron microscopy and histochemical techniques.

The pseudobranch of B. glabrata was recognised as the main site of entry for both Frescon and 4'-chloronicotinanilide rather than a site of action. On the other hand, the liver of S. mossambicus was recognised as a possible site of action for Frescon, which causes mortality by interfering with the normal functions of this organ. These findings were based on results obtained by liquid scintillation spectrometry, other techniques which are based on fundamentally different principles e.g. histochemistry, x-ray microanalysis and autoradiography need to be investigated to present more detailed information on the sites of accumulation and action within the organ.

Although no attempts have been made in this study to present quantitative data, TLC analysis of chloroform extracts of the bile and liver showed that N-tritylmorpholine absorbed by snails and fish and 4'-chloronicotinanilide absorbed by snails remained chemically unchanged while 4'-chloronicotinanilide

absorbed by fish was metabolised to more polar forms. Hence, the high tolerance of fish to 4'-chloronicotinanilide is suggested as being due to the rapid disposal of these metabolites via the bile.

Evidence is presented to show that B. truncatus infected with S. haematobium is more susceptible to Frescon than uninfected snails, despite the fact that infected snails take up less chemical than uninfected ones. These results indicated that the application of molluscicides to defined transmission sites would eliminate or greatly reduce the number of infected snails within the transmission site even when a complete kill is not achieved.

A difference in susceptibility to Frescon, between B. truncatus collected from Frescon-treated and untreated areas of the Gezira, could be shown and this has been tentatively attributed to the differences in penetration rate of the molluscicide between the two collections of snails. It is suggested that methods described could be used subsequently to monitor for appearance of resistance in this or other situations.

The need for development of new and more effective molluscicides is justified by the fact that the use of molluscicide as a single measure or integrated with other control measures is considered to play a vital and continuing role in schistosomiasis control. 4'-chloronicotinanilide and its analogues seem to offer the possibility for truly selective control of snails. The

future development of these compounds should, however, involve laboratory and field trials incorporating different formulations, investigation of field methods for analysis of low concentrations in water and possible routes of commercial synthesis.

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APPENDICES

Appendix 1. Comparison of snail shell size and total snail weight of B. truncatus.

shell size (mm)	total snail wt (mg)	log total snail wt.
2.6	3	0.477
3.1	5	0.699
3.0	5	0.699
3.6	8	0.903
3.8	10	1.000
3.8	11	1.041
4.0	12	1.079
4.3	15	1.176
5.1	25	1.398
5.4	30	1.477
5.5	32	1.505
5.9	40	1.602
6.0	40	1.602
6.0	41	1.613
6.0	42	1.623
6.0	44	1.643
6.2	47	1.672
6.5	53	1.724
6.8	62	1.792
7.0	67	1.826
7.2	75	1.875
7.5	85	1.929
7.9	100	2.000
8.0	108	2.033
8.5	120	2.079
8.7	125	2.097
9.3	150	2.176
9.4	156	2.193
10.0	185	2.267

Appendix 2. Quench calibration data for combusted sample preparations.

cpm = counts per minute
ESR = External Standard Ratio
eff = efficiency

sample (ml)	cpm	ESR	% eff
0	1000771	0.7979	49.18
0.25	1000298	0.7617	46.34
0.50	1000410	0.7454	45.25
0.75	1000763	0.7233	43.89
1.00	1000052	0.7194	43.52
1.00	1000434	0.7156	43.05
1.25	1000309	0.7012	42.47
1.50	1000295	0.6811	41.19
1.75	1000051	0.6645	40.31

Appendix 2a. Quench calibration data for solubilised *Biomphalaria glabrata*.

sample	cpm	ESR	% eff
0.25 ml snail digest	69859	0.7037	38.93
0.50 " " "	65305	0.6654	36.39
0.75 " " "	60056	0.6244	33.47
1.00 " " "	55934	0.5898	31.17
1.25 " " "	52099	0.5401	29.03
1.50 " " "	49159	0.5006	27.39
whole snail	54709	0.5631	30.49
" "	59533	0.6132	33.18
" "	50873	0.5093	28.35

Appendix 2b. Quench calibration data for *Sarotherodon mossambicus*.

Tissue sample & digest vol. (ml).		cpm	ESR	% eff	log % eff
gut	0.50	62644.7	0.6325	34.42	1.5368
	0.75	65472.7	0.6507	35.97	1.5559
	1.00	67754.6	0.6670	37.22	1.5708
	1.25	68748.1	0.6813	37.77	1.5771
	1.50	70300.5	0.6890	38.62	1.5868
kidney	0.25	61786.5	0.6249	33.94	1.5307
	0.75	63281.6	0.6411	34.77	1.5412
	1.00	66568.4	0.6603	36.57	1.5631
	1.25	68402.0	0.6750	37.58	1.5750
	1.50	72774.4	0.7192	39.98	1.6018
liver	0.25	43641.8	0.4114	23.96	1.3795
	0.50	48324.9	0.4725	26.55	1.4241
	0.75	52920.1	0.5315	29.08	1.4636
	1.00	57560.3	0.5794	31.62	1.5000
	1.25	62582.8	0.6301	34.39	1.5364
	1.50	68567.3	0.6829	37.67	1.5760
muscle	0.25	62946.4	0.6261	34.59	1.5390
	0.50	65597.9	0.6548	36.04	1.5568
	0.75	67439.3	0.6700	37.05	1.5688
	1.00	69551.8	0.6823	38.22	1.5823
	1.25	71058.3	0.6979	39.03	1.5920
	1.50	73328.4	0.7132	40.28	1.6051
brain	0.25	67577.7	0.6514	36.14	1.5508
	0.50	68319.0	0.6750	37.53	1.5744
	0.75	69548.7	0.6882	38.22	1.5823
	1.00	70951.8	0.6962	38.98	1.5908
	1.25	72718.6	0.7114	39.95	1.6015
	1.50	74594.3	0.7254	40.98	1.6126

