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CHEMORECEPTION AND FEEDING IN THE GREY FIELD SLUG, DEROCERAS RETICULATUM (MÜLLER), WITH REFERENCE TO MOLLUSCICIDE FORMULATION

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ABSTRACT

Studies on Deroceras reticulatum (Müller), the grey field slug, indicate that this animal is responsive to food odours and can therefore select its food. Olfactory responses to food odours are monitored by distance chemoreceptors while contact chemoreception is responsible for the gustatory stimuli associated with taste. Both types of sensory perception are important in the feeding cycle. The former has been investigated with the aid of a time-lapse camera and the behaviour of slugs monitored in the presence of various food materials and their components. Gustatory responses have been assessed by a feeding assay which permits the quantitive analysis of food ingested. These two aspects of feeding behaviour have been combined to provide a framework for the improved formulation of slug baits for use in agriculture. The two molluscicides metaldehyde and methiocarb have been compared in laboratory and field tests for their efficiency in poisoning slugs and their effects on soil fauna. The field results, which included comparisons between laboratory and commercial metaldehyde formulations, indicate the importance of monitoring the residual population of slugs which remains after baiting. It appears that the true level of bait efficiency may be obscured if tests are concerned only with recording the number of poisoned animals trapped. The addition of a mammal repellent to slug baits does not appear to adversely affect their attraction to slugs but individual

formulations may vary considerably in both their palatability and their effectiveness. The laboratory feeding tests have suggested that Deroceras reticulatum habituates to certain diets and that the feeding response may decline over a few days - this can be restored by presenting a novel food to the animals. The meal size of this species can be manipulated in the laboratory by altering the diet and by the addition of attractive components. Some attempt has been made to define these compounds with a view to improving the consumption of baits by slugs in the field.

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CHEMORECEPTION AND FEEDING IN THE GREY FIELD SLUG *Deroceras reticulatum* (Müller) WITH REFERENCE TO MOLLUSCICIDE FORMULATION

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INTRODUCTION

A large number of slugs on cultivated land is generally indicative that crop yields will be low and the quality of the produce poor. Slugs cannot be totally eliminated but chemical control can reduce the population by up to 90%. A knowledge of the life cycle and habits of the pest is fundamental to any control programme since control mechanisms are greatly dependent on the activity of the animals - slugs must approach bait pellets and ingest the toxicant for most treatments to be effective. The timing of control measures can markedly influence the effectiveness of a treatment since adults and juveniles appear to respond differently to poison baits.

Most slug populations reach their peak numbers in March - April when the spring growth of plant material provides an abundant food supply and temperatures are sufficient to enable the temperature-sensitive eggs to hatch. The peak in populations can vary for each species however, and the succession Arion ater (Linné,1758), Arion subfuscus (Draparnaud,1805), Limax maximus (Linné,1758), Deroceras reticulatum (Müller,1774), Milax sowerby (Férussac,1823) and Arion hortensis Férussac,1819 has been recorded by Barnes & Weil (1944). The vertical distribution in the soil also alters throughout the year though some species are generally more active in the surface layers. Slugs tend to move underground in colder weather but Deroceras reticulatum, the most significant pest species

is perhaps the most mobile and active at low temperature. This potential to survive at low temperature is one of the most exceptional characteristics of slugs - Mellanby (1961) has reported that Deroceras reticulatum will move normally at 0.8°C.

An attempt has been made to survey the literature and outline the life processes which may be important in regulating slug activity and hence influence control. Attention has been focused on Deroceras reticulatum since it is the species responsible for most damage and although there are minor variations in the life cycles and habits of other species, it is probable that the factors regulating activity will be similar for all species of slugs.

1.1 THE PHYSIOLOGY AND ACTIVITY OF SLUGS

SPECIES DESCRIPTION

Deroceras reticulatum (Müller 1774) is the commonest slug in N.W. Europe and a major pest species. In early scientific papers it was referred to, and confused with, Agriolimax agrestis which has since been shown to be a distinct species (Quick 1960), though the name Agriolimax reticulatus which was given to the reticulated or grey field slug under investigation has now been largely superseded by the name Deroceras reticulatum. This latter name will be used throughout this work, even where other authors have cited the older name.

The colour of the body can vary from cream to dark grey but is usually mottled in appearance and the sole of the foot has a darker central area. The animal produces

a white mucus when irritated which most easily distinguishes it from a similar slug Deroceras caruanae (Pollonera ,1891). Adult slugs can reach a length of 5cm when fully extended after a growth period of one and a half years and are thus only of moderate size when compared to other Limacidae which can reach a length of 20cm. D. reticulatum has two generation peaks per year though eggs are laid throughout the year. Hunter (1966) and Hunter & Symonds (1971) recorded that slugs hatching in early summer will produce eggs in late autumn taking perhaps five months to complete development, while those that hatch in autumn take longer - about seven months - to mature. Overlapping generations are common however, and laboratory populations appear to mature earlier than their contemporaries in the wild.

LIFE CYCLE & GROWTH

Eggs are laid in clusters and may lead to an aggregated distribution of the slugs after they hatch. The eggs are very susceptible to low temperatures at first but become increasingly resistant as they develop. Arias & Crowell (1963) found that at 20^{o}C, eggs of Deroceras took an average of 15.5 days to hatch, though a range of 15 - 96 days has been recorded. Growth of newly hatched slugs is erratic and individuals from the same egg batch do not necessarily grow at the same rate. Ridgway (1971) indicated that there were significant changes in the growth rate of Arion ater fed on diets with variable protein concentration and Chatfield (1973), working with the snails Monacha cantiana

(Montagu 1803) and Hygromia striolata (Pfeiffer 1828), also observed that different foods have different growth potentials - continued growth only occurred in snails which were fed green leaf material. Judge (1972) fed newly hatched Deroceras on either pea seedlings or broccoli and found that weight gain was greater and mortality lower in the group reared on peas.

Judge also monitored the stages at which this species laid eggs in the laboratory. He measured the length of developing Deroceras each week at a range of temperatures (40 - 80°F) and found that longevity and maximum growth were much reduced at high temperatures. Eggs were laid at 11 weeks after hatching by slugs maintained at 50 and 60°F when the body length averaged 3.14cm and 2.78cm respectively. Peak egg laying occurred at 18 - 20 weeks when the animals were approximately 4.0cm but slugs reared at 40°F did not lay eggs until 32 weeks after hatching suggesting that development is suppressed by low temperatures.

This lack of uniformity within a population of slugs complicates laboratory tests as the physiological and reproductive state of the animal is known to affect performance in behavioural trials. A complicated control of metabolism is suggested by neuroendocrine studies on molluscs. Subramanyam (1973) injected homogenised nerve ring extract into the pulmonate snail Ariophanta and found signs of a strong hyperglycaemic factor. Extract of the cerebral ganglia indicated that a minor hypoglycaemic factor also exists.

REPRODUCTIVE CONTROL

Although terrestrial molluscs are hermaphrodite, cross-fertilisation occurs and in most species protandry is common to prevent self-fertilization. The reproductive physiology of slugs is dependent on both external factors and neurosecretions.

Environmental factors strongly influence maturation of slugs - a high humidity, for example, is necessary for oviposition in Deroceras reticulatum (Runham & Laryea 1968). Visible light can produce harmful effects in the ovotestis of Deroceras (Henderson & Pelluet 1960) and Helix aspersahas been found to lay eggs under a 15:9 light : dark regime but not under a regime of 9:15 (Stephens & Stephens 1966).

Evidence of hormonal secretion is seen in experiments where slugs have been castrated and implanted with pieces of gonad in the haemocoel so that there is no physical connection (Laviolette 1954, 1956, Runham et al. 1973). Runham et al measured the fate of the transplant in Deroceras by its percent expansion and hormonal secretion by the appearance of secondary sexual characteristics.

The optic tentacles are thought to regulate gametogenesis since their removal causes an increase in the number of eggs produced - this is seen within three to eight weeks in Arion ater (Pelluet & Lane 1961). If extracts of brain and tentacle are given to these animals, the number of eggs returns to normal suggesting that the tentacles in some way inhibit oogenesis. Two different hormones are proposed - one from the brain which controls egg production and one from the tentacles which stimulates sperm production.

The normal situation provides a balance between the two with tentacle hormone appearing first and brain hormone increasing in potency at sexual maturity (Pelluet 1964).

Wattez (1977), working on Arion subfuscus, has found a tendency for infantile gonads to develop along a female line when cultured in isolation, supporting the idea of oocyte inhibition by tentacles. By sampling and sectioning the gonads at regular intervals after tentacle amputation, Wattez & Durchon (1972) were able to show that the female predominance in tentaclectomised animals was due to the removal of the oocyte inhibitor and thus confirm the work of Pelluet & Lane. Takeda (1977) illustrated that the process is similar in Deroceras reticulatum - injections of brain homogenate increase the number of eggs laid, while injections of tentacle homogenate decrease this number. Slugs with amputated tentacles also show an increase in the number of eggs produced. Removal of neurosecretory cells from the cerebral ganglia of Deroceras reticulatum prevents growth and since glucose and galactogen levels are still high, growth stoppage is not due to a shortage of food substances (Wijdenes & Runham 1977). These authors suggested that the freshwater snail Lymnaea secretes a growth hormone from the dorsal body which can promote maturation of the oocytes and differentiation of the female sex organs (Wijdenes 1977 , Wijdenes & Runham 1976).

Boer & Joosse (1975) reviewed the literature on the dorsal body and suggested that it releases a hormone which controls female factors produced by the gonad - neurosecretory cells in the cerebral ganglia control release of a hormone to regulate the production of male factors by

the gonad.

Factors such as crowding can influence metabolic processes - Knights (1979) reports that glycogen content is low in Cepaea nemoralis under stress conditions such as crowding. Seasonal variations occur in the level of various metabolites and it appears that reproductive condition may be of importance in this. The galactogen content of the albumen gland of Ariolimax columbianus is highest in the breeding seasons and lowest after egg laying. A tentacle extract is thought to be involved since removal of the optic tentacle increases galactogen content and the process may be reversed by injection of tentacle extract (Meenakshi & Scheer 1969). Smith (1967) found a sudden appearance of neurosecretory material prior to copulation in Arion ater and Simpson et al (1966) also observed cyclical secretory activity and suggested that water balance may be involved too.

There is some evidence that the reproductive state of a slug can influence susceptibility to poison baits (see p 253).

THE BODY WALL

A knowledge of the structure of the body wall is important since most baits have some contact action and are absorbed through the epithelium. Topical application of chemicals is difficult to assess since the material is quickly sloughed off in the mucus produced by the numerous mucus producing cells. Mucus is produced over the whole body to protect and lubricate epithelial cells and since its secretion is dependent on the hydrostatic pressure of the

blood (Machin 1964) the mucus may have a dual origin with one part being an ultra-filtrate of the blood and the other a secretion from the mucous cells.

Pulmonate skin is metabolically active (Newell 1977) with some respiration undoubtedly taking place through the body wall. Receptor cells are numerous, especially on the ciliated region of the foot which suggest that they are mechanoreceptors though they may also have some other functions. A study of the skin from the dorsal and pedal surfaces of Arion hortensis and Deroceras reticulatum (Newell 1973) indicated that the dorsal epithelium was similar in both species but the foot of Deroceras was more ciliated. This indicates certain parallels with the absorptive epithelia of other animals which would conform with the hypothesis that the skin, in particular the pedal area, is responsible for uptake of many substances.

The structure of the skin differs to some extent in each species with Arion possessing a tougher skin which is more adapted to a subterranean existence and the skin of Deroceras being softer and possessing larger mucous cells to equip it for a more active existence on the surface of the ground.

ACTIVITY

Slugs exhibit a regular cycle of activity which motivates them to leave their shelter in the evening, attain their peak activity a few hours after sunset and to return to their shelter in the early morning. This inherent cycle can be influenced by weather and is most marked in spring and autumn. Closer analysis reveals that peak activity

and maximum numbers of each species occurs at different times throughout the year, though overlap does occur between these population peaks. Beyer & Saari (1978) suggest that even within one species, the lifespan can be affected by the habitat - in a study on Arion subfuscus most of the slugs in an open field were dead by the end of July whereas those in a nearby wood survived longer as the canopy offered shelter.

Factors which initiate the departure from and the return to the homesite have been investigated by several workers and two main schools of thought exist - temperature changes are thought by some to be responsible for slug activity, and light by others, though rainfall and vapour pressure have also been investigated. Dainton (1954a, 1954b) proposed that between 4°C and 20°C, activity is induced by falling temperature and supressed by rising temperature, but between 21°C and 30°C falling temperatures deter movement and rising temperatures encourage movement. She reasoned that since slugs begin their activity before nightfall, light could not be the stimulus for activity and the response to rising and falling temperatures would cause the slugs to avoid extremes and aggregate at an optimum temperature - laboratory tests indicated that this was in the region of 17 - 18°C. The field experiments involved a comparison of vertical temperature range but gave no estimate of horizontal temperature variations between adjacent areas. An animal moving over the surface of the ground could therefore be moving up a temperature gradient which, if Dainton's theory, is to be accepted, would counteract the effect of falling temperature and cause the slug to cease movement. The

temperature also remained well above 10°C in most of her experiments yet slugs are known to be active at much lower temperatures (Mellanby 1961) and the conclusions do not extend to an explanation of activity at low temperatures.

Baker (1973) used an aktograph to record slug activity and supported the idea that temperature initiates activity but found that the return to shelter was more closely linked to a fall in the relative humidity. The restriction imposed by the aktograph will, however, have affected the behaviour of the slugs and the results cannot readily be extrapolated to explain movements in natural conditions. Crawford-Sidebotham (1972) suggested that both temperature and vapour pressure deficit control the activity of slugs.

Other workers have indicated that light cycles are of importance. Karlin (1961) found that light was involved in the activity of Deroceras and whilst falling temperatures promoted movement away from resting places, rising temperatures encouraged movement into more protected areas. He also suggested that the responses to food odours, particularly after rain, may modify the response to light. Getz (1963) investigated light preferences of molluscs and also indicated that light is important in the activity pattern though he had no evidence that it was the trigger to initiate the activity.

Cine records of Deroceras made by Newell (1965, 1966, 1968) show that surface activity is geared to light cycles and independent of temperature between 9°C and 16°C. He has suggested an inherent rhythm which probably acts by phased reversal of gravity response, causing the slugs to

move upwards in the evening when a low light threshold is attained and downwards before sunrise. This inherent rhythm is reinforced by the diurnal light cycle and the response to light has been further investigated by other workers in laboratory experiments which monitor the persistence of the rhythm

Arion ater was found to synchronise its activity with light cycles and since this was not influenced by constant darkness, the rhythm can be considered to be truly endogenous (Lewis 1969). Beiswanger & Prior (1976) used activity wheels to examine the circadian rhythm and found that it persisted in constant darkness even after the tentacles were removed, though the timing of the cycle then became less accurate. Sokolove et al (1977) entrained slugs to different light and dark (L:D) cycles at constant temperatures. At 12:12 L:D, they became active in darkness for a total period of 9-10 hours. Animals under a 16:8 L:D regime, which were subsequently placed in constant darkness, D:D, showed a drift in time of peak activity. Their data suggests that the circadian peak is not always constant and can be entrained and shifted by different 24 hour L:D cycles. Rollo (1978) also found that the activity rhythm of slugs can be entrained by light cycles but he found a difference in the light threshold of each slug species he tested. He suggests that the inability of Dainton to demonstrate a light response in Deroceras may be explained by inadequate light intensity in her experiments.

Moreton (1979) found that the activity cycle in Deroceras caruanae was paralleled by cytological changes so that the slug absorbs digested food in daylight and breaks

it down at night. Other physiological cycles may also exist.

The endogenous activity cycle is most marked in newly caught slugs and deteriorates as the animals are kept in constant temperature or light conditions. This has important implications when animals are being kept in the laboratory for behavioural studies or any test involving an activity response. Slugs which are kept in culture (Stephenson 1961) are unlikely to exhibit the same responses as animals which have been recently collected from the field and results which have been obtained from laboratory reared slugs must be treated with caution. Since young and old slugs react differently, however, it is important to use animals of the same stage of development in behavioural work.

Reproducible results can only be obtained in studies on slugs by imposing a time limit on the length of time the animals are housed in the laboratory so that activity cycles will conform to the natural situation where slugs become active at night and emerge from their resting sites to feed when light and temperatures both decrease. Laboratory conditions must ensure an equable temperature and high humiditiy with a regulated light cycle. The optimum temperature for slugs is 13.5^{o}C - 17.5^{o}C (Ricou 1964) and moisture is certainly important in maintaining activity, yet the thresholds for these and other physical features can vary for different species of slug. The animals may be kept under reverse daylight conditions but it is suggested that those kept for a period of seven days in constant laboratory conditions will have lost much of their inherent activity cycle and should not be used in

behavioural studies.

1.2 HABITAT

Slugs and snails have a widespread distribution and both groups are serious pests throughout Northern Europe, America and parts of Asia. Snails are restricted to calcium rich habitats since they require calcium in their diet for shell growth. Slugs lack the protection offered by a shell and are therefore more prone to desiccation but they are relatively unrestricted by mineral requirements and are also able to work their way into small niches inaccessible to snails.

Slugs are abundant on heavy soils where drainage is poor and moisture levels remain high and although some species show a preference for particular topographical or vegetational features, most slugs have a cosmopolitan distribution. Several associations with tree or plant species have been proposed. Beyer & Saari (1977, 1978) investigated the possible affinity of Arion subfuscus for 13 tree species and although slug numbers were not increased near the trees, there was a slightly higher density near timothy plants (Phleum pratense) and three other slug species - A. fasciatus (Nilsson 1823), Deroceras reticulatum and D. laeve (Müller 1774) - showed a possible association with trees that had a high calcium content in their leaves. South (1965) suggested that D. reticulatum may be associated with cocksfoot (Dactylus glomerata) but indicated that the eggs were laid in batches, often at the base of cocksfoot plants, and the dispersive powers of the slug are poor so a high density in the vicinity of this plant may not be due to any

specific nutritional requirements. Recently, Fog (1979) has investigated the numbers of slugs and snails on decomposing wooden stumps and found a positive correlation between the numbers of snails present and the growth of bacterial colonies on some tree stumps.

Soil structure has a major influence on the presence and the numbers of slugs. Traditional farming methods have involved breaking the soil by ploughing so that the organic matter at the surface is fragmented and combined with the lower layers. The inversion of the soil exposed both eggs and adults to predators and desiccation, thereby reducing the slug population. Modern farming practices tend towards less soil tillage and a greater reliance on chemical treatment with fertilisers so that mechanical disturbance of the soil is minimal. Rollo & Ellis (1974) compared three soil tillage regimes and found that slug attack was most severe in zero tillage plots. Reduced soil disturbance allows the number of slugs to increase and the widespread practice of monoculture accentuates this since the pests are maintained over large areas. The practice of ploughing in waste to increase the moisture holding capacity and organic content of the soil improves the conditions for slugs and plant debris remaining on the the soil surface provides a perfect breeding ground.

An unfortunate paradox therefore exists whereby those farmers who try to conserve soil structure and improve the fertility of their land are the most vulnerable to increasing slug damage in their crops.

1.3 PEST STATUS

Molluscs feed using a tough radula which rasps holes in fruits, grains and tubers and shreds leaves. Slugs are more important than snails as pests in Great Britain, feeding on potatoes, brassicas, soft fruits, wheat, salad crops and legumes and several species of flowering plants e.g. zinnias and marigolds. Deroceras reticulatum is perhaps the major pest as although this slug is smaller than many other pest species, it can breed throughout the year and a rapid increase in the population can occur. Rollo & Wellington (1975) found it to be the most numerous and most destructive slug in Vancouver where official reports state that at least 26 types of crop are attacked by slugs. The M.A.F.F. also report that this is the most common and most injurious slug in Great Britain.

The extent of the damage is expressed in terms of lost yield (acreage or tonnage), lost profit or percentage of crop damaged, but it is difficult to quantify with any degree of accuracy. The damage is often cyclical so although heavy, poorly drained soils are most likely to harbour high populations of slugs, these may not prove to be of pest proportions in some years. One consequence of this is that farmers are deterred from baiting regularly and the population gradually increases in intervening years. High rainfall is probably the main factor influencing outbreaks of slug damage, but temperature, the nature of the preceding crop and local cultural practices are also important.

A population of 50 slugs per metre (500,000 slugs per ha) is considered to be a heavy infestation though not uncommon and Brugger (1974) quotes 1 million

slugs per ha. as a severe infestation. Carrick (1942) suggested that a tolerance level of 250,000 slugs per ha should be accepted for the Scottish potato crop which may have a population ranging from 75,000 to 1,250,000 Deroceras reticulatum per ha. (from Magnenat 1972). It is doubtful however, whether a crop could sustain such a level and still be economically viable and a population of 100,000 slugs per ha. may be a more realistic limit to apply.

Slugs are not only pests of agriculture and garden crops since Limax sp. are frequently found in kitchens and areas where household refuse is deposited. Frömming (1957) reported that Limax flavus Linné, 1758 is a common pest in cellars and in bakeries where it can live entirely on stored flour. When crops are harvested mechanically a problem can arise in sorting the pest from the crop, especially where it is of a similar size. Slugs are often harvested with peas which are cut and vined at night (Wharton & Ensor 1969) and Cepaea nemoralis and C. hortensis (Müller 1774) are likwise a hazard when harvested with blackcurrants (Stringer & Morgan 1969). Cairaschi & Lecomte (1973) also indicate that the snail Theba pisana is a pest at grain harvesting since the snails block the harvesting machines and cause losses of the grain. Although molluscs may not therefore be the major pest, they can necessitate extra sorting of the crop and cause additional costs in harvesting.

Grain hollowing by slugs is a common cause of crop failure in the increasingly important, autumn sown winter wheat, resulting in reduced yield. The leaves are also grazed in late autumn and spring but once the plants reach a

certain height, growth is usually rapid enough to prevent excess damage at this stage.

In Great Britain, the potato crop is one of the worst affected by slugs with the damage in 1974 estimated at £790,000 (Rayner 1975). The exact value is impossible to calculate since costs arising from sorting the damaged crop, wasted labour etc. cannot be determined. A survey of N.A.A.S. advisors (Hunter 1969b) revealed that the damage is located mainly in the eastern counties but considerable local variation occurs - clay and silt soils have the highest level of damage. Approximately 39,000 tons of potatoes were thought to have been reduced in value in 1967 and Rayner believes that this could be halved using poison baits.

Certain varieties of potato are more susceptible to attack than others and Pinder (1974) found that although the numbers of slugs in five potato cultivars were similar, there was a marked difference in the extent of damage. It is not certain whether the skin or the flesh is responsible for this damage but Pinder suggests that biochemical differences may be more important than physical. Warley (1970) could find no consistent relationship between crude fibre content, skin strength and slug attack. He did however, conclude that immature tubers - the least attractive - had a higher sucrose content than mature tubers and that the least susceptible varieties had the highest sugar content. Mature tubers - the most attractive - had a very variable sugar content at harvest. In his study,Redskin proved to be the most susceptible variety and Pink Dell the least.

Stephenson (1965) believes that slugs do not

actually initiate attack on the tubers since they prefer damaged potato and he suggests that attractive substances may be exuded from plant wounds. Thomas (1947) thought that Arion hortensis and Milax budapestensis (Hazay 1881) were responsible for initiating damage while other pest species caused secondary damage. Newell (pers. comm.) however, grew potato plants in sterile soil with the aerial parts protected by polythene bags to protect from infestation by other organisms. The introduction of Deroceras reticulatum into the soil resulted in extensive damage to the potato tubers, showing that Deroceras can initiate attack on potato plants.

Most damage to the potato crop occurs in autumn after the death of the haulm when surface vegetation is limited and the slugs are forced to move underground (Hunter 1969a). Haulm removal also permits increased wetting of the ridge and thereby improves the conditions for the slugs (Warley 1970). Optimum control is therefore achieved by baiting in July or August before the haulm dies off and the slugs move underground.

1.4 CONTROL

NATURAL PREDATORS

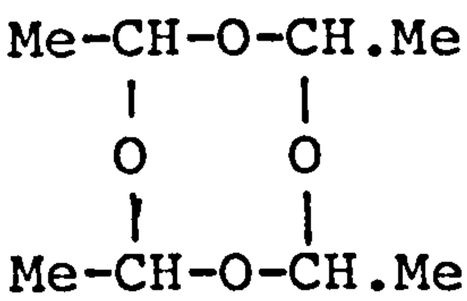
There are many natural predators of slugs and although their contribution in controlling pest populations is unknown, it is unlikely to be very important. The fly, Tetanocera elata, is known to kill slugs (Knutson et al. 1965) and Mead (1979) reports that the initial discovery by Berg in 1965 of such pulmonate - dipteran relationships led to successful contol programmes of many aquatic snails.

Baronio (1974) has recorded that some of the Sciomyzidae (Diptera) and Drillidae (Coleoptera) live almost exclusively on slugs and snails while members of the Lampyridae, Carabidae and Hydrophilidae (Coleoptera) will willingly eat them.

Ocypus olens, a large rove beetle, has been observed to eat almost its own weight of snail tissue each day and field observations from an area where this beetle is well established suggest that the snail population is smaller than other similar areas and that the beetle may thus be controlling the snail population (Fisher et al. 1976). Many birds - jackdaw, mallard, rook, lapwing - are also known to eat slugs as are hedgehogs, shrews and several other small mammals.

Snails have been introduced in some areas to control pest populations of a more harmful mollusc. Species of Gonaxis, Culella and others were used in control programmes against the giant African snail, Achatina fulica Bowdich, which established itself in Hawaii in the 1960's (Mead 1979). More recently, the snail Rumina decollata (Linné 1758) has been used as a predator on experimental sites in California to control populations of Helix aspersa (Fisher - pers. comm.). Although R. decollata may eat senescent leaves of some plants, it causes less damage and devours young Helix by eating through the foot of these animals.

METALDEHYDE



2,4,6,8-tetramethyl-1,3,5,7-tetroxocane

In the late 1930's, the slug killing properties of metaldehyde were accidentally discovered. This is a white crystalline powder of only limited solubility in water (200mg/l at 17°C) which is a polymer of acetaldehyde. Metaldehyde is combined with a carrier such as wheatbran and broadcast as a bait. Pellets containing 5 - 10% metaldehyde are commonly used since binders, stabilisers and fungicides can be readily incorporated into these to increase the life of the bait in the field.

The mode of action of metaldehyde on slugs and snails is not completely known, but it is believed to exert both a topical and an internal poisonous effect. Thomas (1948) first recorded details of the poisoning symptoms and suggested that the effect is threefold:

1. Direct mortality leading to transparency of the gut wall.
2. An anaesthetic effect.
3. An irritant effect.

He observed that high humidity conditions encouraged recovery of slugs though snails were more susceptible to direct toxic action and less likely to recover.

A contact action of metaldehyde is reported by Van den Bruel and Moens (1957), Brugger (1974), Cragg & Vincent (1952), Henderson (1966,1968) and Lange & MacLeod (1941). The molluscicide is most readily absorbed through the sole of the foot, but the L.D. is difficult to determine

experimentally since the chemical is sloughed off by the animals.

Cragg & Vincent (1952) found that the lethal effects were produced in Deroceras reticulatum within one hour with concentrations of 0.0063mg/cm^2 metaldehyde applied to the skin and suggested that contact action is more important than stomach action though this is not generally acknowledged to be the case. More recently, Sharaf & Ghaleb (1978) have devised a laboratory immersion test to assess contact activity of molluscicides which involves grinding the pellets and suspending the resultant powder in water. Snails placed in closed cylinders of molluscicide solution for one hour showed paralysis and loss of muscle tone. A water control ensured that the animals were not killed from the effect of the immersion alone but the solutions interfered with normal respiration and this may have made the animals more susceptible to death by poisoning. The test is therefore of limited value only in assessing the effectiveness of baits for use in the field. A 5% metaldehyde bait was found to have a M.L.C. of 1.45 $\pm$ 0.0025 g/l for Theba pisana while that of a 4% methiocarb bait was 4.20 $\pm$ 0.0044 g/l.

Henderson (1968) obtained a value of 42370 ppm. as the lethal concentration for contact action of metaldehyde, compared with a value of 2027 ppm. for a copper sulphate solution using a dry contact method - ground talc was used as the carrier in place of water since metaldehyde is relatively insoluble. Henderson also developed a method for assessing the activity of metaldehyde as a stomach poison (1966, 1969). The toxicant is normally regurgitated by

the animals, so Henderson incorporated metaldehyde into a cold setting agar gel,injected this into the buccal cavity and passed the slug through a peristaltic mangle. As the slug was passed between the foam rollers of the mangle, the gel was forced into the crop without damage to the animal. A M.L.D. of 85.2 $\pm$ 4.0 μg was obtained for Deroceras reticulatum of 0.3 - 0.6g weight though Hunter & Johnson (1970), using a similar method, obtained a value of 45.71 μg for this species. The main disadvantage of this method is that it gives no indication of the attractiveness of metaldehyde and therefore of how much is likely to be ingested voluntarily.

The fumigant action of metaldehyde is less widely accepted as a method of poisoning. Cragg & Vincent (1952) found no evidence for such poisoning, though Henderson (1970) showed that slugs placed in a closed vessel in which metaldehyde had stood overnight, could be seen to writhe and produce excess mucus. The pneumostome closes when the slug is irritated in this way, so entry of the toxicant must be via the mucus covered skin. This may account for the differential susceptibility of various species to metaldehyde since mucus composition varies between species. Henderson estimated the fumigant action of metaldehyde to have a $L.C._{50}$ of 7.96 ± 0.21 mg/l for Deroceras reticulatum and 8.91 $\pm$ 0.18 mg/l for Arion hortensis.

In 1957 Mayer recorded the symptoms of metaldehyde poisoning in slugs as follows. The slug first shows signs of irritation with muscular contractions of the foot and copious secretion of slime. The animal then becomes

paralysed due to destruction of the nerve plexus and damage to the ganglia. The slug dies from desiccation after losing 40 - 50% of its body weight and can be found in a contracted state. The relative humidity of the environment is thus the most important external factor affecting the success of baiting since in a moist habitat the animals can recover lost water.

Dmitrieva & Shapiro (1975) have observed that one to two minutes after ingesting 50% of a $L.D._{50}$ dose of metaldehyde bait, Deroceras reticulatum enters a phase of hyperactivity during which respiration increases and glycogen and lipid levels fall. This is followed by a 35 minute period of depression when respiration falls. A dose of the full $L.D._{50}$ initiates a phase of hyperactivity lasting 20 minutes and death follows within one and a half hours.

Several authors have suggested that animals with a smaller body weight require a smaller dose to kill them (Daxl 1970, Mayer 1957) but Crawford-Sidebotham (1971) found an age related susceptibility with young slugs less likely to be poisoned than older animals. He suggests that this may be due to the fact that juveniles do not move so far in their nocturnal excursions and are thus less likely to encounter bait pellets.

A differential susceptibility was also apparent in his tests amongst the nine species of slug he compared for vulnerability to both metaldehyde and the carbamate methiocarb. Death through contact action is perhaps more common in larger slugs since these have a greater foot sole area over which to absorb the toxicant. Since there is

some evidence that young and old slugs eat different foods (see P 274), the age variation in susceptibility to baits may be influenced by the attractiveness of the toxicant to young and old animals and the probability that each will ingest the bait.

The actual attractant properties of metaldehyde are disputed though Barnes & Weil (1942) claim that the number of slugs trapped by metaldehyde baits is too high to be accounted for by chance alone. Lange & Sciaroni (1952) believe that at high concentrations metaldehyde is actually repellent to certain molluscs and Fisher & Orth (1975) have suggested that populations of Helix aspersa from regularly baited areas may become resistant to metaldehyde.

The main advantage of metaldehyde is its specificity as toxicity to other invertebrates or to higher organisms is less than other molluscicides and the rapid depolymerisation to acetaldehyde means that residues do not accumulate. The main disadvantage is that metaldehyde efficiency is dependent on weather conditions. Warm, dry weather hastens desiccation of the animals and prevents recovery but slugs tend not to be active in these conditions. Optimum results are obtained by baiting after rain when the animals are active.

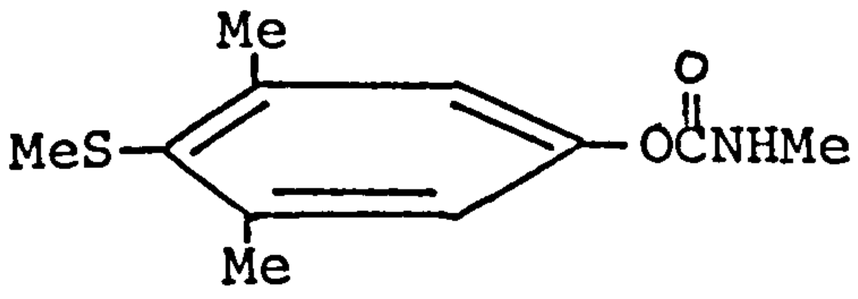
Soil residues of metaldehyde have been calculated by Terriere (1974). At 3lb. per acre, metaldehyde sprays give an initial residue of 1 - 2ppm. which falls to 0.5ppm. after three days, while at 8lb. per acre of groundbait, the initial residue is less than 1ppm. and falls to 0.1ppm. after three days. C_{14} studies show that metaldehyde has completely disappeared in seven days and breakdown is

accelerated by sunlight and high temperatures. Most of the compound depolymerises to acetaldehyde and disappears by leaching, mechanical removal or sublimation from the soil surface.

The lethal dose of metaldehyde is cited by Tilemans & Dormal (1952) as 170 - 200 mg/kg for humans and by Terriere (1974) as 0.25 - 2.5 g/kg (25 - 250 mg/kg) for warm blooded animals in general.

CARBAMATES

Many of the carbamates originally synthesised for insect control have since been found to possess molluscicidal properties and methiocarb (Bayer Ltd- Development Number : Bay 37344) has been the most successful.



4-methylthio-3.5-xylyl methylcarbamate

Carbamates produce marked paralysis or loss of muscle tone (Crowell 1967) but do not cause the sliming response that metaldehyde does. The carbamates Isolan and Zectran have also been extensively tested but have shown residue and toxicity problems. Daxl (1970) reported that Isolan restrained mucus secretion by paralysing the mechanism involved in its release since it blocks the neurotransmitter cholinesterase. High concentrations (600 mg/cm^2) however, were required to improve on metaldehyde kills.

Crawford-Sidebotham(1970) has calculated an Index of Efficiency to compare the carbamate methiocarb, with metaldehyde in a number of laboratory tests involving several species of slug. The index I, was calculated from R_D/R_M where:

$$R_D = \frac{\text{Total number of slugs poisoned with methiocarb}}{\text{Total number of slugs poisoned in methiocarb experiments}}$$

$$R_M = \frac{\text{Total number of slugs poisoned with metaldehyde}}{\text{Total number of slugs poisoned in metaldehyde experiments}}$$

On the morning after baiting,the value of 1.36 for I indicated a slight superiority of the methiocarb formulation but after 48 hours, this value had increased to 2.13 - these figures are an average for the nine species tested. The animals had been placed in a relative humidity of 100% during the intervening 48 hours and a temperature of 15°C so the recovery rate from metaldehyde was exceptionally high. In field conditions where the animals are more exposed, recovery could be expected to be much lower and the difference between the two baits less apparent.

Rorke et al (1974) investigated symptoms of carbamate poisoning in Helix aspersa and found two different types of behaviour suggesting that another mechanism, apart from cholinesterase inhibition, is also involved in the death process. Mallet & Bougaron (1971) observed that methiocarb poisoned animals immediately cease locomotion and that the body lengthens and softens. This softening and swelling of the body is a common sign but the animals do not appear to die immediately - Cardew & Newell (1976) recorded that death from methiocarb in the field may take up to four days.

Hunter & Johnson (1970) determined the $L.D._{50}$ of methiocarb and obtained a value of 21.88 μg for Deroceras reticulatum. Methiocarb is a less specific toxicant than metaldehyde, however, and a number of non-target organisms are susceptible to poisoning. Hermann (1971) estimated that about 5% of the earthworm population was killed by methiocarb, but without an accurate survey of the initial population, this value is difficult to verify. Martin et al (1969) found that methiocarb was highly toxic to birds - the $L.D._{50}$ for a ring-billed gull (Larus delawarensis) is 5 - 10mg/kg. The manufacturers colour the pellets blue since this is supposed to be not easily recognised by birds and they are therefore less likely to ingest the bait. The $L.D._{50}$ for male rats is 130mg/kg (Martin et al 1969) compared with a value of 690mg/kg for metaldehyde (Verschuuren et al 1975). This high toxicity is the major drawback of baiting with methiocarb but the poisoned animals are less susceptible to fluctuations in the weather and recovery in damp conditions is rare.

ALTERNATIVE MEASURES

A number of other chemicals have been used to control pest populations of molluscs in the past. Paris green (copper aceto-arsenite), copper sulphate and calcium arsenate are amongst the most common compounds added to metaldehyde though they have been used alone on a small scale. Increased potency is obtained from a combination of these chemicals but the proportions of each constituent has varied widely in different areas. Combination baits are designed

to improve the number of animals poisoned in the field by incorporating toxicants which can contribute to a number of different poisoning characteristics to the bait. Up until 1965 a 6.25 : 1.25% calcium arsenate : metaldehyde bait was used in the Western areas of the U.S.A. but improved results have since been obtained with a 5.16 : 3.0% mixture (Pappas 1971). Legislative action in some states however, has prohibited the use of arsenicals and replacement baits are being sought.

Combination baits of metaldehyde and a carbamate, Sevin, have been investigated by several people (Crowell 1977, Ruppel 1959, Webley 1964) and a synergistic effect appears to make it superior to either bait individually. Crowell, however, found that a metaldehyde/methiocarb (1% : 2%) bait was less successful than a standard 2% methiocarb formulation since recovery from the combination bait was high. The feasibility of using other insecticides has also been investigated.

Environmental studies by Davis (1968) showed that slugs accumulate organochlorine residues up to 40ppm. and produce the breakdown product TDE (tetrachlorodiphenylethane but this does not appear to kill them (Davis & French 1969). Lower accumulations of DDT (dichlorodiphenyltrichloroethane) have been recorded in snails than in slugs and although the snail Cepaea hortensis readily ingests DDT, it excretes it rapidly (Dindal & Wurzinger 1971). The insecticide Phorate was,however, found to be effective as a slug killer, though field trials showed that it was also repellent (Musick 1972).

Alternative measures of controlling molluscs are available but unsuitable or impracticable for use on a large scale. Hand picking is carried out in areas where labour is inexpensive and Hanna (1966) reports that slugs may be burnt out of infested areas. Mechanical devices such as barriers of bamboo shoots, coal ash, soot, tar, salt, lime etc. are also used in some parts of the world (Malek & Cheng 1974). Barriers of copper (5cm length) were found by Moens et al (1967) to give protection but the efficiency fell after rain when the barrier was covered with a water film. More recently, small containers incorporating an undisclosed attractant which is impregnated on a central disc, have been marketed for use in gardens. The slug or snail is enticed to enter through openings in the container but once inside, becomes trapped and cannot escape, The basic idea is thus similar to the traps used to lure insect pests but these traps are designed for small scale use and are rather impracticable for field usage. Containers of beer sunk into the soil are another method of controlling slugs in gardens as the animals fall into the containers and drown.

Prophylactic measures offer an alternative means of controlling pest populations. Clearing the soil surface removes the shelter and moist conditions favoured by slugs and reduces the risk of populations building up. On an agricultural scale, correct rotation of crops can ensure that susceptible crops are not planted on the same ground in successive years when the populations may be encouraged to increase.

1.5 FORMULATION

The field application of molluscicides necessitates that the chemical be formulated into pellets, sprays, emulsions or granules. Since slugs are basically ground-inhabiting animals, pellets are generally applied but in certain crops and particularly where snails are pests, sprays may be preferred since they can reach a wider area and poison animals that have climbed up trees or plants.

Any successful bait will contain a toxicant, a powerful attractant and an inexpensive diluent or feedstuff which should preferably be attractive itself. In addition, fungicides and binders are required to improve the keeping qualities of the bait. Mini pellets are now marketed for both metaldehyde and methiocarb formulations since these are less susceptible to decay and also present a wider surface area for the same weight of toxicant. Other advantages include reduced transport costs.

Wheatbran is the commonest diluent although any substance of nutritive quality which is acceptable to molluscs can be used. In regions where wheatbran is expensive, a similar bulk substance is substituted - ricebran in the Orient, fresh-pressed orange pulp in California and banana peeling or papaya fruits where these are available as a waste product (Mead 1961). Cattle cake, used tea leaves, coffee grounds and sawdust have all been shown to possess some attractiveness to molluscs and therefore represent possible candidates for carriers (Barnes & Weil 1940). The latter is non-nutritive but the aromatic nature of the wood may be responsible for the

apparent attraction. Speyer (1954) found that a sawdust/ metaldehyde mixture was an effective bait and suggested that dilution of the metaldehyde with such an inert carrier overcame the irritant effects felt when undiluted metaldehyde powder was applied. Sugar seems to increase attractiveness but accelerates deterioration, though Lange & MacLeod (1941) found that molasses, raisins and powdered milk increased attraction and did not report increased decay. Thomas (1948) added 10% dextrose which increased the mortality but not the numbers trapped while the addition of 10% by volume of dried blood to a metaldehyde/bran bait had the reverse effect. Smith & Boswell (1970) have suggested moistening proprietary baits with beer to improve their attractiveness - other incidental reports of slugs being attracted to beer indicate that this could be a promising attractant (Stout 1968, Northern 1970).

Commercial manufacturers of pellets use a variety of attractants to augment the effect of the wheat carrier and amyl acetate is commonly used though probably many flavourants incorporated in human foods would be acceptable. Some inorganic carriers are used in place of wheat to improve the stability and persistence of wheat-based pellets since these swell on contact with moisture and deteriorate rapidly.

Vermiculite or perlite granules provide another type of carrier as the granules can be coated with toxicant and attractants and last extremely well even in moist field conditions. Vermiculite granules are rather brittle however and fragment easily (Lonza Ltd, 1974 -

British Patent 1434899) so a coating of bait uniformly applied to the outer surface of perlite granules provides accurate distribution and ensures that all of the toxicant is readily available to the slugs.

Experiments using gelatin as a bait carrier have been conducted by Stephenson (1970, 1972). Gelatin sheets, treated with formaldehyde to harden them, were cut into discs and placed in open field conditions. They remained intact for 14 days but were not attractive to slugs unless a bran extract was incorporated when they became mouldy. Chemicals to reduce bait deterioration are generally included in commercial pellets though care must be taken to ensure that these secondary chemicals do not alter the attractive qualities. The fungicides Dazomet and Zineb incorporated into baits repelled slugs although deterioration was reduced (Webley 1964) and many compounds indicating promising molluscicidal activity have had to be abandoned because they repel the slugs - the herbicide Ioxynil (3.5-diiodo-4-hydroxy benzonitrile) was tested by Daxl (1971) but found to be markedly repellent to slugs when tested in the field and also to damage the crop when applied at concentrations sufficient to kill slugs. The development of new formulations could overcome such problems if toxic chemicals were perhaps incorporated so that the molluscicide was released only after ingestion of the capsule.