/over

Appendix 2b. continued.

skin	0.25	46930.7	0.4513	25.78	1.4113
	0.50	50726.4	0.5004	27.86	1.4450
	0.75	54431.0	0.5[illegible]0	29.91	1.4[illegible]5
	1.00	58584.5	0.5923	32.19	1.5077
	1.25	62852.2	0.6298	34.54	1.5383
	1.50	69084.4	0.6826	37.95	1.5792
bone	0.25	62526.6	0.6249	34.35	1.5359
	0.50	64986.1	0.6490	35.70	1.5527
	0.75	67753.9	0.6674	37.22	1.5708
	1.00	68761.8	0.6825	37.78	1.5726
	1.25	71129.9	0.7015	39.08	1.5920
	1.50	72521.5	0.7101	39.85	1.6004
eye	0.25	23000.7	0.0815	12.63	1.1014
	0.50	27973.4	0.1605	15.36	1.1864
	0.75	33706.9	0.2592	18.52	1.2676
	1.00	39719.7	0.3636	21.82	1.3389
	1.25	47831.7	0.4718	26.28	1.4196
	1.50	59736.3	0.6077	32.82	1.5161
gill	0.25	37857.2	0.3305	20.81	1.3183
	0.50	42515.7	0.3981	23.35	1.3683
	0.75	45852.2	0.4560	25.18	1.4011
	1.00	50183.5	0.5010	27.56	1.4403
	1.25	55915.0	0.5690	30.73	1.4876
	1.50	63627.8	0.6434	34.95	1.5434

Appendix 3. Susceptibility of Bulinus truncatus and Sarotherodon mossambicus to various concentrations of Frescon and 4'-chloronicotinanilide on 24 hours exposure.

cn = concentration
d = number dead
pr = probit

B. truncatus v. Frescon			S. mossambicus v. Frescon			B. truncatus v. 4'-chloro-nicotin-anilide			S. mossambicus v. 4'-chloro-nicotin-anilide		
cn	d	pr	cn	d	pr	cn	d	pr	cn	d	pr
0.025	3	4.29	0.0165	0	3.56	0.20	0	3.81	30	0	3.74
0.030	3	4.71	0.0170	2	3.94	0.25	3	4.55	31	2	4.23
0.033	4	4.93	0.0175	2	4.30	0.28	5	4.92	32	5	4.70
0.036	6	5.13	0.0180	3	4.66	0.32	6	5.37	33	5	5.15
0.039	6	5.32	0.0185	4	5.00	0.35	8	5.64	34	6	5.59
0.044	8	5.60	0.0190	8	5.34	0.38	8	5.94	35	9	6.02
0.050	8	5.89	0.0195	7	5.67	0.40	9	6.11	36	10	6.44
0.060	9	6.31	0.0200	10	5.99	0.44	9	6.42			
						0.48	10	6.71			

Appendix 4a. Activity of Biomphalaria glabrata.

		Distance moved every 20 minutes(cms)				
	time (min)....	20	40	60	80	100
	snail					
	1	61.2	133.1	90.4	173.4	10.1
6 snails	2	30.5	115.5	160.6	105.6	3.1
kept	3	90.4	85.6	122.7	84.0	13.0
separately	4	80.7	118.1	97.9	181.2	5.4
in a	5	52.8	141.7	155.8	94.7	18.7
petri-dish	6	62.1	110.3	146.2	125.4	50.7
	mean	62.9	117.4	128.9	127.4	16.8
	s.d.	21.1	19.5	30.0	41.1	17.5
	snail					
6 snails	1	24.2	90.1	130.1	79.2	102.3
grouped in	2	46.6	120.6	154.7	126.6	82.4
cylindrical	3	58.4	100.8	136.4	140.0	77.4
tube with	4	59.9	148.7	85.1	168.8	46.6
constant	5	91.1	133.3	92.4	135.7	74.4
flow rate	6	49.2	146.2	100.3	127.3	61.2
(1ml min^{-1})	mean	54.9	123.3	116.5	129.6	74.1
	s.d.	21.9	24.0	27.8	29.1	19.0
	snail					
	1	50.6	70.7	104.2	65.0	38.2
6 snails	2	84.9	118.5	139.4	100.7	123.5
grouped in	3	40.2	60.9	99.6	111.2	103.7
cylindrical	4	66.7	99.4	107.7	73.4	106.8
tube with	5	92.2	41.7	70.1	117.6	100.8
changed	6	66.6	103.6	86.3	93.4	89.9
flow rate	mean	66.9	82.5	101.2	93.6	93.8
	s.d.	19.7	29.3	23.2	20.8	29.3

120	140	160	180	200	220	240	260	280
10.2	10.8	0	12.2	6.5	5.1	0	0	0
30.4	19.0	1.6	25.1	16.5	10.0	15.6	21.1	1.1
12.7	0	24.1	0	12.2	11.3	8.1	9.8	0
22.6	2.0	4.3	10.3	4.9	8.3	14.7	8.7	24.2
16.2	12.4	11.4	0	5.4	12.6	12.2	1.1	12.2
40.3	40.2	9.1	7.9	4.5	15.6	1.9	24.0	0
22.1	14.1	8.4	9.3	8.3	10.5	8.8	10.8	6.3
11.5	14.6	8.8	9.3	4.9	3.6	6.6	10.0	10.0
38.8	0	31.2	24.6	20.1	7.6	9.6	4.0	10.0
40.4	10.2	22.1	40.3	22.9	24.1	20.1	8.2	1.0
19.9	50.5	12.4	17.6	18.4	25.0	16.9	9.9	21.1
4.8	41.0	2.2	4.2	9.7	9.4	5.3	18.2	16.5
17.7	20.3	6.8	15.6	5.0	2.3	9.4	9.6	20.0
18.6	19.9	16.4	27.0	6.2	6.9	7.9	10.4	2.2
23.4	23.7	15.2	21.6	13.7	12.6	11.5	10.1	11.8
13.7	18.9	10.5	12.2	7.7	9.6	5.7	4.6	8.8
11.6	19.9	14.9	76.6	2.0	9.4	13.4	10.2	9.4
38.9	7.4	6.4	1.6	8.4	11.6	20.2	12.4	8.7
44.2	27.8	25.2	28.4	14.9	8.9	6.8	3.6	34.7
45.0	11.7	0	17.6	18.1	2.1	3.4	9.1	7.6
47.9	32.0	18.2	9.4	13.3	10.9	4.4	6.7	8.7
42.2	19.6	18.7	13.4	14.9	11.2	10.1	8.4	6.8
38.3	19.7	13.9	13.0	11.9	9.0	9.7	8.4	12.7
13.4	9.3	9.2	9.3	5.8	3.5	6.3	3.0	10.8