A slow release formulation of sodium pentachlorophenate for use against the freshwater snail Biomphalaria glabrata was explored by Ritchie et al (1969) and found to release 0.75g active substance per hour for 48 hours at 30°C, but the problem of even distribution in air

is more difficult to achieve. Weiss (1973) has suggested that bait could be incorporated into cereal grains which could then be deposited in the soil to improve persistence. Attempts have been made to waterproof bait granules though Judge (1969) suggested that in damp conditions the attractive qualities of bait only persist for 24 hours. Webley (1966) showed that a colourless, odourless and harmless film formed on the surface of moistened bait held in a stream of air carrying chlorosilane vapours. Thomas (1948) formed a metaldehyde/bran/casein glue bait which could be broadcast in biscuit form and retained its effectiveness for three weeks.

The method of application of a molluscicide can result in great differences in the effectiveness of the treatment. Sprays are more effective in some situations and Webley (1964) has recommended that sprays be used for epidemics and groundbait as a long term control measure. Pappas & Carman (1961) found that the dense foliage of Valencia orange trees prevented the bait from effectively killing snails since poisoned animals beneath the branches were able to recover in the sheltered environment there. Sprays of molluscicide enable the toxicant to reach the pests while feeding on the aerial regions of the trees and it is generally assumed that some plants - strawberries, brassicas and beans - receive better control from sprays than pellets (Howitt & Cole 1962).

Metaldehyde sprays are difficult to prepare but grinding the powder with fine talc, diatomaceous earth, or kaolin powder gives a more miscible mixture (Symonds 1975).

Four types of metaldehyde sprays were tested by Moreton (1953) at a level of 100 gallons per acre. A 0.02% solution of metaldehyde and water gave the best results but field observations were complicated by the fact that high concentrations killed directly whereas low concentrations immobilised the animals and exposed them to a more lethal dose.

Seed dressing have been sought since much of the damage to winter wheat is caused by grain hollowing (Gould 1961) and copper oxychloride was found to have some success. A more common method of reducing damage to the seeds however is direct drilling where the pellets are sown with the wheat grains and the slugs can thus be encouraged to feed on the toxicant rather than the crop.

1.6 BAIT DISTRIBUTION

The distribution of bait pellets is a controversial topic in the literature. Hunter & Symonds (1970) devised an equation to calculate the probability of a slug encountering a bait pellet and estimated that proprietary pellets do not attract over more than 4cm. This is based on the dubious assumption that slugs move randomly within the area in which the pellets have been placed but slug movements are erratic and the distribution of the animals tends to be aggregated. As slug activity is dependent on weather, the animals will also encounter the pellets more frequently in damp conditions when they forage over a wider area. Hunter & Symonds suggest that a spacing of 20 - 30 cm pellets is an economically viable level of bait application though Weiss (1973) thought that maximum

efficiency could be obtained at a spacing of 7.5cm. This figure was also quoted by Webley (1970) - 10 pellets per 2.5 feet - but he suggests that a spacing of 15 - 40cm (2 to 5 pellets per 2.5 feet) is sufficient for the more active Deroceras reticulatum. Laboratory results of Miles (1969) indicated that metaldehyde baits were not attractive at 2 feet intervals but decreasing the distance to 1 foot increased the probability of a slug reaching the bait by 100%.

The attractiveness of the bait is a major factor in determining the appropriate distribution of the pellets and the addition of suitable attractants should be able to reduce the level of application by encouraging the slugs to move over a wider area.

Some authors suggest placing the baits under covers to improve the persistence of pellets and to prevent interference from birds and small mammals. Thomas (1948) found that his metaldehyde and casein biscuits were eaten by hedgehogs and proposed that for small scale treatments, baits could be uncovered in the morning to hasten desiccation of poisoned animals and covered at night to encourage the animals to collect the traps. Uncovered baits were found by Webley (1963) to have a better dehydrating action and give improved kills but slugs were preferentially attracted to the moist conditions under the tiles.

The species of slug which is causing damage to a particular crop will also affect the efficiency of baiting at a specific dosage. Webley (1970) found that the return per pellet was less for the more subterranean Arion hortensis and Milax budapestensis as these species are less likely to encounter bait pellets than Deroceras. Warley (1970)

confirmed these results and Mallet & Bougaran (1968) indicated that the attractive distance for Deroceras was 1 metre and that for Arion hortensis was 0.45 - 0.9 metres.

This species difference in the distance of attraction is further complciated by the difference in susceptibility of each species to poison baits, (see p240). Godan (1966) reported that the sensitivity to toxic baits varies between closely related species and that the weight, age, diet and physiological condition can affect their vulnerability to baits. It appears that the timing of bait application is crucial to the success of the treatment - slugs are more susceptible at egg laying and since bait ingestion depends on the activity of the animal, application of baits in spring and autumn produces optimal results. The precise timing must, however, relate to the development of the crop being protected as some stages are more sensitive to àttack than others.

The same situation occurs with some legumes where damage to the seeds is the greatest cause of crop failure. Charlton (1978) found that in a laboratory test, less than 10% of clover seedlings survived to six weeks when slugs were present as the germinating seed was hollowed by the animals. Rowitt (1961) suggested that most damage may be caused by the loss of photosynthetic area in the young plants but once the seedlings reach the true leaf stage, growth is normally rapid enough to prevent excess damage by slugs and crop losses result from the retardation of germinating seeds rather than weak plants. Lutman (1978) investigated the role of slugs in grassland and suggested that they may be important

in reducing the photosynthetic capital at a critical stage and thereby delay the onset of spring growth.

Citrus plantations in California, Florida, Israel and Spain are often badly damaged by snails. Lewis & La Follette (1942) recorded approximately 250 Helix aspersa per tree in summer but more recently, numbers of up to 3000 per tree have been recorded (Pappas - pers. Comm.). Helix aspersa and Theba pisana are the commonest snails and are particularly abundant where overhead sprinklers are used to irrigate the crop. Furness (1977) compared three types of irrigation in citrus orchards and found that damage by Helix was severe where overhead sprinklers were used, less severe with sprinklers under the tree and only mild where furrow irrigation was employed. The snails are capable of migrating from the trees to sheltered areas underneath and can thus protect themselves from desiccation.

The young fruit is damaged when snails rasp at the rind and this can lead to further damage as fungal spores attack the fruit then, causing it to rot. Some marked fruit can be salvaged for turning into juice but the value of the crop is then reduced which can be disastrous in areas which rely on high quality produce.

The action of molluscicides for use against terrestrial molluscs dictates that the animals not only come into contact with the toxicant but also ingest a sufficient quantity to poison them - this requires a positive response from the animal which is thought to be mediated by the chemical sense. The methods in which the animals, notably Deroceras, approach the bait have been investigated in this work by analysing slug movements in the presence of toxicants

and attractive compunds. The feeding habits have been investigated in laboratory tests which aim to examine the normal meal size of Deroceras and investigate how this can be increased so that baits are readily ingested.

The problem of maintaining a feeding stimulus once the animals have begun to eat, has not been investigated before, yet this is essential to ensure that the animals ingest a lethal dose. Attractants have long been used as carriers to augment the attractive qualities of the toxicant as the stimulant characteristics of metaldehyde are still disputed and the carbamates do not appear to possess any attractive qualities. The sensory capabilities of slugs and snails have recently been more thoroughly investigated and the evidence produced suggests that these animals are competent at distinguishing food odours. Chemical attractants have therefore been sought to encourage the animals to move towards bait pellets from a greater distance and to improve the chance that the pellets are actually eaten.

Legislative controls restrict the type of compounds which may be incorporated into the bait and since each chemical added to the bait must undergo extensive toxicity tests, the costs of improving the bait may outweigh the advantages to be gained from improved performance of the pellets. Components of food have therefore been assayed for their ability to stimulate feeding in slugs - measured by the number of animals feeding on the food - and to sustain a feeding response - measured by the volume of food material consumed. Field tests to investigate the more promising attractants under field conditions and to compare commercial

formulations have also been made in an attempt to combine the elements of chemoreception and feeding involved in bait ingestion.

SECTION 2 CHEMORECEPTION IN MOLLUSCS

2.1 EVIDENCE FOR A CHEMICAL SENSE

CHEMORECEPTION - AQUATIC MOLLUSCS

Various behavioural studies have been made to determine the presence of a chemosensitive response in molluscs. Nassarius obsoletus (Prosobranchia) shows a proboscis search reaction (PSR) in the presence of food, which was utilised by Carr (1967a, 1967b) to determine the response-inducing components of shrimp extract. The mouth opening response (MOR) of Aplysia (Opisthobranchia) has been used as an index of the effectiveness of amino acids to initiate a chemoreceptive response (Jahan-Parwar 1972) and the escape reaction of Tegula funebralis (Prosobranchia) in the presence of starfish extract has been shown by Burke (1964). Acmaea limulata and A. scutum (Prosobranchia) respond to distant predatory starfish scent by moving up a vertical surface. The chemoreceptors in this animal seem to be located in the mantle margin since cauterisation of the osphradium or ctenidium does not eliminate the response (Phillips 1975).

Fasciolaria tulipa (Prosobranchia) shows escape or capture behaviour in response to odour of conspecifics depending on whether the motive is cannibalism or copulation (Snyder & Snyder 1971). The cue is not visual since an enclosed individual induces no response,while a sponge soaked in water from F. tulipa tanks does. Experiments with Aplysia confirmed that chemoreception also occurs in this species since animals preferentially entered the arm of a

Y tube which contained food and aggregated when water containing Aplysia scent was passed into a bath of animals. This suggests that pheromonal cues may be important in the breeding season when the animals need to congregate (Audesirk 1975, 1977). A similar aggregated response to pheromone release has been shown in Biomphalaria glabrata Say. (Simpson et al 1973).

Bovbjerg (1965) has suggested that herbivores may lack distance chemoreception as there is no selective pressure for it since their food supply is abundant. He could find no evidence for it in Stagnicola reflexa (Pulmonata - Basommatophora) which has an abundant food supply in its environment. This contrasts with the work of Jahan-Parwar (1972, 1975), however, which indicates that Aplysia can detect amino acid constituents of food that it normally eats.

Kempendorff (1942) has noticed an age difference in the response of Helisoma nigricans (Pulmonata - Basommatophora) to species odour. Older snails responded best while young animals often behaved passively, though this is an escape reaction and not therefore related in any way to sexual responses of mature snails. The chemoreceptive sense may thus develop in molluscs as they grow.

CHEMORECEPTION - TERRESTRIAL MOLLUSCS

Terrestrial molluscs can also be shown to possess a definite chemoreceptive sense. The terrestrial environment has a high selective advantage for auditory and visual senses but chemoreception is important in sexual behaviour especially where the animals have a solitary existence . Most

chemoreceptive organs show common properties e.g. the presence of phosphate splitting enzymes such as alkaline phosphates (Hodgson 1965).

Chemoreception in slugs and snails has been documented since 1891 when Moquin-Tandon reported that slugs move towards nutritive substances. Limax maximus was observed to continuously orientate towards a damaged apple and bean pods. Cautery of the tentacles eliminated the powers of orientation and dissection of Testacella tentacles revealed the enormous olfactory nerve with gangliform papilla at the tip. He concluded that the sense of smell lies in the terminal bud on the tentacles. Griffiths (1892) indicated that the tentacles in Helix pomatia Linné 1758 and H. aspersa were the site of olfactory organs and not the pedal gland as had previously been thought. He smeared the edge of a slab with non-irritating chemicals such as methyl alcohol, ether and ethyl acetate and noted that the animals with no tentacles gradually approached the edges of the slabs while intact animals turned away from the edge. Other workers had used irritating substances such as turpentine which irritated all sensitive tissues so that intact and tentaclectomised animals both avoided them.

Taylor (1894) confirmed that the olfactory ganglia are at the distal end of the tentacles and that odours are definitely attractive in food finding. He suggested that the exhalation of strong odours implies that an animal can perceive smell and that the ganglia in rhinophores of molluscs vary in size depending on the

keeness of the smell. Cooke et al. (1895) also noted that many terrestrial species of mollusc exhale a disagreeable smell and believed that they must therefore be able to perceive odour. This suggestion does not, however, conform to any accepted theory of chemoreception and the disagreeable smell could simply be a defence mechanism.

In 1898, Adams noticed that Limax maximus could orientate towards food remains on a plate and reorientate itself as the plate was moved. There was no visual stimulus since the night was dark and long grass obscured vision from the soil surface. Kiekebush (1953), in a more extensive analysis of the chemical sense of Helix pomatia, demonstrated a different timelag in the responses for chemicals applied to the body (6 seconds), the edge of the foot (10 seconds) and the tip of the foot (25 seconds). Sugar, bitter and salt compounds seemed to have fairly different taste qualities for snails, producing a response at concentrations of 5.45 - 10.9%, 0.024 - 0.49% and 0.08 - 0.21% respectively and removal of the oral lappets reduced susceptibility.

Kittel (1956) attempted to show a difference between taste and smell in Arion empiricorum (Arion ater) and Limax cinereoniger Wolf 1803 by monitoring their behaviour to fungi. A typical response reaction was seen as animals were placed in a path near fungi, with tentacles protruded and locomotion in a spiral path. The distance of attraction was limited to a radius of 5 - 40cm. and the two species only differed in their speed of approach - 1.2mm/second for A. empiricorum and 3.7 mm/second for L. cinereoniger. The posterior tentacles seemed to be responsible for

direction until the animal got to within a few centimetres of the fungi when the oral lappets seemed to come into play. The behaviour of the slugs with amputated tentacles was studied and although their appetite was unaffected, the attraction distance dropped from 40 to 3.5cm. The difference was negligible when only one tentacle was amputated. Amputation of the terminal "bouton" region only limited attraction to 10 - 20cm. indicating that this is the main sensory area. Ricou (1961) and Frömming (1954) have also found fungi to be attractive to slugs at distances of up to 7 metres.

These observations demonstrate conclusively that slugs and snails have a well developed chemical sense. Organs for detection of chemical signals are present in the head region of molluscs although this does not exclude the possibility of chemical sensors on other parts of the body. Chemical cues are involved in food finding, homing and trail following but two separate sensory mechanisms are involved - contact and distance chemoreception. Contact chemoreception is associated with gustatory stimuli and probably involves the anterior tentacles and oral lappets bordering the mouth - these are considered to be evolutionary remnants of a third pair of tentacles which have fused to form the lower borders of the mouth (Carrick 1939). Distance chemoreception primarily involves the posterior tentacles and a moving slug or snail can frequently be observed to raise the anterior of the body, turn the head through approx. 90^{o} and sweep the tentacles in a wide arc. This ability to detect food at a distance

is of great advantage to an animal which moves relatively slowly. Similar searching movements are thought to be involved in homing behaviour.

2.2 SENSE ORGANS

Molluscs respond to light, temperature, humidity, wind and chemical changes in their environment. Receptor cells throughout the body provide some information but specific sense organs play the dominant role in monitoring external fluctuations and relaying them to the CNS. The sense organs are generally situated anteriorally so that they can sample the environment but the precise nature of the receptor will depend on the habits of the animal.

Chemoreception is evolutionarily the most primitive form of distance perception. It is especially important in aquatic animals where vision may be limited by depth or murky waters. Aquatic gastropods generally possess an osphradium which samples the water as it enters the mantle cavity and functions as a chemoreceptor. This has disappeared in pulmonates, though for many years it was believed that terrestrial molluscs also sensed their environment on inspiration and the chemical sense was assumed to be located at the pneumostone (Piéron 1908).

The entire osphradium bears nerve endings (Bailey & Laverack 1966) but they are especially numerous in the grooves which suggests that turbidity reception is unlikely to be a chief function of this organ. Tegula funebralis and Buccinum undatum (Prosobranchia) show no

appreciable difference in their behavioural response to chemical stimulation when all or part of the osphradium is removed (Bailey & Laverack 1966, Burke 1964). It may therfore be that the osphradium has a more specific function e.g. monitoring pheromones or excretory products.

Opisthobranch gastropods have a pair of rhinopores - modified head tentacles - which seem to be sensitive to both tactile and chemical stimuli. These are clubbed and plicate to increase the sensory area (Morton 1967) but Audesirk (1975) has shown that they are not the only site of chemoreception since orientated movements of Aplysia were only destroyed by removal of both rhinophores and tentacles - the tentacles, in fact, appeared to be of greater importance.

Light is important in regulating slug activity though it is highly improbable that the animals use visual cues in their food finding behaviour. Stylommatophoran slugs possess a pair of eyes at the tip of the posterior tentacles which are capable of perceiving changes in light density but not capable of image formation. The eye of Deroceras reticulatum is a closed vesicle, the front of which functions as a cornea, while the posterior half has a photoreceptive area. An accessory retina lines a diverticulum of the main retina and is thought to function as an infra-red receptor (Newell & Newell 1968). Hermann (1968) showed that optic guidance was impaired in blinded Otala lactea but unaffected in animals exposed to similar trauma and other workers have indicated that light is important for accurate entrainment of slugs to light cycles

(see P_{II}).

The gastropod statocysts, situated on the pedal ganglia, are also known to have a receptor function (Wolff 1969). These are also closed vesicles but formed as ectodermal invaginations and innervated by the cerebral ganglia. The inner sensory epithelium and calcareous statoliths respond to stimulation of a specific area and the animal orientates with respect to gravity. Numerous tactile receptors also occur all over the body.

The major sensory structures in the Pulmonates are the tentacles, in particular the longer posterior or superior tentacles of Stylommatophorans. The tentacle tip of these forms two raised zones with the eye situated in the furrow between them. A tentacular or digitate ganglion is also present but lacking in the largely aquatic Basommatophorans which have eyes at the base of their single pair of tentacles. Townsend (1974b) has indicated that chemoreception in the Basommatophoran, Biomphalaria glabrata, is centred at the base of the tentacle since he recorded a delay in response after stimulation at the tip. He suggested that this allows the ciliary current to pass from the tip to the base of the tentacle and carry the odour with it. Kempendorff (1942) similarly suggested that the tentacle base is the main site of taste perception in Helisoma nigricans.

The tentacle tip is thought to be the most responsive region to stimuli in Stylommatophorans. Wallis & Wright (1971) have identified a primary receptor system in the tentacle tip of Arion ater where bipolar cells lie

below the surface of the epithelium and send out distal processes which pass up between columnar epithelial cells. Two main types of receptor cell are found - one in which the dendrite terminates in a cup, from the centre of which arises a group of cilia and the other in which the nerve ending terminates in a hillock from which cilia arise. Axonal processes are connected via the large ganglion in the tip of the tentacle to the tentacular groove.

Electrophysiological recordings by Wallis & Wright (1971) indicated that the sheath and tentacle tip were extra sensitive to tactile stimuli and the authors proposed both phasic and tonic components of the receptor. Phasic receptors adapt rapidly and have a low stimulus threshold while tonic receptors have a higher threshold and a longer period after discharge. Ichinose (1968), however, found no increase in the electrophysiological activity in the tentacular nerve of Achatina fulica when the posterior tentacle tip was stimulated with a tactile stimulus, though an increase was recorded in the five chemotactile nerves which run alongside the tentacular nerve and terminate in the procerbrum.

Two types of muscle are responsible for contraction and retraction of the tentacles - an interwoven muscular net in the subepithelial tissue and discrete bands of muscle running the entire length and attached to the distal ganglion by muscle straps (Rogers 1968). Interlocking muscle cells may account for some contraction but the more numerous paramyosinic smooth muscle cells are capable of maintaining the highest muscle tone. The epithelium on the distal portion of the tentacle is smooth in contrast with

the tubercles on the rest of the tentacles and large mucous cells ensure that the surface is continually moist.

In Arion ater the fully extended posterior tentacle is 1.25cm. long and 0.125cm. wide with an average of 150,000 dendrites per mm^2. Of the four types of free nerve ending identified, at least one is thought to be mechanoreceptive (Wright 1972). Charles (1966) cites a thermoreceptive function for tentacles of Aplysia and possible involvement in the compass orientation of Nassarius by magnetic or electrostatic field perception. The histological and behavioural evidence however, suggests that the posterior tentacles are mainly responsible for the reception of chemical stimuli. Their anterior position on the top of the head and bilateral disposition ensures that they are advantageously sited for sampling the environment.

The participation of each gastropod sense organ in the reception of chemical stimuli is, however, difficult to demonstrate experimentally. Tentaclectomy does not always result in abolition of the stimulus response and many workers have argued that the operation itself may damage the animal to such a degree that the results are invalid. Goldschmeding & Jager (1973) have experimented with seven different types of tentacle amputation on Lymnaea stagnalis (Pulmonata) including a sham operated control group. The number of eating cycles per ten minute exposure to sucrose was recorded for each animal and since operated snails were able to continue normal eating cycles, they concluded that the tentacles and tentacular nerves do not play a major role in the detection

of chemical stimuli. Townsend (1973.) showed that a functional osphradium was not essential for directional movement towards a food source in Biomphalaria glabrata but when intact and tentaclectomised animals were compared (1974b), the intact snails performed better. Zigzagging movements of the animals as they approached the food were unaffected in the operated group of snails. He suggested that the tentacles perform a sampling and conducting function, offering increased spread of receptivity and facilitation of directional information reception.

Observations by Suzuki (1967) on the behavioural responses of Ezohelix flexibilis (Pulmonata) to food odours have indicated that while animals with one or both tentacles amputated showed some response to food, intact animals performed significantly better. In a three part investigation of the posterior tentacles of Achatina fulica (Pulmonata), Ichinose (1968) studied their anatomy, behaviour and electrophysiology. The anatomy indicated a similar structure to other Stylommatophorans and olfactory ability was investigated by recording the behaviour of eight animals in a four-choice radially symmetrical maze. Vials of vegetable juice and distilled water (control) were placed at various points around the animals and 68% showed a positive chemotaxis in 354 tests. When the epithelium of the tentacle tip was removed however, only 25% of the snails in 294 trials indicated a positive chemotaxis. These results suggest that the tentacles, in particular the epithelium of the distal end, are the most important sensory areas for

distance chemoreception.

2.3 HOMING

In addition to their role in the food finding behaviour of molluscs, the tentacles also appear to be involved in other chemosensory responses such as homing and trail following. Many molluscs exhibit homing - they return to a particular part of their habitat after foraging activities. This phenomenon is well known in limpets where marking shells has shown that they return to a specific scar on the rock surface at low tides. In gastropods, it has been most succinctly demonstrated with time lapse photography (Cook 1979a, 1979b, 1980, Gelperin 1974, Newell 1965a, 1965b, McCormack 1970). Other workers have allowed slugs and snails to move over moistened paper and recorded the tracks with kaolin powder or ink. Duval (1972) monitored homing in Deroceras reticulatum and observed that as the circuitous movements of nightime ceased, the animals adopted a straight course to shelter, which was often the nearest region of shadow. The homesite was not necessarily the same place each day and the tendency to home seemed more pronounced in dry weather. Rollo & Wellington (in press) also found that homing was more common in dry weather and that approach was more direct. It is advantageous to return to a known shelter in summer months when heat could cause dehydration of slugs still seeking shelter in the morning.

McCormack (1970) made track traces and activity flow charts for each slug he tested and found a range of

tracks from simple loops through to figures of eight and non-classifiable paths. He attributed homing to learned visual stimuli since the preferred homing shelters he set up could be rotated through 180° and Limax maximus would still reorientate itself. Newell (1965) indicated that Deroceras reticulatum returns to the same home on a number of consecutive days and showed that locomotor activity was interspersed with sexual activity, resting and feeding.

The accuracy of the homing response was shown by Edelstam & Palmer (1950) using Helix pomatia. These snails could home with an angular error of less than 30° on distances under 40 metres, though the response was inhibited by adverse conditions. They suggested that olfactory cues were responsible and that memory may also serve to orientate the snails. Gelperin (1974) demonstrated the importance of olfactory cues by severing the olfactory nerve in Limax maximus and preventing homing. Similar severing of the optic nerve had no effect on the success of homing and neurophysiological evidence confirmed the olfactory function of the digitate ganglion. He thus attributed the homing response to olfaction and although he found no evidence of slime trail following, he did not indicate that he specifically excluded the possibility of this in his test procedure.

Cook, however, working with Limax pseudoflavus Evans 1978, (formerly known as L. grossui) has suggested that trail following may be involved in homing. He has proposed an olfactory beacon for the normal method of homing with trail following reserved as an additional

possibility (1977) though the sensory sites for the two are separate. Optic tentacle removal reduced homing while removal of the anterior tentacles impaired the accuracy of trail following but had no effect on homing (1979a).

Both Gelperin (1974) and Cook (1980) have indicated that slugs home upwind. Cook has proposed the following explanation for homing: "If a slug perceives a food source upwind as it leaves home, it will proceed to this and either move to a different upwind home or move downwind from the food until it detects its original home and then turn upwind to this. If it does not detect food though, it will search downwind and home upwind. This results in consistent upwind homing although the slug can leave home either upwind or downwind." Such an explanation is perhaps over precise since a large proportion of molluscs observed in laboratory trials do not conform to any pattern and environmental or topographical factors may override the homing instinct. Gelperin for example, found that of 42 paths, 50% showed no homing, 8% homed downwind and 42% homed upwind. Southwick & Southwick (1969) carried out a mark and recapture field trial with Achatina fulica and found that after experimental displacement of 5 - 30 metres, 52.2% returned home within 60 hours.

Rollo & Wellington (in press) have shown accurate diurnal homing in Ariolimax columbianus by plotting their movements in laboratory cages for almost two months. The animals had preferred shelters within the cages and although one slug consistently returned to the same shelter for 49 nights with a break of only one night, most animals showed greater

flexibility and used different shelters during this period. The authors suggest that the animals home using an olfactory beacon but other factors may be involved and the response is unlikely to be a simple reaction to odour. Topographical features of the laboratory cage, for example, tend to encourage the slugs to follow a path near a wall. They also suggest that a pheromone may be deposited in the faeces and serve as a recognition signal but their work does not indicate that this can be distinguished from a marker in the mucus.

2.4 CHEMICAL SIGNALS IN AGGREGATION AND TRAIL FOLLOWING

Several workers have suggested that molluscs release chemical substances which act as signals to members of the same, or closely related species. Cameron & Carter (1979) have indicated that Cepaea nemoralis and C. hortensis were more active and showed faster growth rates when housed individually and that crowding or placing in containers pretreated with mucous trails reduced activity. They suggest the possibility of growth inhibiting pheromones to regulate population density - a feature which has also been proposed in the aquatic snails Biomphalaria glabrata and Fossaria cubensis (Levy et al 1973).

Rollo & Wellington (1979) have observed aggressive behaviour in terrestrial slugs, particularly in hot weather when food and shelter are reduced. Deroceras reticulatum however, was less aggressive than most of the

species examined. Takeda & Tsuruoaka (1979) have described a "headwart" in the terrestrial snail Euhadra peliomphala which is situated between the optic tentacles. Extracts from this and adjacent tissue were placed on the floor of a container of snails and activity estimated using a scale of 0 - 10, where 10 signified copulation. The animals placed near the extract exhibited precopulatory behaviour within ten minutes. The response was more marked when groups of snails were tested together than when animals were tested individually.

Behavioural work on Veronicella ameghina and V. floridana has indicated that these slugs may also release pheromones which cause them to aggregate in particular places. Dundee et al (1975) used circular dishes and circular stone discs distributed within the dishes to provide resting sites for the slugs. The circular nature of the apparatus eliminated the possibility of attraction to angles or edges and after 48 hours, most of the slugs had aggregated under one particular disc. After 72 hours all 20 slugs were under this disc and further experiments indicated that sand taken from under this disc was sufficient to cause aggregation, even with new discs. Conditioned sand placed on top of a disc caused the animals to aggregate in the vicinity but not directly underneath, though after a week, they had all dispersed. This work indicates that an aggregative pheromone is produced and is volatile since it evaporates when placed on top of a surface but may be trapped underneath.

Audesirk & Audesirk (1977) have observed that

the sea hare, Aplysia californica forms large breeding aggregations in summer and autumn. The authors have distinguished neurons in the cerebral ganglia which show excitatory responses to the odour of conspecifics but not to the odour of food algae.

Trail following in molluscs is probably associated with contact chemoreception. This is almost certainly mediated by pheromones since the animals appear to follow only trails laid by conspecifics. Townsend (1974a) observed that Biomphalaria glabrata "tracker" snails could follow trails laid by another individual of the same species but not trails laid by Lymnaea. The trails were polarised and the information persisted for 10 - 30 minutes. The snail Physa has also been shown to lay trails in Y tube experiments (Wells & Buckley 1972). Individuals tended to use the same arm of the Y tube when surfacing to replenish their air supply, but scrubbing the tubes removed the mucous trails and abolished the preference for a particular arm of the tube. Again the information in the trails persisted for about 30 minutes.

The terrestrial snail Achatina fulica will follow trails laid by members of its own species but not those of Otala vermiculata (Chase et al 1978). These animals also orientated preferentially towards airstreams passing through containers of other Achatina rather than air from Helix aperta. Otala snails, however, appeared to have less pronounced preferences for odours of conspecifics. Responses in Achatina were dependent on whether the snails had been housed together individually or collectively before the test,

suggesting that pheromones were involved and that the trail following response may be important in bringing animals together for copulation.

This pheromone has been further investigated by comparing pedal and salivary gland extracts (Chase & Boulanger 1978). In a two choice olfactometer test, Achatina preferentially selected the arm bearing the pedal gland extract and preliminary analysis indicated that the substance responsible for the attraction was a lipid, which is consistent with analysis of other pheromones. Since young snails exhibited a positive response to conspecific odour, the authors suggest that the pheromone is not necessarily involved in reproduction but may be useful for aggregating the animals in suitable habitats.

These reports add further support to the hypothesis that molluscs can distinguish and respond to specific odours. The evidence has been accumulated from behavioural work on many species of molluscs and points to a highly developed chemical sense in these animals.

2.5 ODOUR THEORY

Chemoreception has been defined by Beets (1977) as a reversible physical interaction between two molecular species - the epithelium and the stimulant molecules. It involves the absorption of odour molecules onto the aqueous layer which covers the receptor endings and the depolarisation of the cell membrane at the chemoreceptive surface. This initiates the electrical stimulus which alerts the olfactory centre in the central nervous system. Although research in

olfaction is generally concerned with vertebrate or insect receptors, the basic mechanism is likely to be similar in molluscs since mucus provides the necessary aqueous medium. Several theories have been proposed to explain the mechanism of odour sensation and it is clear that certain characteristics are common to odorous compounds.

The stereochemical theory of odour highlights the structure activity relationships (SAR) between the odour molecule and the receptor membrane. A "lock and key" mechanism ensures that only molecules which fit a particular receptor cell are absorbed. Amoore et al (1964) have suggested seven primary odours which can be combined to produce every known odour - camphoraceous, musky, floral, pepperminty, ethereal, pungent and putrid. For the first five, the geometry of the molecule is responsible for the acceptance or rejection by the surface membrane while for the latter two odours, the charge of the molecule is more important. Pungent molecules are electrophilic - they are produced by compounds with a positive charge and a strong affinity for electrons - while putrid molecules are nucleophilic - they are produced by compounds with a negative charge and a strong affinity for the nuclei of adjacent atoms. Size and shape descriptions have been calculated and camphoraceous molecules for example are roughly spherical with a diameter of approximately 7 Ångstroms (0.7nm.).

Wright & Burgess (1975) attribute the sensation of odour to molecular vibration patterns. The odour molecule combines with an olfactory pigment molecule of matched frequency and the vibration frequency of the latter is altered.

Davies (1965) believes that the partition coefficient of substances between the air-water and water-lipid interfaces is important and that the cross-sectional area of the molecule will determine its acceptance by the receptor. His puncturing theory suggests that gaps in the cell membrane, caused by odour molecules being desorbed, allow K^+ and Na^+ to exchange and initiate the nervous impulse.

Sensory adaptation can happen when all the receptor sites are saturated or blocked by odorant molecules. This occurs with continuous exposure to an odour and the molecules which are normally desorbed from the receptor surface remain there preventing "recovery" of the receptors. Another factor affecting acceptance of odorant molecules is the mucus which bathes all receptors. The air-mucus partition coefficient is perhaps one of the thresholds affecting the acceptance of a substance but other theories suggest that mucus may act as a reservoir of inorganic ions involved in the electrical stimulation of the cell or that mucus may simply have a protective function forming a barrier between receptor and external environment.

Stephenson (1979) has proposed that the chemoreceptive capabilities of Deroceras reticulatum are explained by the contact between plant solutes and the mucus of the slug. He suggests that plant attractants are dissolved in the surface waters of leaves and that an aqueous bridge forms between this and the mucus on sense organs - solutes could flow across this bridge to gustatory receptors, probably associated with the lips. This hypothesis however, is based on photographic evidence not yet substantiated by other

workers and the "channels" in the head region, which Stephenson proposes to be pathways for the mucus to flow towards the tentacles, may simply be folds of skin. The entire body is also lubricated by mucus and solutes could thus be absorbed through the body wall anywhere on the animal and would indicate that many regions could be chemoreceptive.

Ritter (1979) has proposed that the more accepted theories of chemoreception can be combined to produce a general outline of chemoreception:

"When an odour molecule approaches a receptor molecule, the receptor and effector are reciprocally distorted and this distortion may constitute (part of) the recognition signal in the receptor. The molecular shape of the odorant is highly important and should fit the shape of the receptor like a socket and pit. At least three features are involved in the reaction between the stimulant molecule and acceptor : attachment, activation and conductivity changes in the cell membrane of the receptor. Synchronous throbbing of the odour molecule and the receptor in the frequency range of 50 - 400 cm^{-1} adds to the informational content of the signal."

The interpretation of these theories provides a guideline which can be used to predict whether a compound is likely to be odorous and how it can be altered to make it acceptable. Any odorous compound must be sufficiently volatile to release molecules to the air and have some solubility in water and lipid to penetrate the chemoreceptor epithelium. The potency of compounds can often be increased

by adding branches to straight chain atoms and strong odours are frequently associated with a chain length of 4 - 8 carbon atoms. Shifting the position of a functional group on a small benzene ring alters the odour as does the conversion of a substance to its optical isomer, emphasising the importance of molecular shape in determining sensitivity.

Dravnieks (1968) has formulated an equation which represents the types of compound likely to be odorous:

$C_aH_bO_cN_dS_eSe_fCl_gBr_hI_i$ where a is less than 10
b is less than 22
c to f is between 0 and 2
g to i is between 0 and 6

The arrangement of the molecule is important and the main physical determinants of odour are molecular size, the shape of the molecule and the position of any functional groups. Other characteristics such as the electron donor strength of molecules may influence the threshold of detection. Dravnieks (1968) suggested that, for a series of odorants in different classes, the threshold varied with the ability to form a charge-transfer complex. Vapour pressure differences account for part of the threshold variations in compounds of similar structure as a decrease in vapour pressure produces an increased partition coefficient and alters the distribution and release of odour molecules into the air.

Roderick (1966) has summarised other features of odours - higher members in a homologous series are generally less odorous and unsaturation enhances odour, especially where the double or triple bond is near a polar group. The intensity of a mixture of odours can show independant,

additive, subtractive or synergistic effects but it is often the impurities in a compound which give it the characteristic odour.

The odour molecule produces an electrical change within the cell which alters the rate of firing of the resting receptor membrane - this can be monitored by electrophysiological techniques which involve implanting electrodes in nerve cells or attaching them to receptive membranes. The electro-olfactogram (EOG) obtained by monitoring electrical events which occur after stimulus emission can be analysed into several components and indicates the activity of the stimulant. This electrophysiological approach has been popular in olfactory studies of molluscs since the nerve cells tend to be large and easily identifiable. Recordings have been made from the slugs Limax maximus (Gelperin 1974, Gelperin et al 1978) and Ariolimax californicus (Senseman 1976, 1977a) and from the snails Achatina fulica (Ichinose 1968) and Ezohelix flexibilis (Suzuki 1967). Both Helix aspersa and H. pomatia are also commonly used in studies of the central nervous system.

Neurophysiological studies suggest that substances producing a negative EOG excite receptor cells while substances producing a positive EOG inhibit receptor cells (Gesteland 1964, Suzuki 1967). The negative EOG is obtained when a puff of air containing an attractive food odour or component of this is applied to the sensory epithelium - a negative potential change is recorded between the sensory surface and a point on the olfactory

nerve. Gelperin (1974) exposed the isolated digitate ganglia to amyl acetate and obtained a large action potential and a negative EOG. A puff of moist air gave no response though potato and carrot odours gave a response which could be increased in size by more rapid application of odorant-laden air. In such a test it is necessary to take into account the sensory capabilities of the nervous system under study and to be able to isolate a semi-intact preparation (sensory epithelium, olfactory nerve and olfactory ganglia) which can be shown to mimic the response of the entire animal.

The feasibility of a successful neurophysiological study of olfaction in Deroceras reticulatum was investigated but since this slug is much smaller than the molluscan species normally used, it was considered that the equipment required would need to be more sensitive and the facilities available were unsuitable. Also, the project under investigation was ultimately concerned with the responses of the complete animal in the field and it was concluded that a behavioural approach to the problem of olfactory responses in slugs may provide results which are more immediately applicable to the problem of pest control. Electrophysiological studies can, however, produce valuable evidence of attractancy in compounds and are a useful screening tool.

2.6 BEHAVIOURAL RESPONSES TO ODOUR

The behavioural approach to chemoreception adopted in this study involved monitoring movement and

behaviour of the animals in the presence of food or chemicals. Exposure to attractive stimuli results in an orientated response which can be exploited to assess the degree of attraction of that compound at a particular concentration. The orientated response can be either chemotactic (response to odour molecules) or anemotactic (response to wind current) though it is probable that both methods are employed by molluscs to reach an odour source. Positive chemotaxis occurs when the organism senses a concentration gradient of odour molecules and follows this from a low to a high intensity. Shorey (1976) has subdivided this further into chemotropotaxis which occurs when simultaneous comparison of different odour concentrations at two points in space is involved and chemoklinotaxis which occurs when the organism scans across the field continuously and successive comparisons of odour intensities are involved. The former can operate only in the immediate vicinity of the odour source where the concentration of odour molecules is high but the latter enables the organism to orientate over several metres.

The response to odours is influenced by the physical features prevailing at that time. Kalmus (1942) found that slugs exhibited positive anemotaxis to a gentle wind but a stronger force caused a negative response. In still air, a point source of odorant emits irregular bursts of odour molecules which Wilson (1970) has suggested form a hemispherical concentration gradient. In moving air, the plume created downstream is semiellipsoidal in shape while the active air space above a terrestrial trail is

contained within a semicylindrical zone. Animals can thus perceive odour molecules at a distance from the source and orientate themselves towards the centre of the stimulus by comparing intensities and steering a course between the lateral gradients. Gelperin (1974) believes that slugs and snails can respond to odours even at the fringes of the "active space", if the initial behavioural response triggered by the odour is movement upwind.

As an animal moves forward, the response is further complicated since it must continue to sample the air to obtain cues for orientation. Molluscs show a characteristic zigzag reaction as they move; for they react to reduced gradients at the boundary of the 'active space' and steer towards the centre. The bilateral disposition of the major sense organs, the tentacles, aids in such orientating and Shorey (1976) has suggested that the cyclical deviations towards the areas of low concentrations prevents sensory adaptation of the receptors.

2.7 THE NATURE OF CHEMOSTIMULANT COMPOUNDS

Work on insects suggests that two types of receptor exist - odour generalists which respond to a wide range of odours at low concentration and odour specialists which have a higher threshold and a narrower range (Boeckh et al 1965). Most sensory cells appear to have primary or secondary processes attached to increase reception of molecules but only a small amount of chemical is required to trigger a response - the human threshold is 2×10^{-7} moles (Roderick 1966), and movement towards an odour is

initiated by 10^{-5} -10^{-6} molecules in many invertebrates (Carthy 1958).

Investigations into the type and concentration of chemicals that stimulate a response in molluscs have been concerned with components of their natural food. Chemical feeding signals for aquatic molluscs seem to be mainly amino acids or their derivatives with L-isomers being more effective than D-isomers. Few of the compounds are volatile but most are small molecules and strongly polar so that they are soluble. Cyclic or steroid molecules are important in reproductive behaviour and indicate a specificity in molecular recognition (Bardach 1975). Bullia (Prosobranchia) has been shown to respond to components from its natural food and trimethylamine is particularly attractive though other quaternary compounds are effective in producing a response (Brown 1961). This indicates the correlation between olfactory sensitivity and the chemical structure of the stimulants.

Carr et al (1974) fractionated extracts of eight species of marine animal and deduced that the major stimulants for Nassarius obsoletus were probably peptides and proteins. Purified human serum albumen induced a 50% positive proboscis search reaction at 1.2 x 10^{-9}M in Nassarius (Gurin & Carr 1971) and Carr (1967a, 1967b) had earlier defined the components of shrimp extract which produce a PSR response as heat stable, soluble in polar solvents, non-volatile, of low molecular weight and alkali soluble. The attractive substances for the Schistosome vector Australorbis glabratus (Pulmonata - Basommatophora) also had some of these characteristics, being heat stable and water soluble (Etges 1962). Individuals

which responded positively to food extracts could avoid wheatgerm contaminated with $BaSO_4$, ZnO and $CuCO_3$.

Several amino acids which are constituents of the natural food of Aplysia were effective in eliciting a response in concentrations as low as 10^{-6} to 10^{-7}M for glutamic acid and 10^{-5} to 10^{-6} for aspartic acid. These two amino acids also act as stimulants for Buccinum undatum (Bailey & Laverack 1966). Other amino acids evoke escape response or sexual behaviour in Aplysia and the most sensitive receptors in the anterior tentacular groove are 100 - 1000 times more sensitive to food substances than other stimuli. Behavioural and electrophysiological data give a correlation in the threshold level for individual compounds though the latter are 5 to 15 times higher than behavioural thresholds (Jahan-Parwar 1972, 1975).

Information on the nature of chemicals that are attractive to terrestrial molluscs is more sparse since several factors may complicate the response to odours in air. The attractant chemicals may, however, be similar to those suggested by experiments with aquatic molluscs since the chemoreceptive process is essentially the same for both groups of animals. Although slugs and snails will feed on a wide variety of foods (see section 3), it is possible to select the preferred plants and highlight chemicals which are common to these. For example, the pyrazines found in potatoes and carrots and the sinigrins, which characterise the Cruciferae and produce isothiocyanates, may be proposed as possible attractants. Many of the fruits favoured by terrestrial molluscs have esters dominating the odour (eg.

amyl acetate, ethyl butyrate) and some carboxylic acids - C_6 caproic, C_8 caprylic and C_{10} capric - are considered to be particularly attractive.