Appendix 4b. Activity of Bulinus truncatus.

		Distance moved every 20 minutes(cms)				
time (min)....		20	40	60	80	100
	snail					
	1	44.9	43.4	77.7	55.5	34.7
6 snails	2	30.4	49.9	31.4	47.4	18.5
kept	3	15.5	80.3	40.4	96.7	51.2
separately	4	29.0	55.7	55.9	80.6	45.4
in a	5	38.9	70.2	57.8	62.3	24.7
petri-dish	6	27.2	55.6	71.0	61.7	42.9
	mean	31.0	59.2	55.7	67.4	36.2
	s.d.	10.1	13.6	17.6	18.1	12.7
	snail					
6 snails	1	35.6	60.9	112.3	96.0	30.9
grouped in	2	21.2	66.4	98.7	105.2	24.6
cylindrical	3	34.0	69.7	79.9	39.9	32.0
tube with	4	43.0	109.4	50.1	60.4	26.7
constant	5	50.1	90.9	74.3	59.5	6.2
flow rate	6	31.5	88.8	69.9	71.0	21.9
(1ml min^{-1})	mean	35.9	81.0	80.9	72.0	23.7
	s.d.	9.9	18.5	22.0	24.5	9.4
	snail					
	1	42.6	80.6	84.9	95.5	78.6
6 snails	2	28.9	97.4	59.6	72.6	52.3
grouped in	3	20.7	54.9	47.3	84.1	48.3
cylindrical	4	27.1	42.5	60.2	60.5	49.2
tube with	5	51.1	60.4	90.4	100.7	75.4
changed	6	39.6	64.9	84.1	129.2	88.8
flow rate	mean	35.0	66.8	71.1	90.4	65.4
	s.d.	11.3	19.5	17.6	24.0	17.6

120	140	160	180	200	220	240	260	280
0	6.7	5.6	2.1	2.4	0	2.1	1.6	0
10.1	15.4	3.2	0	0	0	19.4	4.4	0
7.6	5.6	12.2	4.4	0	0	6.4	3.7	0
8.7	0	33.4	0	0	0	3.2	14.7	1.7
2.9	9.7	15.5	0	0	0	2.2	13.4	1.1
7.0	19.9	17.8	0	0	0	1.3	1.3	0
6.1	9.6	14.6	1.1	0.4	0	5.8	6.5	0.5
3.8	7.2	10.8	1.8	1.0	0	6.9	6.0	0.7
33.2	9.0	24.6	18.7	5.4	0	0	4.6	0
15.6	13.9	18.7	11.4	3.2	0	2.8	0	19.7
7.2	45.4	21.8	19.4	9.4	0	0.	0	0
16.2	31.6	29.2	21.6	16.5	0	0	0	6.2
18.4	14.4	6.4	44.2	4.5	0	2.6	0	18.0
14.7	11.1	0	2.6	0	0	0	6.6	0
17.6	20.9	16.8	19.7	6.5	0	0.9	1.9	7.3
8.6	14.4	11.3	13.9	5.8	0	1.4	3.0	9.3
49.6	17.8	6.1	4.4	28.1	6.3	0	7.5	0
37.2	2.1	0	0	9.4	14.4	6.4	0	0
29.6	14.1	1.6	2.7	2.7	9.2	8.5	0	1.7
23.4	18.0	8.2	6.1	1.4	8.7	3.8	0	0
38.0	11.3	0	5.3	4.7	3.2	0	8.7	0
11.8	9.9	7.0	3.8	3.7	37.0	3.2	6.8	0
31.6	12.2	3.8	3.7	8.3	13.1	3.7	3.8	0.3
13.1	5.9	3.7	2.2	10.1	12.3	3.4	4.2	0.7

Appendix 5. Adsorption of Frescon on polythene tubing.

A fresh 0.005 ppm solution of ^{3}H-Frescon (157.8 cpm/ml) was run through a peristaltic pump fitted with new polythene tubing. The activity of the solution (cpm/ml) on passing through the tubing, was measured at various time intervals.

Time after start of pumping	cpm per ml			Mean cpm/ml	% adsorbed
	I	II	III		
10 min	114.6	113.9	111.1	113.2	28.3
30 min	111.8	115.5	113.8	113.7	27.9
1 hr	113.2	117.8	116.3	115.8	26.6
2 hrs	119.5	119.8	118.0	119.1	24.5
3 hrs	123.6	124.1	127.3	125.0	20.8
4 hrs	129.0	124.9	124.1	126.0	20.2
6 hrs	124.8	120.8	128.5	124.7	21.0
12 hrs	120.1	124.1	122.5	122.2	22.5