It is known from work on insect attractants, however, that secondary plant products and chemicals present in trace amounts may be responsible for the attraction of the plant and evaluation of chemical constituents on an ad hoc basis can be time consuming and unrewarding. The responses of slugs to macerated plant tissue or synthesised flavourants was therefore assessed so that more information on the chemostimulant properties of the whole food, rather than individual components, could be compiled. Orientating and locomotor responses to food and baits were recorded using a time lapse camera in conjunction with feeding tests (section 4) and field trials (section 5). It was hoped, in this way, to determine whether a substance capable of stimulating the animal to feed could also initiate directed locomotion to the source of the food.

2.8 EXPERIMENTAL APPARATUS

Unfortunately, there is no adequate method for quantifying odour and odour classification is largely a psychological discipline which suffers from semantic problems. The number of odours an individual can discriminate depends on training and inherent ability but olfactory responses in simpler organisms can be more objective and behavioural responses provide a means of analysis. Odour responses are evaluated with the aid of an olfactometer - an apparatus capable of delivering a standard quantity of odorant or of

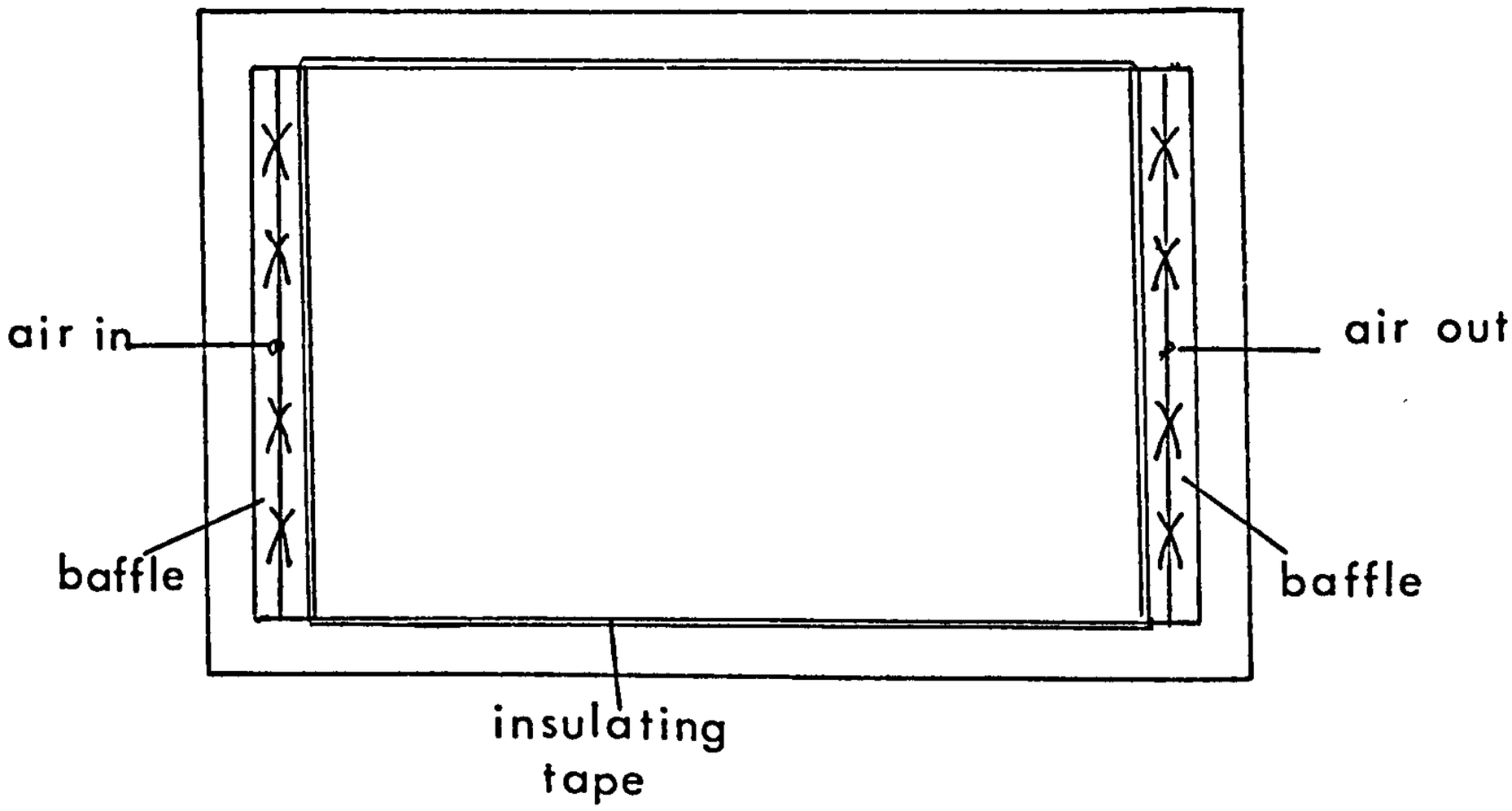
monitoring behavioural response to odour.

Schneider (1968) has outlined the main problems in olfactory research as the need to control stimulus quality and quantity and to register the reaction of the organism. An olfactometer allows controlled release of odour molecules into an airstream which is a basic step in defining the threshold of response. The acceptance or rejection of an odour can be assessed by monitoring movement towards,or away from,the source or by searching movements of the chemoreceptors. The basic olfactometer for use with invertebrates is a simple Y tube which allows air to diffuse along the two equal arms from two different sources and requires that the animal choose which arm to enter - a light source is often used as a stimulus for the animal to move. More advanced forms incorporate a moving airstream and temperature,or humidity,variables but it is frequently necessary to adapt an apparatus to fit the sensory capabilities of the test organism. Dethier (1947) quotes the ideal requirements of an olfactometer as follows:

1) The environment should be as normal as possible.
2) The results should be attributable to the chemical stimulus only.
3) The results should be quickly obtainable.
4) The results should be obtainable with a homogenous or a heterogenous population.

Y tubes have been used with snails, notably Physa (Wells and Buckley 1972) but these can only be used for simple experiments and more complex types have been designed for use in mollusc research. Mallet & Bougaron

FIG. I

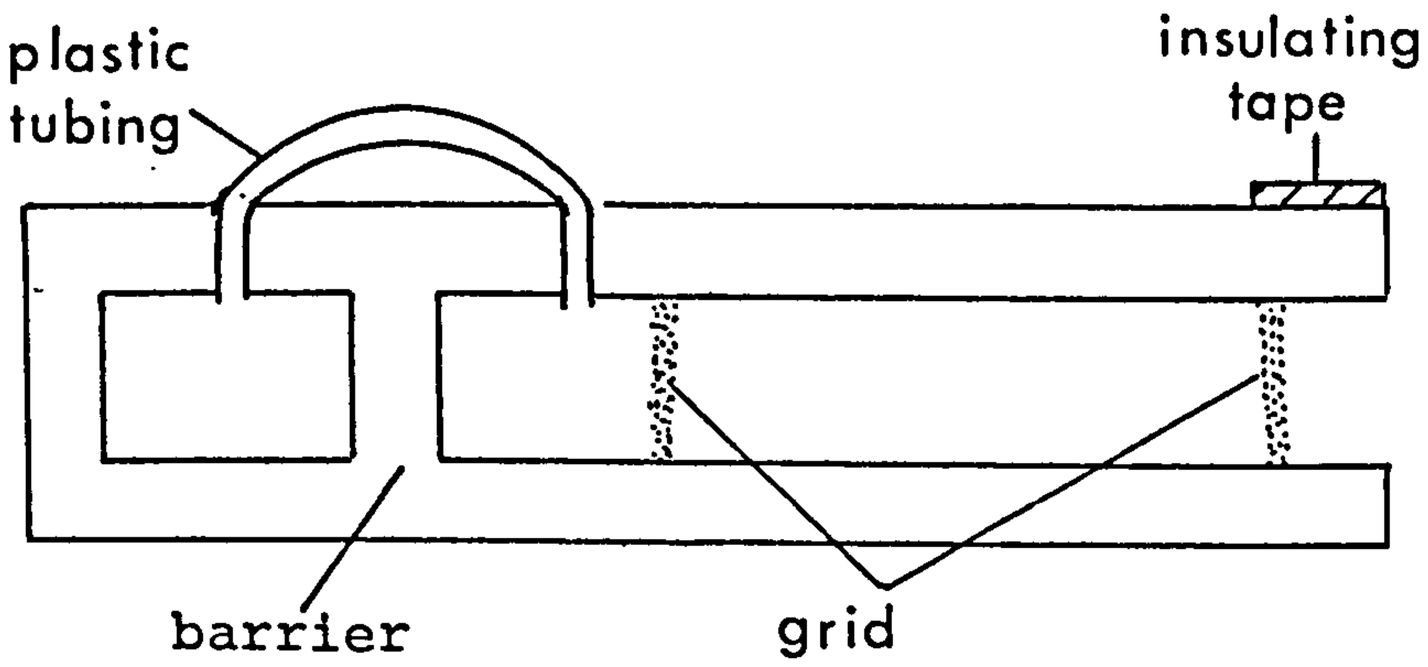


PERSPEX ARENA WITH BAFFLE AT EITHER END TO ENSURE

SMOOTH AIR FLOW

Removable cover (not shown) enables arena to be cleaned between trails and insulating tape forms airtight seal

FIG. 2



DETAIL OF BAFFLE AT EITHER END OF ARENA

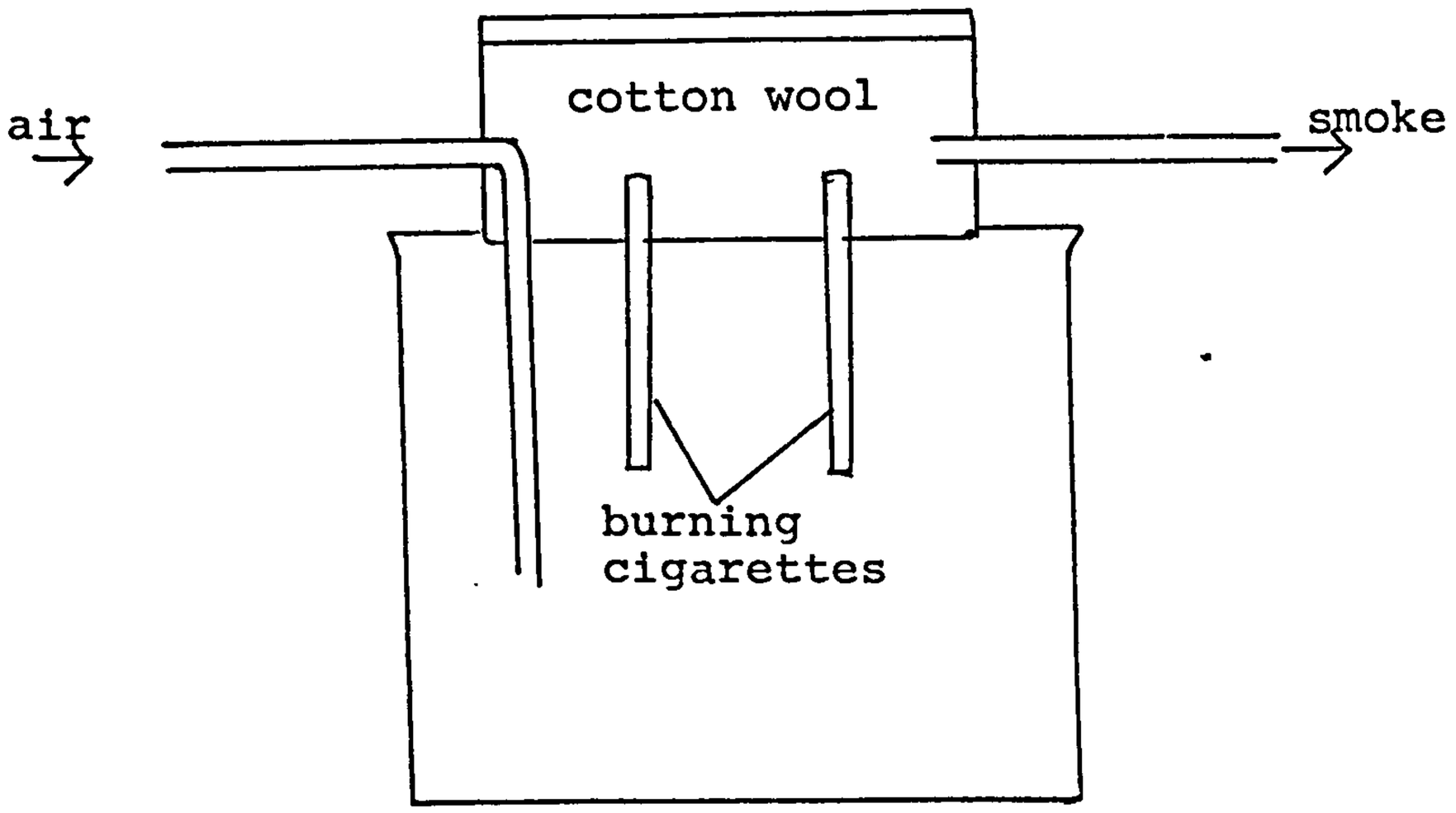
The barrier in each baffle forces air into the plastic tubing which connects the two sides . This constriction of airflow ensures that air enters the arena at a constant rate.

(1968) produced a radial olfactometer which allowed slugs to select one of six exits leading to either an odorous substance or a control. Suzuki (1967), working on Ezohelix flexibilis, used a large glass box with two air inlets for odourised and deodourised air and a central outlet - the movement of snails to either air inlet was observed and percentages of migrated animals provided a comparison of response to each air source.

The olfactometer used in this trial to monitor slug behaviour in the presence of food materials or their components was based on a design used by Cardew (pers.comm.). The apparatus (see Fig I) was modified to prevent air leakage and to allow suction of air through the system. A perspex arena (50 x 75 x 4cm) with a baffle at both ends provided an apparatus which could be used to simultaneously introduce two odour streams into opposite ends or 'pull' a stream through the arena using suction from the opposite baffle. Each baffle (see Fig 2) was partitioned by a perspex barrier so that air could only pass from one side to the other through eight narrow tubes. This constrained the flow and the stream then passed through two grids, with a pore diameter of 2mm, so that it entered the arena at a constant rate over the entire width of chamber. This prevented the formation of 'dead' pockets of air in each corner of the apparatus.

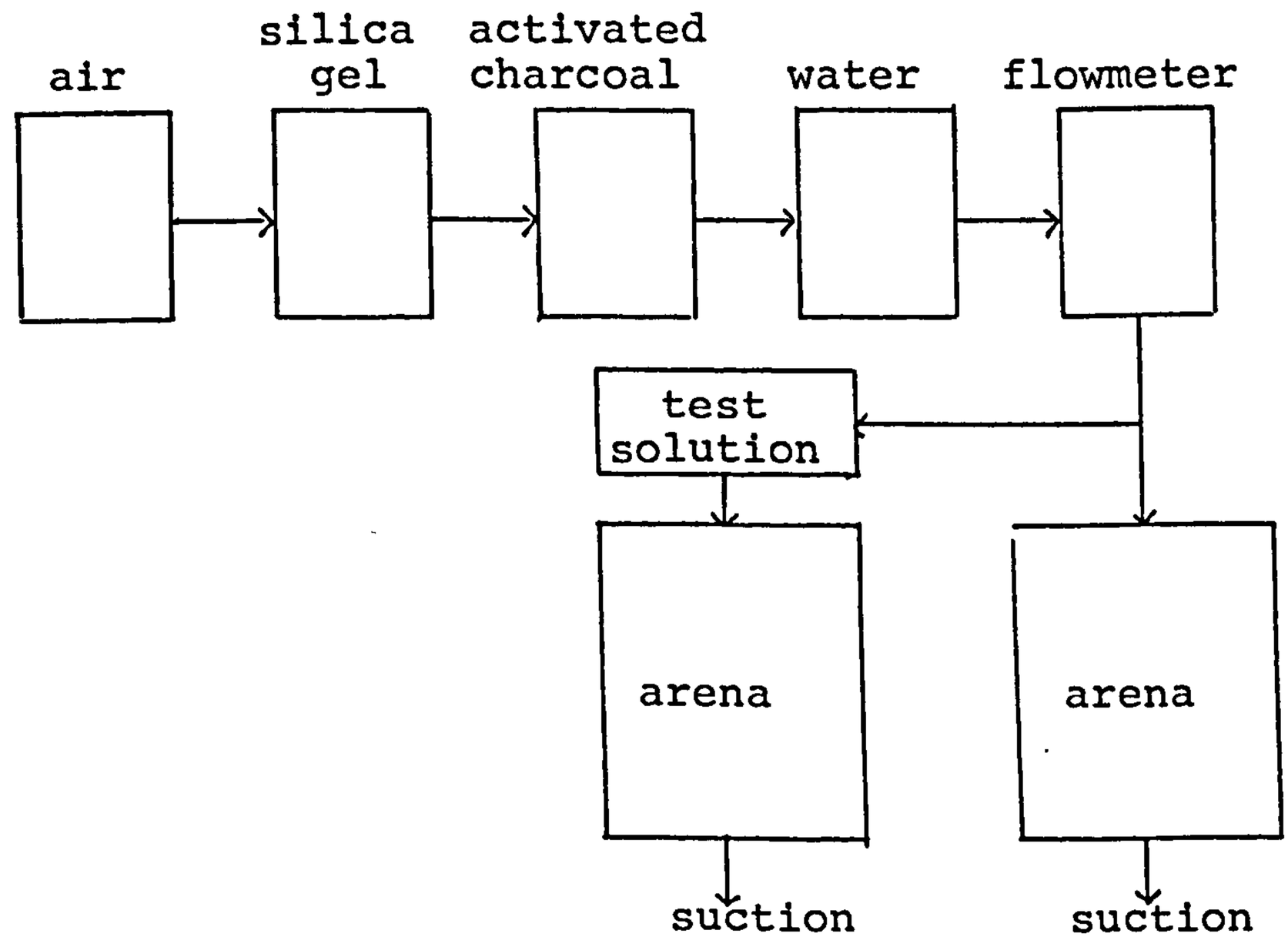
Airflow through the system was measured using a small smoke generator which produced plumes of white smoke (see Fig 3). The smoke front, generated by two cigarettes or cigars, was easily visible which enabled the rate of flow to be calculated and proved that the baffles were effective

FIG. 3



APPARATUS USED TO GENERATE SMOKE FOR CALIBRATION OF AIRFLOW THROUGH ARENA

FIG. 4



ARRANGEMENT OF APPARATUS USED IN TESTS TO MONITOR SLUGS

in producing a constant stream. In the last 20cm of the arena the smoke front tended to become convex as the flow at the sides was disrupted but this could be improved by drawing the airstream through the end of the arena. The smoke also illustrated that some turbulence in the airflow occurred in the height of the apparatus but a depth of 4cm was necessary to accommodate snails in the arena. Placing an object in the path of an airstream immediately disrupts the air front so the ideal situation of uninterrupted flow was impossible to attain as the presence of the slug or snail caused turbulence.

Air from a cylinder (1987 pounds per square inch gas) was passed through silica gel to remove all moisture, through activated charcoal to absorb any impurities and then through distilled water to humidify it. A flowmeter (G.A. Platon Ltd. Basingstoke - Range 0 - 1200cc/minute) monitored the rate at which the air passed into the arena and suction at the opposite end was provided by a small suction pump attached to a tap. Slugs respond positively to wind currents so it was necessary to compromise between an airflow which was sufficiently strong to typify a moving airstream and one in which the anemotactic response of the animal became the dominant factor. Animals were therefore allowed to move in airstreams with a flow rate of 10 - 100cm/sec and monitored for orientated responses to the odourless air. A flow rate of 10 - 20cm/sec produced a positive anemotactic response in Deroceras reticulatum and was maintained in subsequent tests with odorous compounds.

Responses to food or artificial odours were assessed by either passing the airstream through a solution of the test material before entering the arena or by placing the test material in the arena itself and examining the response of the animals in still or moving air. Connecting tubes to the arena were a flexible plastic of 2.5mm. bore and a layer of insulating tape on the upper wall of the arena provided an airtight fit for the removable cover which facilitated cleaning between trials. Fig 4 shows the arrangement of the apparatus used.

2.9 TIME LAPSE PHOTOGRAPHY

The film record provided an accurate means of analysing slug movements as it produced a permanent record of the path followed by the animal in the presence of food material.

The cine camera initially used to monitor behavioural responses of molluscs was a Vinten time lapse camera with a wide angle Kern - Paillard 25mm F 1.4 lens. The system operated at 4 frames per minute with a synchronised flash (Bowens Mondite 200) providing the illumination. Newell (1965a) has shown that short bursts from an electronic flash do not affect the normal activity of slugs. The 16mm reversal film (Ilford Mark V) provided 20 hours of recording on a 100ft spool.

A time lapse video system was later obtained since this enabled tapes to be analysed and edited speedily and avoided completely the need for external flash. The video system was also less expensive to run and the tapes

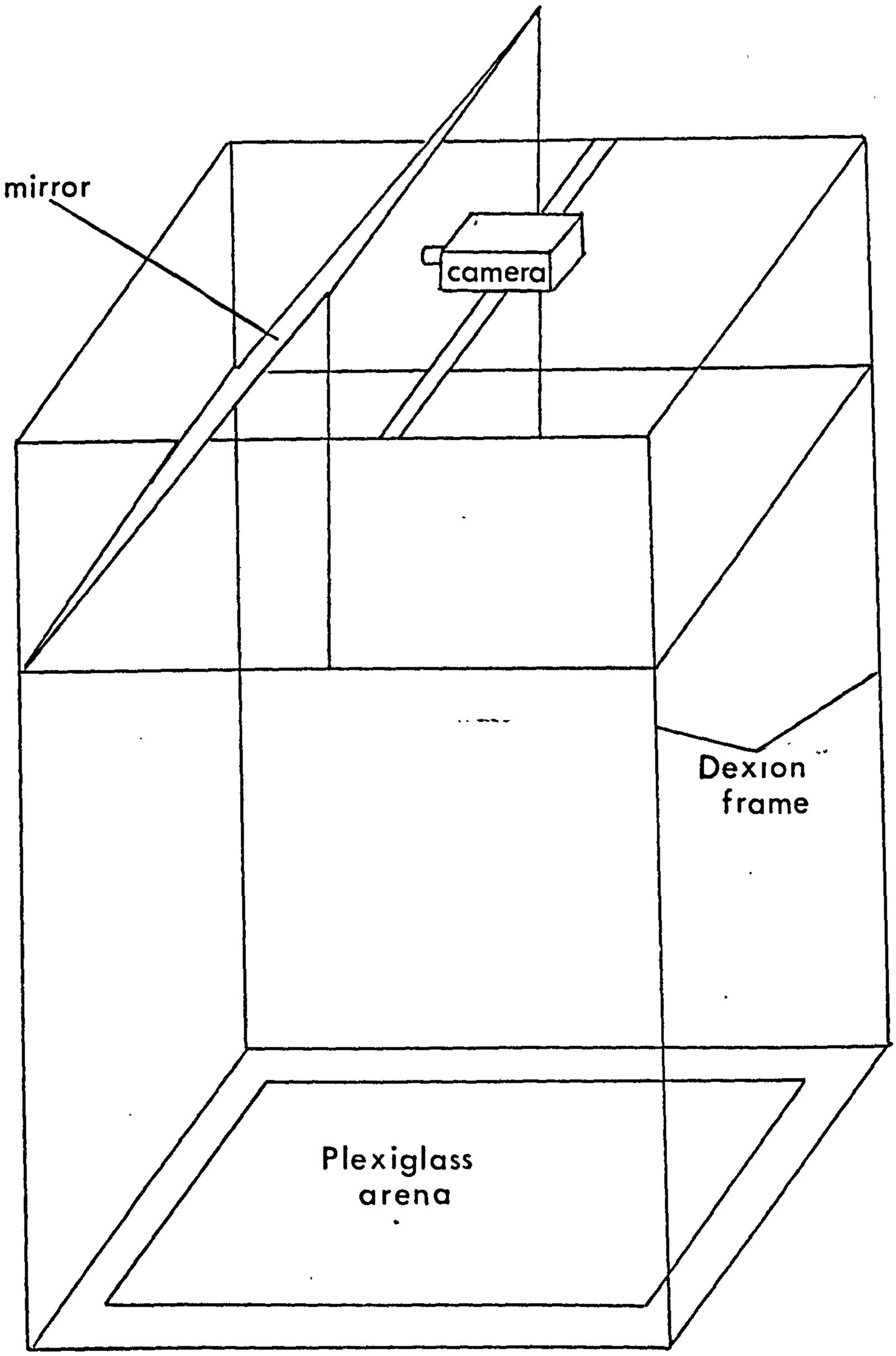
could be erased and reused to minimise cost. A security camera, recorder and monitor (Shibaden SB 408K camera, Shibaden SV 612K recorder and Shibaden VM 903B 9 inch monitor) was obtained and the camera tube replaced by a silicon tube which operated at low light intensity. The animals could therefore be observed without interference but the quality of the recording was lower than that obtained with the normal cine camera. It was considered however, that the sacrifice in resolution was compensated for by the easier recording and faster analysis possible with the video system.

The perspex arena was placed on the floor with the camera directly above. A distance of approximately 2 metres provided a suitable height for the camera to include the entire area of the arena in which the behavioural tests took place. With the cine system a beam across the room was sufficient to support the camera and time control box. The video camera however, had a narrow base which made it unstable and it was necessary to provide a more secure support. A large frame was therefore built to house the camera, using Dexion pieces to form a shell of 2m x 1.5m x 0.75m with appropriate cross members to strengthen the structure.

During use, the camera tube heats up and ash which forms as a result may fall onto the end of the tube when the camera is held vertically, forming permanent blind spots on the area of the tube through which the image must pass. To prevent this damage to the tube, it was necessary to hold the camera horizontally so that any ash would fall onto the lower surface of the tube and not affect the reception at

FIG. 5

SUPPORTING SYSTEM FOR VIDEO



the end. A system was therefore needed to enable the camera to be held horizontally and yet permit the area beneath the camera to be filmed. A mirror was incorporated into the system so that the camera filmed the image rather than the arena itself. A front-silvered mirror (61 x 37cm) was supported by the Dexion frame at an angle of 45° to the horizontal. Fig 5 is a diagram of the apparatus and supporting system used to record responses of slugs in the presence of food.

2.10 EXPERIMENTAL METHOD

Large sheets of filter paper (Whatman No. 4 - 46 x 57cm) or a layer of peat 2cm deep were placed on the floor of the arena to provide some optical contrast for recording purposes and to maintain a high humidity during filming which could last up to ten hours. The filter paper sheets could then be developed to provide a second means of analysis. This was done by sprinkling fine charcoal over the entire sheet and washing excess charcoal off - the mucous trail of the animal remains as a black line where charcoal adheres to the mucus on the paper. After each test, the filter paper was removed and the floor wiped clean to remove traces of mucus which could affect subsequent tests. Where peat had been used as the base, the top layer was removed and the remaining peat combined with some fresh peat to provide a clean surface for further tests.

In most tests a salt barrier was placed at the perimeter of the arena to contain the animals. This was particularly necessary where they were being observed over

a period of several hours to prevent the animals from moving outside the range of the camera. A small clock was placed on the perspex cover of the arena with the face towards the camera so that the passage of time was recorded on the film.

The presence of a number of slugs in the arena may well have affected the response of an individual since the odour of conspecifics is known to affect behaviour. Placing just one animal in the arena, however, was very expensive in time and film since many slugs showed no response. The problem was to some extent resolved by having a large arena where a small animal such as Deroceras could move some length without interference from other slugs. Where slug paths crossed, however, trail following may have dominated the response to introduced odours or food items, so interference factors were taken into consideration in these multiple animal tests.

Tests were made at laboratory temperature, generally 16 - 20^{o}C, with newly collected animals. Each animal was used in one trial only to minimise learning. Before a test, the substrate on the floor of the arena was moistened with distilled water to facilitate movement of the animals and to provide a high humidity during the time the animals were in the arena. Food items or experimental chemicals were placed in position on the moist floor. The distribution of food items was symmetrical - either two items placed equidistant from the centre, four items placed in a line along the centre of the arena, four items placed in a square around the centre or eight items in a radial

plan. In all these patterns of food distribution the animals were placed centrally. Occasionally, a single test chemical was centrally placed and the animals distributed around this.

The position of both food and animal were estimated rather than mark the surface of the peat or paper since this could possibly affect the responses of the animal. Immediately the slugs were placed in the arena, they moved some distance in the direction in which they faced. Since this probably had no association with food or chemical items in the arena but was more likely due to disturbances caused by moving the animals, a central zone of 5cm. diameter was allowed for the animal to orientate. All path lengths were therefore measured from the edge of a 5cm. circle around the starting position of each animal.

Hamilton (I977) compared the index of directness of paths made by snails,using the equation $d = d_r / d_p$ where d_p is the length of path travelled and d_r is the resultant distance. This gives all paths a value of between 0 and I.0 with the low value being associated with circuitous paths and the high value with straight paths. He appreciated also that many animals, including snails, exhibit initial searching movements while they orientate themselves and allowed a 'criterion distance' for the animals to make these random movements. Path lengths were therefore measured from this criterion distance and not from the origin of the path.

Track lengths were measured using a map measurer to trace the path of each slug on the 'developed' filter paper. The developed I6mm films were projected onto a screen

and the video tapes replayed on the monitor. These photographic records of slug movements enabled the path lengths to be checked and provided the only means of analysis for tests on the peat substrate. The film records also gave additional information on the length of time apportioned by each animal to feeding, resting and locomotion and the speed of approach to food could be calculated.

The presentation of the test chemical to the slugs varied with the type of compound so that essential oils,for example,were allowed to evaporate from a central point source and solids such as bran were placed in piles within the arena. Pickett & Stephenson (I980) tested vacuum distilled components of lettuce, maize, dandelion, carrot and pea by allowing Deroceras to move over paper streaked with the extract. The length of slug trail which coincided with the plant extract suggested that volatiles from lettuce, carrot and dandelion were the most attractive and the authors used gas chromatography techniques in an attempt to define the precise chemicals invoved - preliminary results indicated that unsaturated C_6 alcohols were responsible. The trail-following bioassay does not,however, distinguish between olfactory and gustatory attractants since the animals are continuously in contact with the chemical. The test gives no indication that the plant material can initiate movement or that the slugs are orientating towards an odour.

It is perhaps more useful therefore to have a point source of odour so that orientation to the test material can be assessed, This approach can then highlight the distance over which odours are attractive and provide more concrete evidence of directed locomotion.

2.II CHEMOSENSORY RESPONSES OF SLUGS

Many tests were made with a number of fruit flavourants serving as either a point source of odour or combined with bran to compare the enticement of this food with and without additional attractants. The results obtained, however, were often erratic and the number of positive responses low. A number of features of slug behaviour were recorded though. The trail following reported by some authors (Section 2.4) was confirmed in Deroceras reticulatum and indicates that these animals may release a pheromone into their mucous trail. Observations on the movements of slugs over a I2 or 24 hour period showed that the animals do not remain for long at a source of food but that they may leave and return to this point later. The return route was rarely along the outward trail so the animal must reorientate using the olfactory stimulus of the food or possibly using a marker it has deposited on the food as an olfactory cue.

The distance of attraction was investigated by marking concentric rings on the cover of the arena and observing the pattern of approach to centrally placed odorants or foods. The erratic nature of the locomotory responses of slugs made it difficult, however, to quantitatively estimate the attractant properties of test compounds and most tests were inconclusive. The behavioural response was used as a screen however, and showed promising results with beer or a by-product of this, pressed yeast. The tracks recorded in some of the trials with beer are presented in this chapter since this compound was subsequently used in feeding tests (Section 4).

2.I2 OBSERVATIONS ON SLUG MOVEMENT TOWARDS BEER

Matthews (I977) observed that Limax maximus could orientate towards the odour of flat beer when odour-laden air was passed through a Plexiglass tunnel. The number of positive responses to beer was higher than to a water control but, as with many behavioural tests involving slugs, the number of unresponsive animals was high. Monitoring the path of an animal provides some indication of the relative attractiveness of different foods or compounds placed in the vicinity since it can be assumed that the more attractive compounds will entice the animal to approach more directly and perhaps more speedily. Observations on the attractiveness of beer were made in conjunction with feeding tests.

A radial plan of eight bait points with an approximate radius of 28cm was arranged so that four bran points moistened with water alternated with four bran points moistened with beer. Six Deroceras were placed centrally and movement monitored for 30 minutes in still air. Tracks were recorded and analysed both by 'developing' the filter paper and tracing movement on the video monitor. The results are presented in Table I, and Fig. 6 is a typical picture of the 'developed' paper.

Ten trials with these two foods/attractants involved 60 slugs. Of these , 47 exhibited a positive response and moved to one of the baits - eight of these slugs left the food within the 30 minute period but none moved to another food point. The bran moistened with beer attracted twice as many slugs as the bran moistened with water though the directness of approach was similar for the two foods.

TABLE I OBSERVATIONS ON SLUG MOVEMENT TOWARDS BEER

TRIAL	+ve	d BRAN + WATER	+ve	d BRAN + BEER
I	2	0.96, 0.94	4	0.83, 0.85, 0.7I, 0.52
2	0	-	5	0.67, 0.72, 0.77, 0.9I, 0.9I
3	0	-	5	0.68, 0.75, I.00, 0.95, 0.57
4	2	0.57, 0.82	3	0.9I, 0.75, 0.84
5	3	0.96, 0.79, 0.95	3	0.86, 0.6I, 0.96
6	I	0.83	3	0.92, 0.74, 0.53
7	2	0.45, 0.75	0	-
8	2	0.85, 0.6I	I	0.69
9	2	0.86, 0.88	4	0.88, 0.8I, 0.39, 0.87
IO	I	0.77	4	0.59, 0.87, 0.74, 0.88
TOTAL	I5		32	
MEAN		0.80		0.77

d = Index of directness

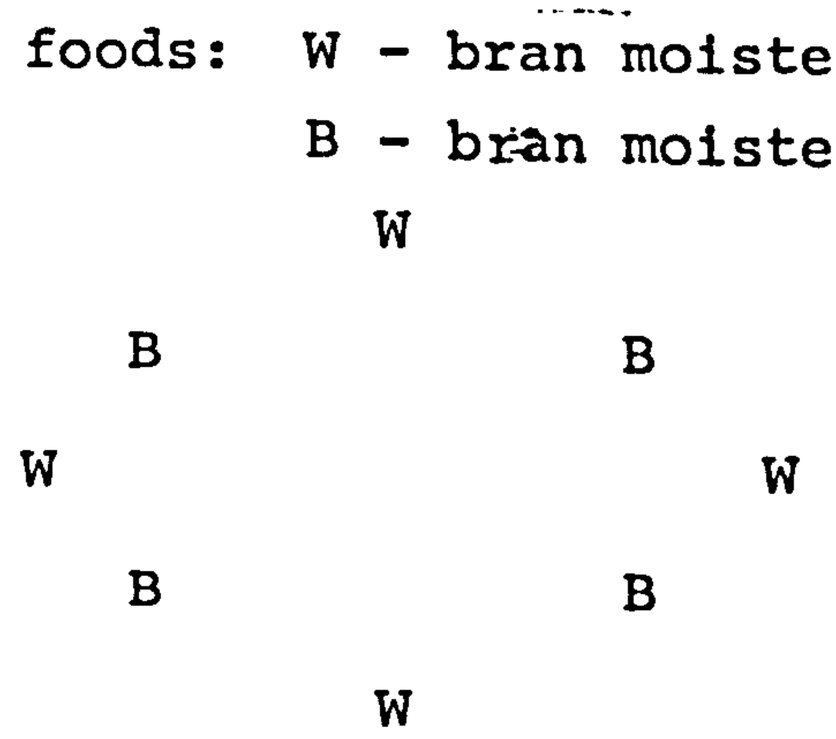
$$= \frac{\text{resultant distance travelled}}{\text{path length}}$$

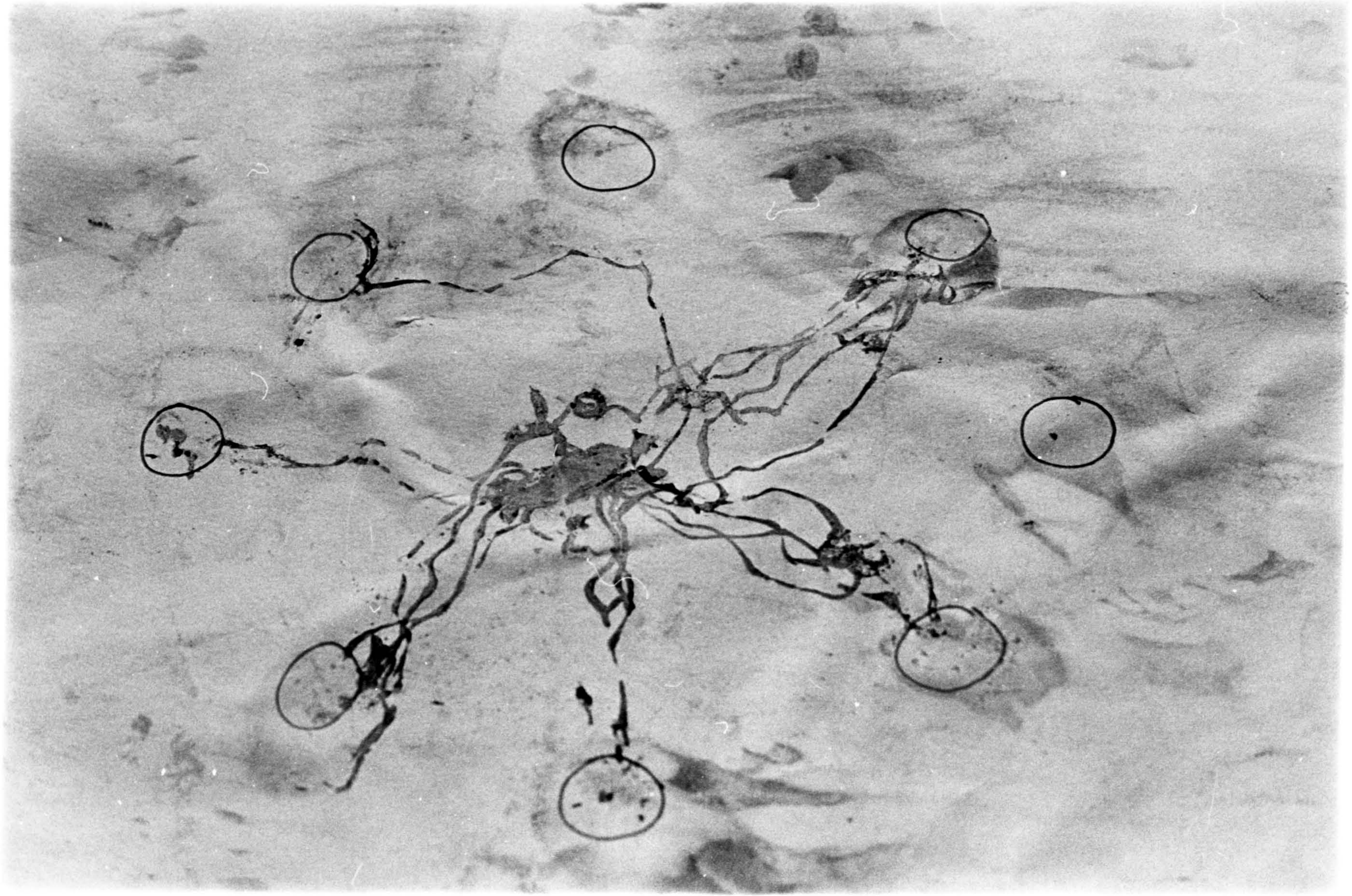
BLANK IN ORIGINAL

FIG. 6

MUCOUS TRAILS LAID BY SIX SLUGS WHEN PRESENTED WITH A RADIAL PLAN OF ATTRACTIVE FOODS

Plan of foods: W - bran moistened with water
B - bran moistened with beer

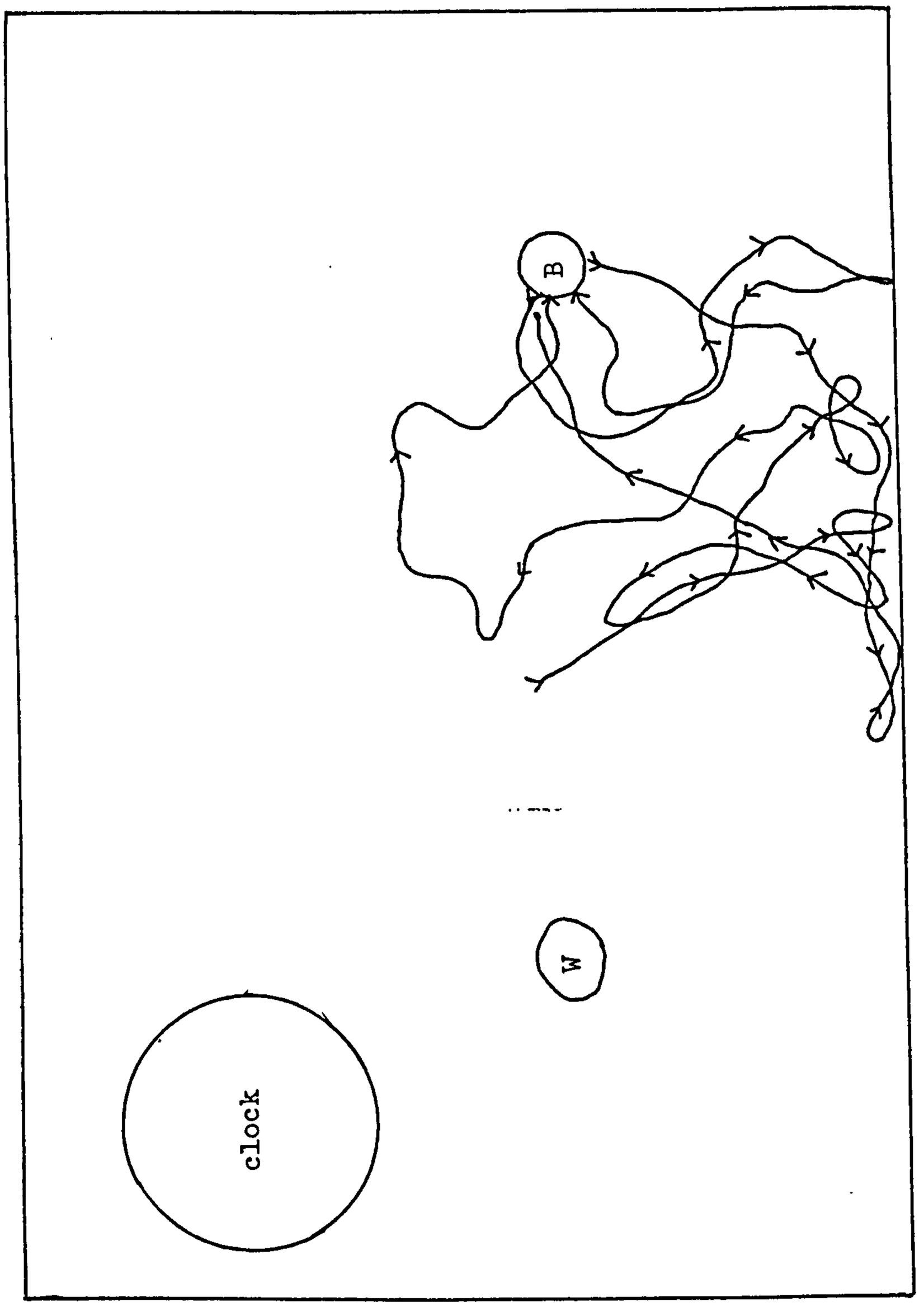




The results suggest that beer is attractive to slugs over a distance of at least 28cm and could be used to augment the attractive properties of bran. Fig. 6 shows that the animals left and returned to the food a number of times in the 30 minute test period but all four of the beer moistened bait points were visited by the slugs. Figs. 7, 8 & 9 show the tracks of individual slugs placed in the arena with two bait points only, and indicate that many other tests with beer were less conclusive. The three tracks suggest very different responses to the two foods offered as, under identical conditions, one animal approached the bran + beer, one the bran + water and the third approached neither food.