Appendix 6. Uptake of Frescon by Bulinus truncatus.
dpm = disintegrations per minute

time (hrs)	snail wt (mg)	cpm	dpm	amount ($\mu g \times 10^{-6}$)	$\Sigma\mu g / \Sigma mg$ ($\times 10^{-6}$)
1	46	125.8	315.1	6400	
	42	83.1	211.5	4300	
	25	155.1	414.2	8400	
	13	63.5	163.2	3300	
	36	71.6	184.6	3800	
	95	237.4	610.0	12400	150
2	16	128.4	350.4	7100	
	32	132.4	352.7	7200	
	24	177.3	464.2	9400	
	108	396.1	1060.3	21600	
	47	288.0	730.2	14800	
	6	125.3	335.6	6800	287
3	24	159.5	405.4	8200	
	53	336.6	863.2	17500	
	104	477.7	1272.7	25900	
	62	461.8	1221.1	24800	
	24	210.7	556.3	11300	
	67	495.2	1309.1	26600	342
4	108	751.5	2035.4	41400	
	48	463.5	1251.5	25300	
	30	385.9	1144.2	23300	
	28	302.2	863.1	17500	
	24	200.8	566.6	11500	
	15	129.1	348.4	7100	499
5	75	911.2	2344.3	47700	
	32	466.0	1261.5	25600	
	22	288.7	780.5	15900	
	108	905.9	2434.0	49500	
	53	729.7	1989.2	40400	
	43	579.2	1546.8	31400	530

/over

Appendix 6. continued.

6	81	734.5	1930.0	39200	
	65	628.3	1707.6	34700	
	42	485.1	1374.5	27900	
	40	399.3	1100.2	22400	
	19	372.0	1000.2	20300	
	12	267.8	715.6	14500	
	3	195.7	533.2	8800	640
8	78	994.6	2832.6	60076	
	64	654.3	1734.1	36779	
	53	461.3	1319.4	27983	
	35	385.0	1053.9	22352	
	21	244.3	676.8	14354	
	4	168.1	456.0	9671	671
10	85	822.5	2201.9	44800	
	73	794.3	2123.3	43200	
	15	534.1	1562.8	31800	
	7	261.9	778.0	14800	
	3	179.9	490.5	10000	790
12	115	1538.4	4408.4	89600	
	108	1267.3	3719.9	75600	
	57	1150.6	3366.8	68400	
	42	745.1	2148.6	43700	
	28	750.8	2254.5	45800	
	24	520.0	1511.5	30700	
	16	327.5	938.0	19100	956
24	67	1858.1	5198.9	110263	
	46	1654.5	4874.3	103380	
	42	806.8	2391.0	50709	
	27	639.3	1764.4	37422	
	22	504.5	1361.2	28870	
	13	373.4	1006.3	21343	1112

/over

Appendix 6. continued.

36	70	1496.7	4102.0	87000	
	67	1663.0	4829.1	102422	
	42	1246.4	3656.6	77554	
	27	1025.5	2721.7	57726	
	23	675.7	1940.0	41146	
	12	428.1	1200.0	24449	1624
48	57	1866.5	5111.4	108408	
	40	1032.5	2882.6	61137	
	36	966.4	2766.6	58678	
	32	801.6	2379.3	50464	
	13	450.0	1204.2	25541	
	6	283.2	801.3	16995	1745
72	85	2685.7	7507.8	159235	
	67	1643.3	4662.6	98890	
	42	1495.7	4211.0	89312	
	32	1245.2	3603.3	76422	
	13	381.7	1078.1	22865	
	23	515.6	1385.4	29383	1817
96	70	3069.4	7764.6	164681	
	55	2712.6	7471.0	158454	
	46	1875.6	5165.8	109561	
	29	1038.4	2791.2	59199	
	26	449.5	1202.9	25512	
	15	312.6	871.6	18486	2221

Appendix 7. Uptake of Frescon by Sarotherodon mossambicus.

time (hrs)	cpm	dpm	amount ($\mu g x 10^{-6}$)	amount per mg ($\mu g x 10^{-6}$)
3	276.5	679.9	14018	
	247.2	690.7	14241	
	214.6	647.4	13347	
	260.3	723.3	14912	
	240.3	653.9	13481	350
4	335.7	932.4	19223	
	321.2	877.1	18084	
	371.7	729.0	15029	
	257.9	692.7	14282	
	327.8	904.7	18653	426
6	413.0	1143.7	23580	
	327.8	903.9	18637	
	368.4	997.0	20556	
	346.5	964.0	19876	
	406.3	1081.8	22304	525
18	530.4	1687.3	34870	
	480.7	1280.3	26397	
	502.3	1600.0	32989	785
24	546.9	1581.8	32612	
	663.3	1786.3	36828	
	634.1	1750.1	36082	879
48	797.4	2214.7	45662	
	758.0	2057.0	42409	
	854.4	2300.9	47439	
	716.3	1953.0	40265	
	714.8	2031.9	41893	1080
72	1185.4	3435.3	70828	
	781.6	2086.3	43014	
	903.5	2580.4	53202	
	805.3	2261.2	46620	
	1155.1	3302.1	68082	1409

Appendix 8. Uptake of 4'-chloronicotinanilide by Bulinus truncatus.

time (hrs)	snail wt (mg)	cpm	dpm	amount ($\mu g \times 10^{-6}$)	$\Sigma\mu g / \Sigma mg$ ($\times 10^{-6}$)
2	104	188.8	539.5	12986	
	75	116.7	344.4	8289	
	67	94.3	264.9	6376	
	36	74.1	190.1	4576	
	15	74.0	197.5	4755	
	12	52.4	139.6	3361	
	5	37.2	99.8	2403	136
4	69	283.0	843.7	20308	
	84	371.5	1033.6	24879	
	60	233.8	651.9	15691	
	57	194.7	529.0	12733	
	32	155.3	423.9	10204	
	24	108.8	308.5	7354	
	11	71.9	202.9	4884	285
6	77	308.8	873.4	21022	
	75	348.3	940.1	22639	
	46	212.9	579.1	13939	
	33	144.2	394.1	9486	
	28	158.9	444.2	10692	
	15	163.2	436.4	10505	
	4	51.5	139.7	3363	330
8	77	484.2	1290.1	31055	
	75	460.0	1271.9	293150	
	53	272.9	714.0	17186	
	32	193.9	536.3	12908	
	31	163.7	450.5	10844	
	7	84.0	276.7	5202	388
24	85	753.4	2078.4	50030	
	77	827.2	2258.9	54374	
	67	662.1	1702.9	40990	
	34	451.1	1199.0	28860	
	23	340.9	894.9	21540	685

/over

Appendix 8. continued.

48	18	208.7	929.1	22364	
	38	391.6	1208.8	29097	
	30	459.9	1327.5	31955	
	65	751.9	2091.0	50333	
	79	850.0	2424.2	58352	833
74	20	440.6	1269.9	30569	
	35	743.8	2272.6	54703	
	32	786.4	2983.2	71809	
	46	1042.0	2921.9	70333	
	85	1424.0	4350.9	104729	
	135	2361.0	6442.3	164700	1112
96	11	207.5	560.7	13497	
	19	392.5	1115.7	26857	
	36	873.2	2406.0	57913	
	91	1933.8	5454.3	131289	
	87	1396.5	4049.1	97466	1338
120	3	192.9	597.3	14377	
	9	222.3	556.7	13401	
	35	705.7	2002.3	48197	
	25	918.7	2475.9	59598	
	37	1291.5	3191.2	76816	
	9	1622.4	4330.6	104242	
	10	1654.6	4545.9	109424	1444

Appendix 9. Uptake of 4'-chloronicotinanilide by Sarotherodon mossambicus.

time (hrs)	cpm	dpm	amount ($\mu g \times 10^{-6}$)	amount per mg ($\mu g \times 10^{-6}$)
2	33.6	92.1	2218	
	32.3	86.4	2079	
	47.9	120.1	2891	
	41.0	111.4	2681	
	43.2	117.3	2824	
	39.9	103.8	2499	63
4	105.4	289.9	6979	
	62.7	177.9	4282	
	78.2	219.5	5283	
	77.1	220.1	5298	
	104.3	289.6	6970	
	82.7	216.2	5203	142
6	144.4	349.3	8409	
	120.3	315.1	7584	
	139.6	335.1	8067	
	166.6	444.5	10700	
	180.9	481.7	11594	232
8	251.2	655.9	15789	
	194.7	523.3	12597	
	226.0	825.8	19878	
	278.6	730.4	17580	
	308.9	815.8	19638	
	261.8	673.3	16208	424
18	379.0	1074.9	25878	
	340.3	913.4	21985	
	306.6	861.7	20741	
	343.2	887.8	21693	
	319.5	901.2	21369	558

/over

Appendix 9. continued.

24	427.0	1180.9	28426	
	346.7	917.3	22079	
	501.9	1417.5	34121	
	320.7	871.3	20973	
	545.6	1378.3	33166	694
48	425.7	1188.5	28608	
	435.4	1208.8	29096	
	533.2	1371.0	33000	
	433.8	1212.7	29190	749
72	502.1	1419.2	34107	
	513.4	1429.9	34186	
	527.2	1436.0	35600	
	416.5	1121.9	27004	
	550.0	1433.0	34909	829

Appendix 10. Loss of Frescon by Eulinus truncatus.

time (hrs)	snail wt (mg)	cpm	dpm	amount ($\mu g \times 10^{-6}$)	$\Sigma\mu g / \Sigma mg$ ($\times 10^{-6}$)
0	70	381.4	1035.8	21355	
	85	409.9	1129.9	23295	
	45	192.7	544.3	11221	
	22	185.7	497.1	10250	
	16	133.7	389.4	8029	
	53	241.8	712.0	14679	
	13	111.4	299.9	6183	
	4	99.7	281.6	5806	331
18	95	253.2	704.8	14530	
	40	209.1	571.0	11773	
	62	222.0	634.6	13085	
	32	161.0	434.6	8959	
	36	164.0	422.2	9116	
	8	68.8	187.5	3866	225
24	83	272.2	856.8	17666	
	40	173.4	474.5	9782	
	50	194.5	476.6	9826	
	18	114.2	304.3	6273	
	8	78.5	328.9	4719	
	6	54.7	158.6	3270	
	3	30.9	86.2	1771	216
48	67	191.4	600.5	12382	
	92	237.2	633.4	13059	
	81	222.4	639.8	13190	
	81	227.3	652.0	12442	
	40	120.9	327.2	6746	
	18	50.7	146.5	3020	
	15	47.7	132.4	2729	
	11	36.7	100.4	2070	163

/over

Appendix 10. continued.

72	39	89.2	245.3	5057
	34	78.4	228.1	4703
	57	121.4	349.9	7214
	24	60.0	174.9	3607
	28	64.1	212.1	4374
	9	28.5	77.0	1588
	7	21.0	56.7	1169
96	63	100.0	278.1	5734
	61	128.0	344.7	7107
	50	83.7	245.7	5065
	11	28.4	80.7	1664
	15	45.0	118.4	2441
144	85	106.4	296.0	6103
	22	35.5	96.8	1995
	45	64.8	179.7	3704
	18	30.6	85.4	1761
	15	33.4	85.9	1771
	11	18.2	53.1	1095
165	104	102.9	293.5	6051
	40	62.3	180.3	3717
	30	39.3	114.0	2348
	20	23.2	80.2	1654

	140
	110
	84
	71

Appendix 11. Loss of Frescon by Sarotherodon mossambicus.