Tests in a larger arena placed in the field suggested that the attractant properties of the beer by-product,pressed yeast,may extend to over 40cm. The inconsistencies in the olfactory response necessitate that these results be complemented by tests to confirm that this compound is a suitable attractant for use in commercial baits and the feeding tests presented later do show this. Figs. IO and II provide further evidence of the erratic nature of the olfactory response. In Fig. IO, with a central point source of beer, the slug travelled over II metres in its foraging activities and encountered the odorant source twice during the six hour trial. Fig. II shows the mucous trail formed by another slug which travelled just I60cm under the same conditions. The circuitous track of the former ensures that the animal samples a large area of the test arena while the slug track shown in Fig. II covers only a small sector of the test area.

FIG 7 TRACK RECORDED FROM SLUG PLACED FOR 24 HOURS IN ARENA WITH BRAN + WATER & BARN + BEER



TOTAL DISTANCE TRAVELLED IN 24 HOURS - 510cm

ACTIVITY CYCLE OF ANIMAL:

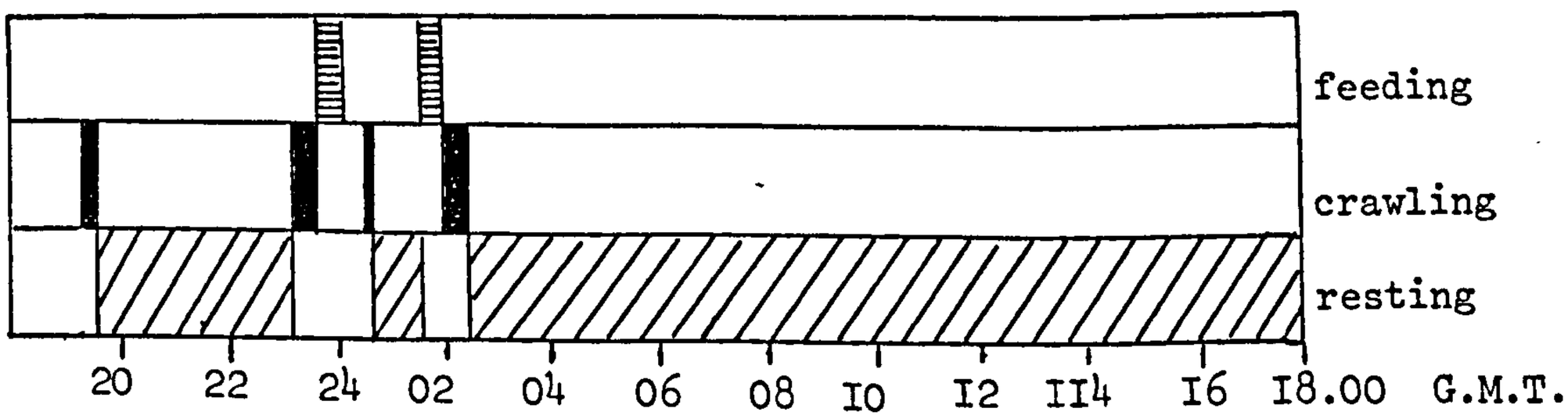
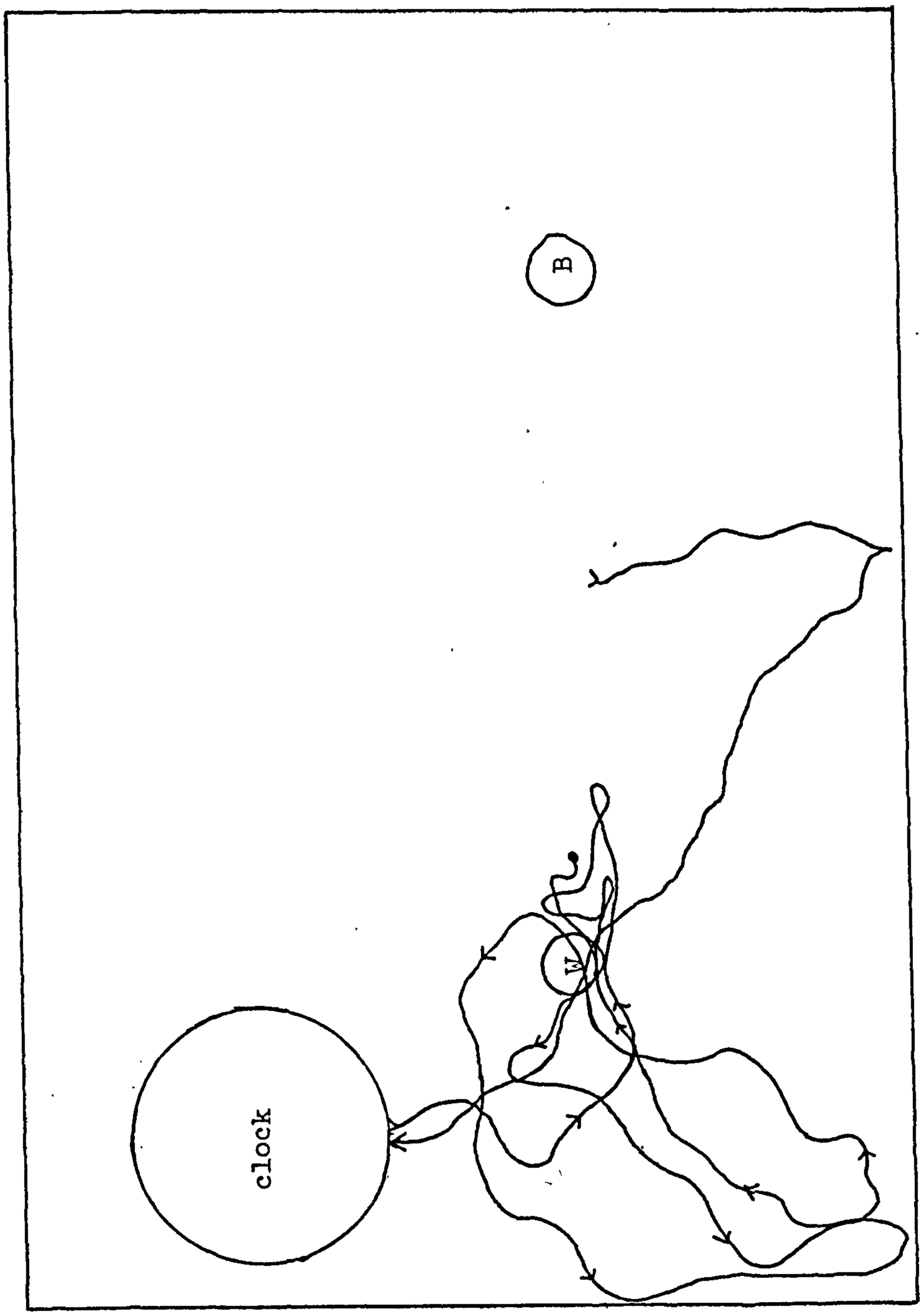


FIG. 8 TRACK RECORDED FROM SLUG PLACED FOR 24 HOURS IN ARENA WITH BRAN + WATER & BRAN + BEER



TOTAL DISTANCE TRAVELLED IN 24 HOURS - 560cm

ACTIVITY CYCLE OF ANIMAL :

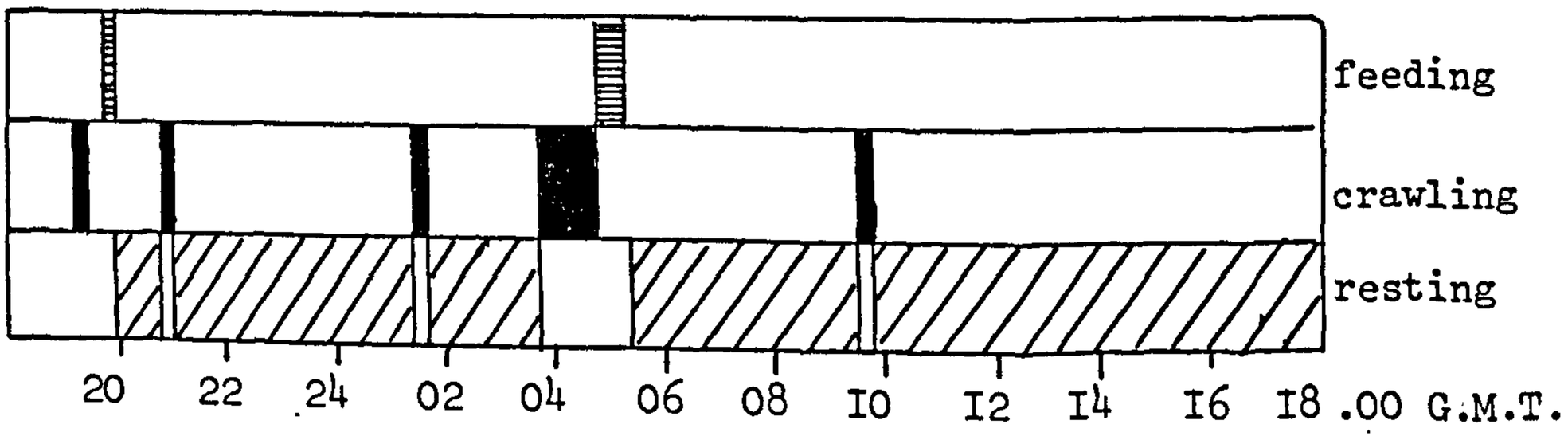
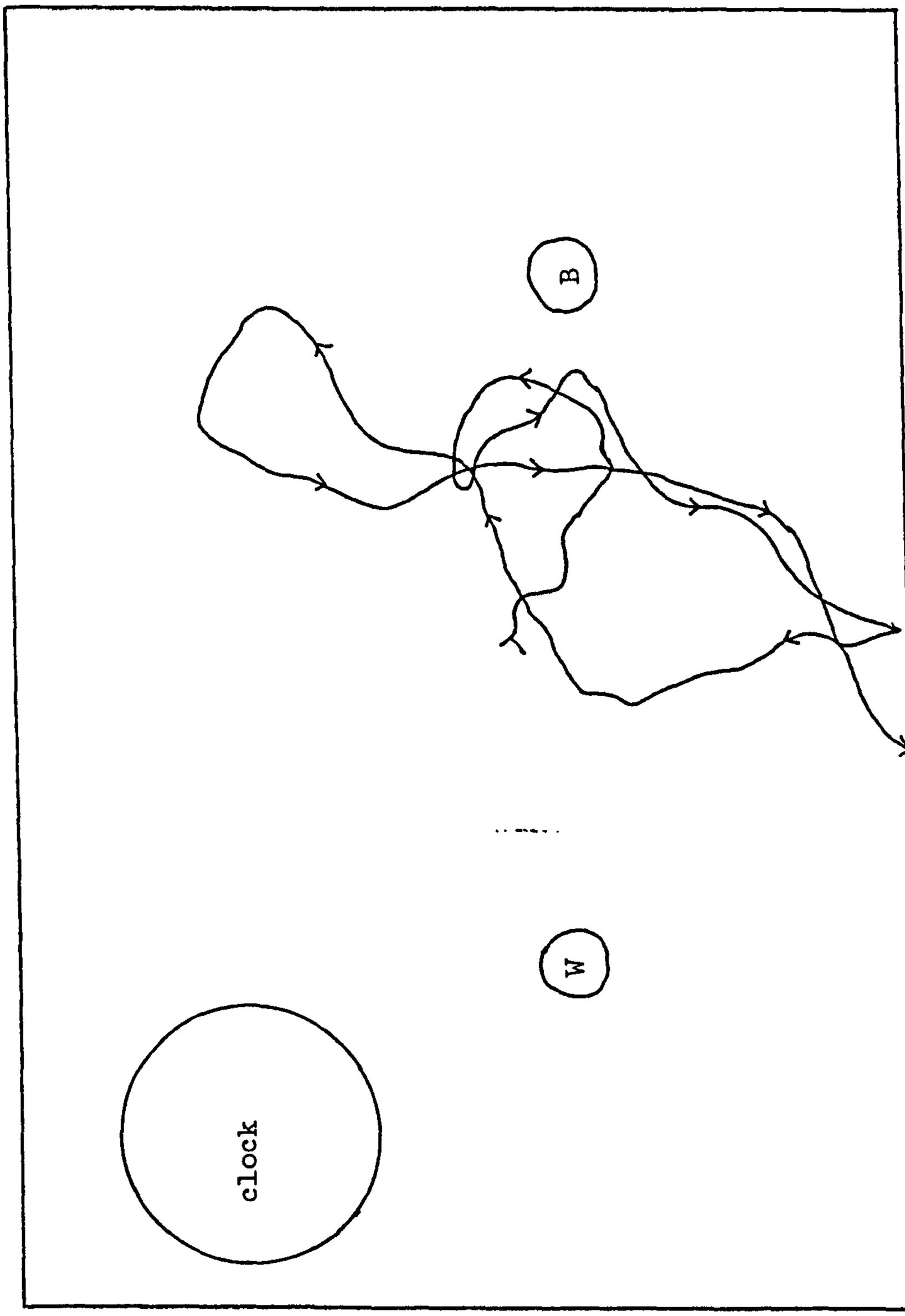


FIG. 9 TRACK RECORDED FROM SLUG PLACED FOR 24 HOURS IN ARENA WITH BRAN + WATER & BRAN + BEER



W - bran + water

B - bran + beer

TOTAL DISTANCE TRAVELLED IN 24 HOURS - 370cm

ACTIVITY CYCLE OF ANIMAL :

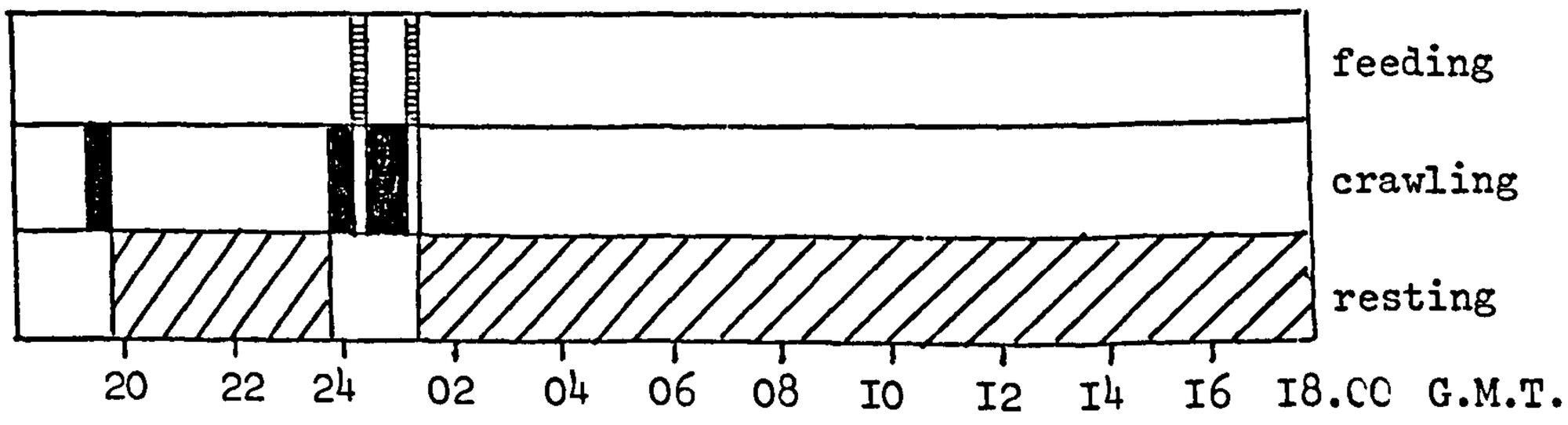
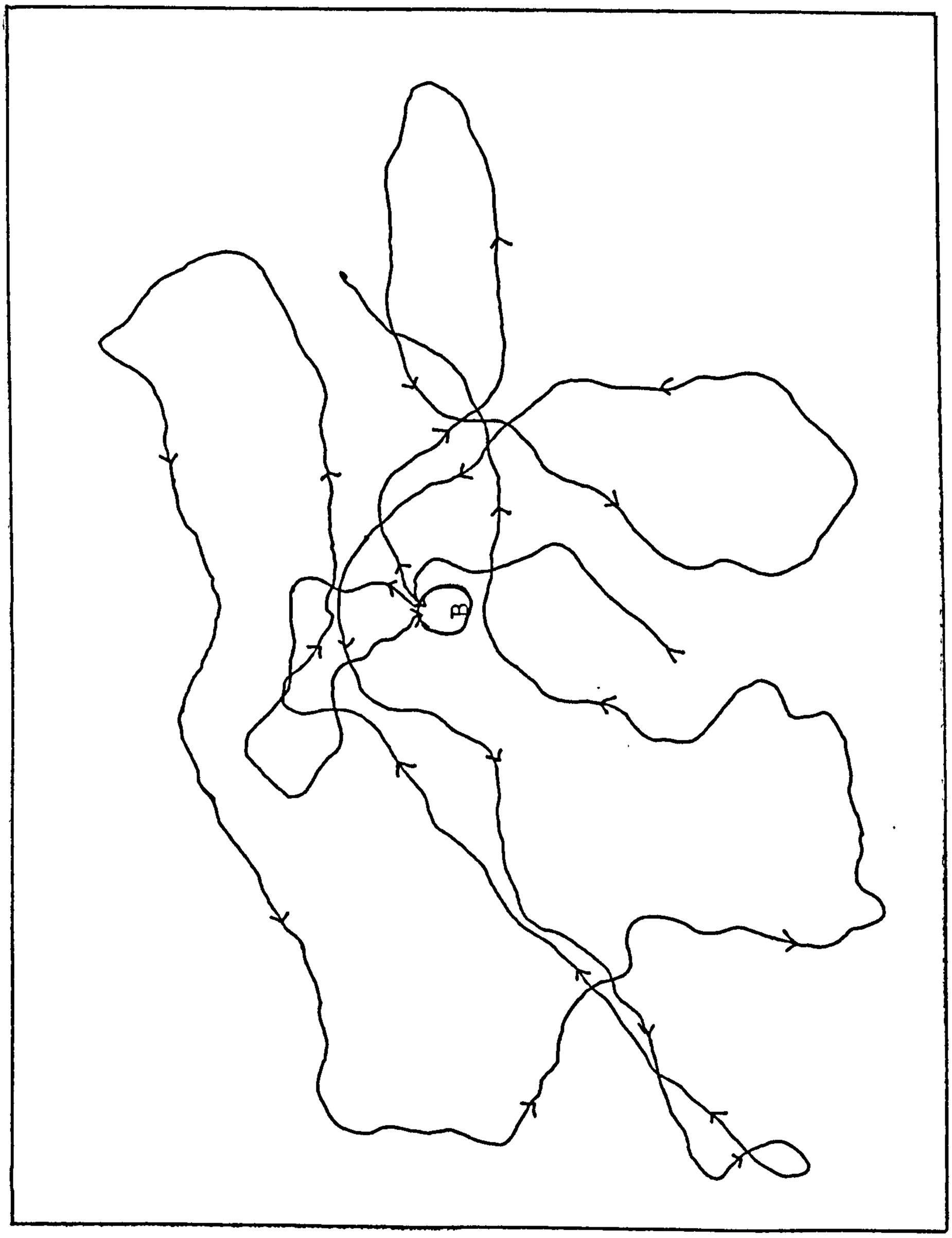


FIG. IO TRACK RECORDED FROM SLUG PLACED IN ARENA FOR SIX HOURS WITH CENTRAL POINT SOUCE OF BEER

TOTAL DISTANCE MOVED BY SLUG IN 6 HOURS - II00cm.