time (hrs)	cpm	dpm	amount ($\mu g x 10^{-6}$)	amount per mg ($\mu g x 10^{-6}$)
0	335.7	932.4	19223	
	321.2	877.1	18084	
	271.7	729.0	15029	
	257.9	692.7	14282	
	327.8	904.7	18653	426
14	228.8	604.9	12472	
	185.7	476.5	9823	
	240.0	718.3	14809	
	150.1	417.6	8610	282
24	106.6	280.5	5783	
	118.5	325.4	6710	
	155.8	400.8	8264	
	108.4	287.1	5932	167
48	98.9	279.2	5756	
	78.1	226.9	4678	
	72.8	192.1	3961	
	81.3	232.0	4783	120
72	63.3	180.6	3724	
	53.7	145.3	2995	
	39.8	111.0	2288	
	45.9	123.9	2554	72
120	45.9	121.5	2505	
	35.1	92.5	1908	
	45.6	123.2	2540	
	30.9	81.2	1673	54
168	16.6	45.0	926	
	29.5	82.6	1702	
	23.6	65.3	1346	
	19.4	51.7	1063	31

Appendix 12. Loss of 4'-chloronicotinanilide by Bulinus truncatus.

time (hrs)	snail wt (mg)	cpm	dpm	amount ($\mu g \times 10^{-6}$)	$\Sigma\mu g / \Sigma mg$ ($\times 10^{-6}$)
0	69	283.0	843.7	20308	
	84	371.5	1033.6	24879	
	60	233.8	651.9	15691	
	57	194.7	529.0	12733	
	32	155.3	423.9	10204	
	24	108.8	308.5	7354	
	11	71.9	202.9	4884	285
3	95	243.0	684.3	16473	
	80	177.9	490.6	11809	
	51	145.6	406.3	9780	
	28	119.2	325.0	7823	
	20	111.0	290.0	6981	
	12	87.0	235.1	5659	205
18	85	223.9	618.5	14887	
	75	165.7	432.7	10415	
	53	125.0	348.1	8378	
	18	63.0	177.1	4260	
	12	50.7	140.0	3370	170
48	80	78.3	213.8	5147	
	77	68.9	191.0	4597	
	104	104.2	282.4	6798	
	19	45.7	128.2	3086	
	22	54.3	124.5	2996	75
96	85	64.7	179.5	4322	
	46	35.6	97.7	2303	
	67	59.1	158.3	3811	
	26	20.1	55.2	1330	
	30	25.0	66.6	1603	
	7	15.1	39.9	961	55

/over

Appendix 12. continued.

144	95	62.2	176.6	4250	
	75	52.8	142.4	3428	
	72	40.5	114.5	2757	
	18	16.8	47.8	1150	
	24	18.7	53.6	1291	
	10	10.5	27.7	667	46

Appendix 13. Loss of 4'-chloronicotinanilide by Sarotherodon mossambicus.

time (hrs)	cpm	dpm	amount ($\mu g x 10^{-6}$)	amount per mg ($\mu g x 10^{-6}$)
0	277.7	775.0	6340	
	268.5	713.6	5830	
	254.1	683.8	5587	
	242.8	647.9	5293	
	238.3	667.6	5455	
	267.3	741.8	6060	144
18	147.8	411.0	3358	
	167.5	447.1	3653	
	156.7	436.7	3568	
	158.5	420.5	3436	
	153.8	427.5	3493	
	180.2	477.8	3903	89
36	116.9	316.2	2584	
	117.2	315.9	2581	
	127.8	441.3	2606	
	126.7	444.0	2628	65
72	48.1	124.7	1019	
	72.8	192.1	1570	
	53.8	147.9	1208	
	76.3	196.1	1602	
	64.6	173.1	1415	
	43.0	117.8	962	32
120	27.2	72.6	593	
	32.7	90.5	740	
	22.9	59.7	488	
	42.4	106.8	873	
	23.1	65.8	538	16

Appendix 14. Uptake of water by Bulinus truncatus

time (mins)	snail wt (mg)	cpm	dpm	amount taken (mg)	amount taken per mg^{-1} (μg mg^{-1})
2	62.7	1175.4	3228.0	18359.2	292.8
	43.4	641.3	1759.0	10004.6	230.5
	43.3	449.5	1217.0	6921.5	207.9
	46.1	683.6	1843.0	10482.2	227.4
	54.6	1035.3	2770.5	15757.4	288.6
	42.6	579.5	1571.4	8937.1	209.8
4	63.4	1186.6	3294.3	16561.5	261.2
	25.2	583.5	1562.6	7855.9	311.7
	33.5	778.0	2119.5	10655.6	318.1
	32.7	811.7	2231.0	11215.9	343.0
6	40.8	1039.4	2883.2	16398.0	401.9
	42.7	1178.9	3141.1	17865.2	418.4
	61.4	1413.7	2928.1	22341.2	363.9
	42.4	1186.3	3202.0	18211.1	429.5
	43.2	996.0	2709.2	15408.3	356.7
	35.0	1042.0	2769.4	15751.2	450.0
8	21.5	574.8	1535.0	7721.8	359.2
	34.9	1022.8	2797.5	14064.3	403.0
	41.9	1226.8	2971.2	14937.4	356.5
	69.4	2126.2	5882.9	29575.2	426.2
10	62.7	1657.4	4641.4	26398.0	421.0
	60.4	1787.0	4834.4	27495.5	455.2
	59.5	1553.0	4166.7	23697.9	398.3
	32.7	1034.6	2804.3	15949.5	487.8
	38.4	1065.7	2717.4	15455.4	402.5
	39.4	1330.0	3666.1	20851.0	529.2

/over

Appendix 14. continued.

12	24.6	832.3	2093.9	10526.9	427.2
	77.7	2389.0	6806.9	34220.9	440.4
	24.6	766.5	2074.4	10428.8	423.9
	41.0	1279.3	3592.1	18058.7	440.5
15	45.0	1440.1	3918.7	22287.4	495.3
	42.3	1337.7	3540.4	20136.0	476.0
	32.4	1099.1	3044.9	17317.8	534.5
	58.9	1823.4	5124.4	29145.2	494.8
	39.7	1231.2	3399.4	19334.2	487.0
	30.5	900.3	2430.9	13826.0	453.3
20	31.7	906.6	2420.8	13768.5	434.3
	29.6	974.3	2609.2	14839.9	501.3
	47.4	1375.5	3635.4	20676.2	436.2
	43.8	1206.1	3284.5	18680.6	426.5
	51.3	1488.9	4085.6	23236.9	453.0
	31.2	925.9	2418.5	13755.0	440.9

Appendix 15. Uptake of water by Sarotherodon mossambicus.

time (mins)	fry wt (mg)	cpm	dpm	amount taken (mg)	amount taken per mg^{-1} (μg mg^{-1})
2	14.2	129.7	353.3	1776.4	125.1
	14.6	100.6	272.8	1371.4	93.9
	11.0	73.4	202.2	1016.7	92.4
	9.0	84.7	234.5	1178.7	131.0
4	13.9	179.3	506.9	2548.2	183.3
	16.6	169.4	463.7	2331.3	140.4
	10.7	132.6	354.7	1783.3	166.7
	10.4	131.8	352.7	1773.2	170.5
6	23.1	320.7	871.3	4955.5	214.5
	29.0	399.5	1031.3	5865.4	202.3
	42.3	583.8	1554.4	8840.6	209.0
	25.8	413.3	1068.9	6079.3	235.6
8	14.8	282.1	752.4	3782.9	256.4
	15.6	242.7	648.8	3261.6	209.1
	12.9	219.4	577.7	2904.2	225.1
12	14.9	342.9	842.2	4234.1	284.2
	14.2	315.9	869.0	4368.6	307.6
	15.2	279.4	765.4	3848.2	253.2
15	31.9	708.2	1819.3	10347.0	344.4
	20.9	446.4	1216.6	6919.5	331.1
	31.7	518.9	1401.1	7968.8	251.4
	31.8	647.1	1757.4	9995.1	314.3
20	30.4	608.0	1657.7	9428.2	310.5
	27.5	566.7	1571.3	8936.7	325.4
	37.8	952.0	2617.7	14888.0	393.9
	34.6	756.7	2058.2	11706.3	338.2

Appendix 16. Calculated and obtained values for uptake of Frescon and 4'-chloronicotinanilide by B. truncatus and S. mossambicus

		Time (hrs) 1	2	3	4
B. truncatus v Frescon	expected (µg)	68	135	203	270
	obtained (µg)	150	287	342	499
B. truncatus v 4'-chloronicotinanilide	expected (µg)	-	100	-	200
	obtained (µg)	-	136	-	285
S. mossambicus v Frescon	expected (µg)	-	-	44	89
	obtained (µg)	-	-	350	426
S. mossambicus v 4'-chloronicotinanilide	expected (µg)	-	33	-	66
	obtained (µg)	-	63	-	142

Appendix 17. Susceptibility of Bulinus truncatus to Frescon; data for snails either infected or non-infected with Schistosoma haematobium.

conc. (ppm)	Infected B. truncatus		Non-infected B. truncatus	
	No. dead out of 10	probit	No. dead out of 10	probit
0.018	2	4.29	0	-
0.022	4	4.72	2	4.04
0.025	5	5.00	2	4.35
0.032	7	5.53	5	4.95
0.040	8	6.01	7	5.50
0.044	9	6.21	8	5.73
0.048	9	6.40	8	5.94
0.055	10	6.69	9	6.28
0.062	10	6.95	10	6.57

Appendix 18. Uptake of Frescon by Bulinus truncatus, either infected or non-infected with Schistosoma haematobium.

Time (hrs)	Infected B. truncatus wt (mg)	amount ($\mu g \times 10^{-6}$)	$\Sigma\mu g/\Sigma mg$ ($\times 10^{-6}$)	Non-infected B. truncatus wt (mg)	amount ($\mu g \times 10^{-6}$)	$\Sigma\mu g/\Sigma mg$ ($\times 10^{-6}$)
2	10	3332		17	3143	
	22	2990		20	6096	
	61	6415		28	8384	
	47	8617		37	10285	
	33	11096		46	16065	
	71	9235	171	68	19623	212
4	14	8132		19	7707	
	18	7769		35	17557	
	36	11325		31	10730	
	45	11985		50	15168	
	58	10856		65	23587	
	102	14822	238	83	22545	342
6	14	2956		30	16842	
	30	19002		29	14537	
	36	19394		48	22068	
	42	15692		47	20212	
	53	22930		64	40710	
	76	24179	414	75	48477	561
8	20	9658		27	19051	
	36	13687		32	25900	
	46	20819		63	50986	
	53	29403		79	51955	
	63	36407				
	74	38399	510			737
10	25	23445		18	18657	
	28	26372		26	20449	
	40	28305		41	31012	
	51	35210		51	64474	
	72	26845		47	22573	
	89	46007	610	95	79339	848

Appendix 19. Susceptibility of Bulinus truncatus; collected from two different locations in the Gezira Scheme, to various concentrations of Frescon on 24 hours exposure.

d = number dead out of ten
pr = probit

Experiment in June 1977					Experiment in Sept 1977				
conc. (ppm)	treated area		untreated area		conc. (ppm)	treated area		untreated area		untreated area	
	d	pr	d	pr		d	pr	d	pr	d	pr
0.022	0	-	2	4.29	0.025	0	-	0	-	3	4.29
0.026	2	4.13	3	4.68	0.030	1	3.53	4	4.50	3	4.71
0.032	4	4.71	7	5.18	0.033	1	3.87	4	4.76	4	4.93
0.038	4	5.18	8	5.58	0.036	1	4.18	4	5.00	6	5.13
0.044	8	5.59	8	5.93	0.039	4	4.46	5	5.22	6	5.32
0.050	9	5.94	9	6.24	0.044	5	4.89	7	5.55	8	5.60
0.056	9	6.25	9	6.51	0.050	7	5.35	9	5.90	8	5.89
0.062	9.	6.54	10	-	0.060	8	6.00	9	6.40	9	6.31

Appendix 20. Uptake of Frescon by Bulinus truncatus collected from two different locations in the Gezira Scheme. First experiment, treated area Bulinus truncatus.

Time (hrs)	snail wt (mg)	cpm	dpm	amount ($\mu g x 10^{-4}$)	$\Sigma \mu g / \Sigma mg$ ($x10^{-6}$)
1	17	40.8	106.9	22	
	42	66.5	173.8	35	
	57	82.3	214.9	44	
	96	138.5	361.3	73	
	120	147.2	377.5	77	
	170	224.1	614.4	125	75
2	13	57.2	152.3	31	
	42	72.8	192.1	39	
	48	111.0	285.7	58	
	67	204.3	530.1	108	
	122	240.2	638.7	130	
	150	287.5	752.7	153	117
3	5	31.9	85.8	17	
	15	83.7	229.6	47	
	42	179.6	470.2	71	
	45	246.7	664.1	135	
	67	341.8	909.6	185	
	104	361.6	966.8	197	244
4	24	172.5	449.9	91	
	32	225.4	583.6	119	
	67	274.9	711.9	145	
	70	451.7	1191.2	242	
	104	685.1	1777.9	361	
	155	814.7	2178.6	443	310
5	12	217.7	591.8	120	
	32	249.1	640.8	130	
	67	299.0	782.8	159	
	75	403.5	1081.6	220	
	104	784.8	2034.3	414	
	138	1029.0	2636.4	536	361

/over

Appendix 20. continued. Untreated area Bulinus truncatus.

Time (hrs)	snail wt (mg)	cpm	dpm	amount ($\mu g \times 10^{-4}$)	$\Sigma\mu g / \Sigma mg$ ($\times 10^{-6}$)
1	13	63.5	163.2	33	
	25	71.6	184.6	38	
	36	83.1	211.5	43	
	42	125.8	315.1	64	
	46	155.1	414.2	84	
	95	237.4	610.0	124	150
2	6	125.3	335.6	68	
	16	128.4	350.4	71	
	24	132.4	352.7	72	
	32	177.3	464.2	94	
	47	288.0	730.2	148	
	108	396.1	1060.3	216	287
3	24	159.5	405.4	82	
	24	210.7	556.3	113	
	53	336.6	863.2	175	
	62	477.7	1272.7	259	
	67	461.8	1221.1	248	
	104	495.2	1309.1	266	342
4	6	121.8	322.0	65	
	24	182.7	479.1	97	
	32	288.9	772.9	157	
	53	296.6	785.6	160	
	67	438.5	1140.7	232	
	104	870.3	2263.5	460	409
5	24	288.7	780.5	159	
	38	466.0	1261.5	256	
	45	579.2	1546.8	314	
	60	729.7	1989.2	404	
	77	905.9	2434.0	495	
	120	911.2	2344.3	477	530

/over

Appendix 20. continued. Second experiment, treated area *Bulinus truncatus*.

Time (hrs)	snail wt (mg)	cpm	dpm	amount ($\mu g \times 10^{-4}$)	$\Sigma\mu g / \Sigma mg$ ($\times 10^{-6}$)
4	17	150.3	418.8	85	
	24	211.2	573.6	117	
	67	334.4	921.0	187	
	71	395.4	1135.2	231	
	77	441.9	1262.7	257	
	104	497.0	1386.3	282	311
6	8	140.3	379.3	77	
	17	238.4	634.5	129	
	36	288.3	838.4	170	
	67	445.2	1281.3	260	
	95	381.3	1029.3	209	
	129	883.0	2510.0	510	390
8	10	163.5	451.0	92	
	16	211.5	590.5	120	
	32	280.3	774.4	157	
	46	383.3	1033.0	212	
	67	440.5	1171.2	238	
	75	503.2	1437.8	292	
	125	848.3	2442.7	497	451
10	12	237.1	653.8	133	
	20	264.4	765.1	156	
	24	480.3	1321.2	269	
	65	751.4	2191.9	446	
	88	812.7	2219.3	451	
	108	896.9	2762.4	562	570
12	9	196.2	609.8	124	
	20	359.2	883.5	180	
	24	421.6	1170.9	238	
	67	1062.3	2907.9	591	
	100	1211.8	3529.6	718	
	129	1478.1	4130.2	840	695

/over

Appendix 20. continued. Second experiment, untreated area *Bulinus truncatus*.

Time (hrs)	snail wt (mg)	cpm	dpm	amount ($\mu g \times 10^{-4}$)	$\Sigma\mu g/\Sigma mg$ ($\times 10^{-6}$)
4	8	129.1	348.4	71	
	15	200.8	566.6	115	
	24	302.2	863.1	175	
	30	385.9	1144.2	233	
	50	463.5	1251.5	254	
	108	751.5	2035.4	414	499
6	3	159.7	533.2	88	
	12	267.8	715.6	145	
	19	372.0	1000.2	203	
	40	399.3	1100.2	224	
	42	485.1	1374.5	279	
	65	628.3	1707.6	347	
	81	734.5	1930.0	392	617
8	8	171.6	477.6	97	
	17	272.6	801.4	163	
	22	451.0	1233.6	251	
	42	692.1	1928.9	392	
	93	887.2	2398.3	488	
	98	1038.3	2858.5	581	671
10	3	179.9	490.5	100	
	16	261.9	728.0	148	
	22	534.1	1562.8	318	
	81	794.3	2123.3	432	
	97	822.5	2201.9	448	791
12	10	327.5	938.0	191	
	38	520.0	1511.5	307	
	39	745.1	2148.6	437	
	42	750.8	2254.5	458	
	53	1150.6	3356.8	684	
	85	1267.3	3719.9	756	
	114	1538.4	4408.4	896	932