ACTIVITY PATTERN OF ANIMAL

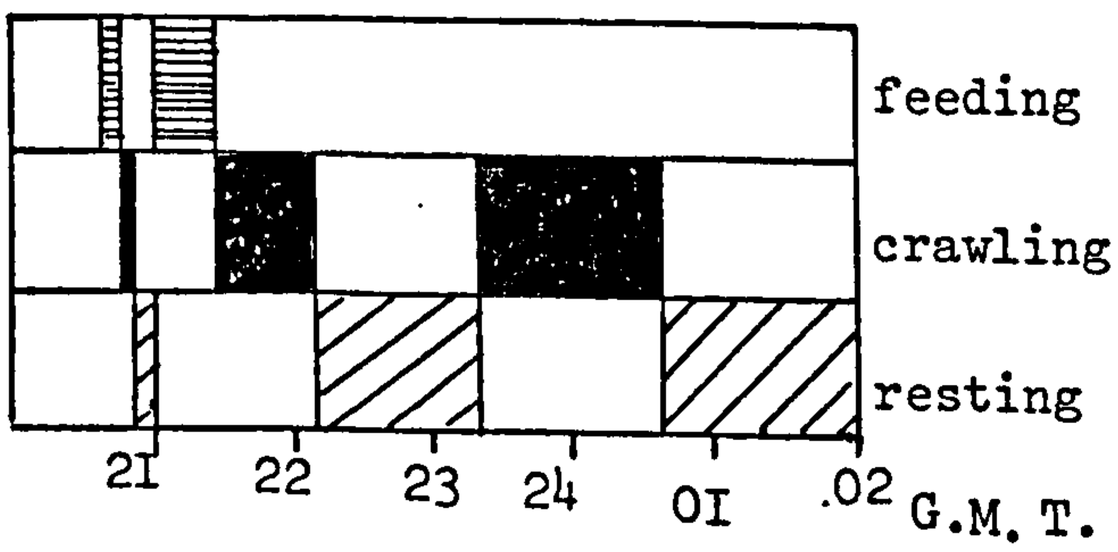
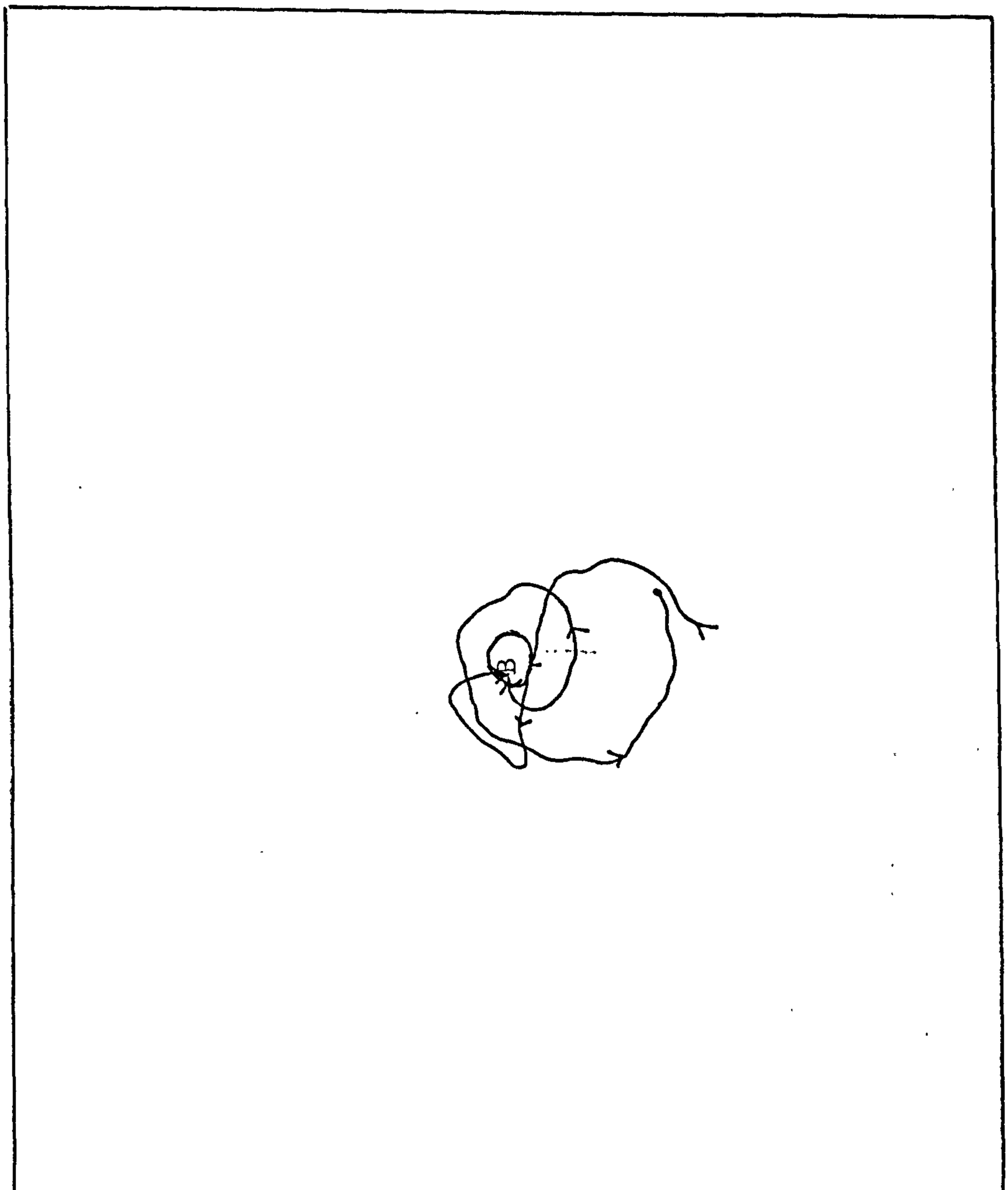


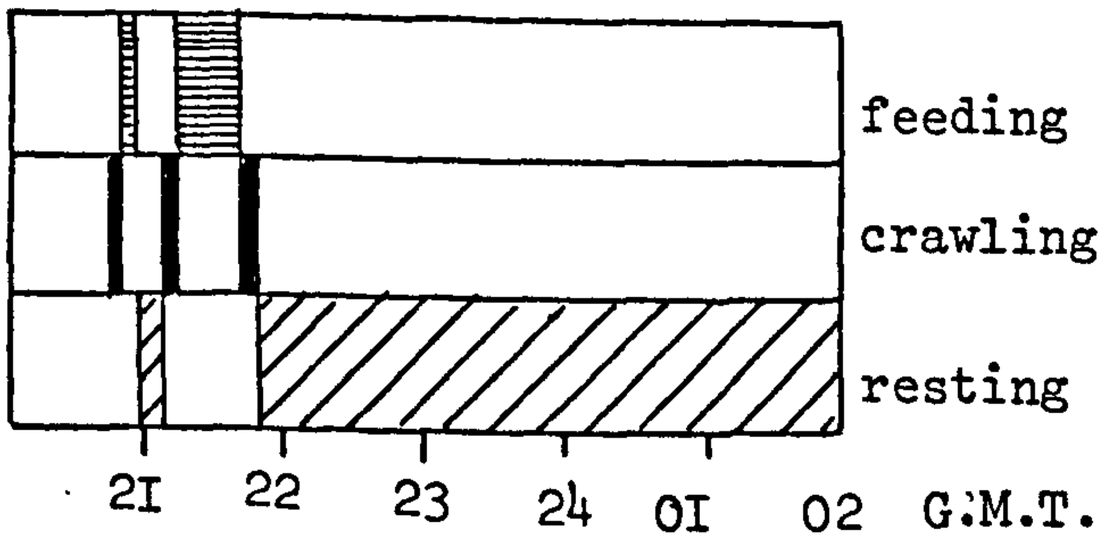
FIG. II TRACK RECORDED FROM SLUG PLACED IN ARENA FOR SIX HOURS WITH CENTRAL POINT SOURCE OF BEER

TOTAL DISTANCE MOVED BY SLUG IN 6 HOURS - 160cm



B - bran + beer

ACTIVITY PATTERN OF ANIMAL



2.I3 CONCLUSIONS

Behavioural tests using an olfactometer to investigate movement of slugs in the presence of test compounds have shown many inconsistencies. Such a system, however, provides a useful screen to highlight potentially attractive chemicals and has indicated that beer will entice slugs from a distance of 40cm. Such a response to poison baits would improve their efficiency and enable application rates to be lowered. Tests to compare bran + beer with bran + water have shown that the former attracts twice as many slugs although the directness of approach to the two foods does not differ. In the field, the slug is surrounded by attractive odours and the approach to an odour source may be more haphazard. The animal seems capable, however, of preventing confusion of its olfactory system and can distinguish individual odours and orientate to them. A combination of distance attraction (olfactory response) and contact attraction (gustatory response) is required for ingestion of a compound to occur although chance encounter of an attractive food can also initiate feeding. The feeding trials reported in Sections 3 and 4 provide more substantial evidence of the attraction of slugs to beer reported in this Section.

SECTION 3 FEEDING

INTRODUCTION

It is well known that slugs will feed on many foods though the features which cause them to recognise and begin to ingest the material are less clear. It may be that the preferred foods have a single attractive chemical which dominates the odour but since food odours are complex, it is more realistic to assume that the animals can recognise several components of the odour individually. This has many advantages to the animal as there is an adaptive value in being able to recognise and utilise a wide range of foods. Attractant chemicals are produced by most plants but are not the only factor which determines the acceptability of food as the attractive odour may be counteracted by a compound which is repellent to the animals. The presence of hairs or spines on a leaf surface can also deter feeding since texture is important in determining the acceptability of food.

In 1939, Cameron suggested that the omnivorous habits of slugs and their low level of sensory response indicated that these animals were indiscriminate feeders. More recently, definite food preferences have been shown to exist in molluscs which suggest that they are in fact capable of a high degree of selection in their food finding behaviour. Cates & Orians (1975) have suggested that generalised herbivores prefer early successional plants such as annuals and biennials e.g. Shepherd's purse (Capsella bursa-pastoris) to later successional or climax plants e.g. Maidenhair fern

(Adiantum pedantum). They investigated the palatability of 100 plant species to Ariolimax columbianus and Arion ater and found that the early successional plants were even more readily eaten. These plants have fewer chemical defences so the herbivores which feed on them save time and energy in detoxification processes required by animals feeding on many higher plants. Many other reports of food selection in molluscs indicate that these animals can actively choose which plants they eat and their wide range of potential foods does not necessarily mean that they are indiscriminate feeders.

3.1 FEEDING

Feeding activity of terrestrial molluscs has been examined by observing food selection, by quantitive analysis using collected faecal material and more recently by electrophysiological techniques. The early work of Cooke et al (1895) and Gain (1891) produced a list of palatable plant products which has been extended by Frömming (1939, 1940, 1957) and Duval (1971). This work has established that slugs will feed on a wide variety of plant material but show definite preferences for certain foods - brassicas, wheat, strawberries, lettuce. This evidence of food selection has been further investigated by Cates (1975) who found that the slug Ariolimax columbianus could distinguish between two morphs of wild ginger. Jones (1962) and Crawford-Sidebotham (1971) recorded that Deroceras reticulatum was amongst those animals that can distinguish between the cyanogenic and acyanogenic leaves

of birds-foot trefoil (Lotus corniculatus) and wild white clover (Trifolium repens), selecting the latter form in preference to the more toxic cyanogenic leaves. Oleson (1979) claims that the snail Trichia hispida (Linné 1758) can select between two forms of the heterostylous plant Primula elatior.

Gut content analysis (Chatfield 1973, 1976, Pallant 1969) can be used to identify fragments of acceptable plants and provide an indirect measure of preferred foods. This may be coupled with a quantitative analysis of faeces produced by slugs fed on a particular food which can enable the energy budget of an animal to be calculated (Jennings & Barkham 1979, Mason 1970, Pallant 1970a, 1970b, 1971, 1974, 1975). Slugs have a high assimilation efficiency (A.E.) compared with most herbivores since they possess a wide range of enzymes and can digest celluloses. Pallant (1970a) obtained an assimilation fraction of 0.784 in the laboratory for Deroceras reticulatum while Jensen (1975) estimated that the A.E. of a field population of Arion ater was 76.30%.

Different food materials appear to have different growth potentials since Limax flavus was found to have an A.E. of 47.66 - 90.41% and 60.18 - 93.83% on potato although both foods have about 16j/mg dry weight (Davidson 1976). These high values for assimilation may not relate directly to field situations since food is readily available to animals in the laboratory and their energy expenditure is low. Richardson (1975) found that Cepaea nemoralis had a higher A.E. (71.73%) on artifical food than on the natural food in its sand dune habitat (30 - 44%) emphasising the

caution required when extrapolating laboratory test results to encompass activities of a normal population. These assimilation estimates, which are calculated from the difference between ingested and egested food, have another disadvantage for studies on Deroceras at least. Pallant (1970b) has observed that this slug produces two types of faecal matter - one is a mucus with brown fluid and small granules which appears to come from the digestive gland and the other is material which has passed directly from the stomach to the intestine. Discrepancies arise over whether to include both of these components in the faecal weight.

More recently, electrophysiological recordings of the snail Helisoma (Fountain 1974), the slug Limax maximus (Gelperin 1975, 1976, 1977, Gelperin & Forsythe 1976, Gelperin et al 1978, Senseman 1977c, 1978b) and the slug Ariolimax californicus (Senseman 1976, 1977a, 1977b) have provided further information on feeding activity. These authors have outlined a feeding motor programme (FMP) which can be elicited by chemoreceptive input or electrical stimulation of the olfactory system. The FMP coordinates rhythmic output from the buccal and cerebral ganglia and recordings from these and metacerebral giant cells in the CNS provide evidence of feeding activity.

An isolated preparation of the buccal mass, buccal ganglia and CNS shows a similar electrical response to that obtained by stimulation of the intact animal and allows greater stimulus control by reducing interference from peripheral nerve systems within the animal. Muscles

of the buccal mass are responsible for the protraction and retraction of the radula while the lips aid in grasping the food and recordings from these mouthparts, made with a force transducer, have provided further information on feeding responses in slugs (Gelperin et al 1978, Senseman 1978a). The animals have been observed over a short period (2 - 60 minutes) and the graphic output from either force transducer or electrical recorder analysed into components of the feeding response. These experimental methods have enormous potential for providing details of central control of motor neurons to feeding muscles but can give no information on the mechanisms involved in food selection.

An alternative method of estimating feeding activity is to offer a choice of food to the animal and allow it to select which food it prefers. By measuring the food consumed over a known period, a quantitive estimate of meal size can be made and foods compared for their ability to initiate and sustain feeding. The major complication with slugs and snails is that they deposit mucus and faeces on their food as they eat and although the material can be wiped clean before weighing, residues may distort the true value of food consumed. The extent of feeding can also be estimated by calculating the area of leaf disc eaten (Judge 1977) or the number of wheat grains damaged per unit of time (Hunter 1968, Newell 1965a).

The main requirements of a feeding bioassay are that the amount of food consumed can be accurately assessed and more importantly, that the animals will eat the food regularly and in sufficient quantities for the

decrease to be recorded. Natural plant products were therefore initially examined for their ability to be used as a food bioassay material. Weight and/or area loss was recorded for a number of food substances in a series of feeding tests each lasting 24 hours.

3.2 EXPERIMENTAL PROCEDURE

All feeding tests were made in plastic seed boxes lined with single thickness of blue paper towel. The boxes were 13 x 20 x 3cm. and had tight fitting lids to prevent escape of the animals. A small hole was drilled in either end of each box to allow air to circulate. Prior to each feeding trial, the paper towel was moistened to provide conditions of high humidity and two weighed test food materials were placed equidistant from the centre of the box. One animal was placed in each feeding box for the duration of the feeding test. Slugs were kept in a constant temperature room set at 10^{o}C though the temperature tended to rise to 12^{o} or 13^{o}C. The animals were starved for 24 hours before each test in an attempt to standardise the feeding response of a group of slugs and then allowed to feed on the test material for 24 hours. The food was then removed, weighed or scored for feeding activity and the boxes cleaned. Thorough cleaning of the boxes was required between each trial to remove traces of food, faeces and mucus which could influence the response of animals in subsequent tests. Each test involved ten slugs and the results are an average value for the group.

Carrot

Discs of carrot (Daucus carota) were cut, blotted between sheets of kitchen towel and fed to four species of slugs - Arion hortensis, Milax budapestensis, Deroceras reticulatum and D. caruanae. A group of control discs were kept under similar conditions of moisture and humidity to compare natural weight loss due to evaporation of water from the carrot. After 24 hours, this control group had the highest loss (24.83%) despite the high humidity conditions in the feeding boxes. Weight loss from the discs fed to the slugs were:

Deroceras caruanae	4.49%
Milax budapestensis	6.15%
Arion hortensis	6.89%
Deroceras reticulatum	12.13%

The high evaporative loss indicated by the control discs made this food unsuitable for use in a feeding bioassay for slugs. Mucus deposits on the carrot discs fed to slugs were not removed by blotting the discs before reweighing and this must account for the rather small weight loss recorded since the food was readily eaten.

Wheat pellets

Pellets of wheatbran formulated in the laboratory were moistened with water of fruit flavourants and placed in small foil dishes in the feeding boxes. Each animal received a choice diet of a plain pellet - wheat moistened with water - and a flavoured pellet - wheat moistened with either apple, orange, strawberry or mandarin

flavourants. Unfortunately, due to a malfunction in the constant temperature control the temperature was not maintained at the correct level and the animals died from the high temperature. The pellets however, proved unsuitable for a bioassay as they readily lost their shape in the moist conditions of the feeding boxes and the animals removed pieces of food from the foil dishes so that reweighing the dishes did not provide a true indication of the amount of food consumed.

Dried peas

Freeze-dried peas (Pisum sativum) offered an alternative substrate for a feeding test since the material is firm and can be hydrated in test solutions. Dried peas were weighed, hydrated, fed to slugs for 24 hours, dried and then reweighed. The amount eaten by each animal could thus be calculated. It was found however, that although peas are one of the many crops attacked by slugs, the palatability of the freeze-dried peas was low and radula marks, indicating feeding, were visible on only a few peas. The amount eaten was therefore insufficient and too inconsistent to be used as a reliable estimate of feeding activity.

Paper

Small squares of paper were cut, soaked in water or potentially attractive test solutions and fed to starved slugs. Slugs will often eat the paper lining the boxes in which they are housed but feeding activity proved too low

to consider using paper as a food substrate. The dried paper squares showed a weight gain in many cases indicating that solutes and mucus were absorbed and in only a few tests was there evidence of feeding. Area loss from the paper proved difficult to measure since the gastropod radula scrapes at the substrate but frequently does not remove the whole thickness of the material. When tracing the outline of the residual food, there is therefore no definite, clear line to follow and the border may be indistinct. Ambiguities can thus occur where only the surface layers of the paper have been rasped away.

Lettuce

Large outer leaves from lettuce (Lactuca sativa) were soaked in test solutions and blotted between sheets of kitchen towel. Discs of 8cm. diameter were cut using a soil corer and a choice of two discs presented to each slug in a feeding box. Control discs were placed in boxes without any slugs. Both weight and area loss were measured after a period of 24 hours so that the most consistent method of determining meal size could be found.

Wetting and blotting the leaf discs destroyed the natural texture so that the lettuce became flaccid and unpalatable to the animals. The control discs however, lost little weight and therefore provided a suitable standard.

Area loss was measured using a planimeter which allows the residual area to be calculated by tracing the perimeter and this can then be subtracted from the original area. Again, however, a problem arose in deciding whether

half eaten leaf thicknesses should be included in the final calculations. Some experiments were made using weight loss from lettuce discs as an index of feeding. Control discs generally lost less than 10% of their weight but as an individual figure occasionally rose above this value, a control group was included in all trials. There was some evidence that animals ate more food when presented with a larger volume.

Potato

Potato (Solanum tuberosum) is a highly palatable food for slugs but unsuitable for weight loss determinations over a 24 hour period as evaporative loss is high. Freeze-dried potato cubes were therefore prepared so that these could be offered to the animals in feeding tests. Pieces of potato (1cm. x 1cm.) were freeze-dried in a vacuum pump cryoliser to remove any water, weighed, rehydrated in test solutions and fed to slugs. The freeze-drying process however, caused the texture and palatability of the potato to be altered so that it resembled polystyrene. The original texture and palatability were not restored upon rehydrating and the material blackened easily. It was then unacceptable to slugs so potato proved to be yet another food substance that was unsuitable for use in a feeding biassay.

Agar

A convenient assay material was finally found in powdered agar which could be reconstituted with test solutions to provide a material of specific hardness. BDH fine agar

powder (Product 33004) proved to be acceptable to slugs as a food material provided that a bran extract was incorporated into the mixture. The agar thus acted as an inert carrier with no attractiveness of its own, since plain agar was unpalatable, and was therefore suitable for comparing the stimulating properties of added ingredients.

100ml. distilled water was added to 5g wheatbran or 5g bran flakes, the mixture stirred and allowed to stand for 15 minutes. The solution was filtered through No. 1 Whatman filter paper and the extract collected over ice. The volume of the extract was made up to 100 ml. and added to 1g. agar powder in a conical flask. The solution was heated to boiling point, stirring continuously, and the thickened bran/agar mixture poured into trays of 20 x 13 x 1cm to set. When cool, the agar sheet was cut into discs of 1.2 cm. diameter using a No.8 cork borer.

At this concentration of agar powder, the discs were hard enough to retain their shape yet soft enough to encourage the animals to feed. Senseman (1977a) found that food hardness determined meal size in Ariolimax columbianus and that he could manipulate the amount eaten by varying the concentration of feeding stimulant and the hardness of the food. Agar based diets therefore provide an ideal food substrate and have, in fact, been used by several workers to rear slugs or snails in the laboratory. Ridgway (1971) and Wright (1973) kept Arion ater on an artificial diet and found that growth of this species was normal though reproductive performance was poor. Both of these authors

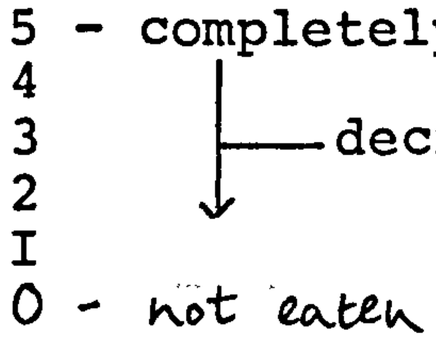
have formulated an agar based diet which contained vitamin and mineral additives for the complete nutrition of the slug but since the animals in this work were normally fed natural plant material between feeding tests, it was not considered necessary to provide a complete balanced diet for the duration of the feeding test. Some of the additives to the agar appeared to be nutritionally adequate themselves as slugs maintained on this food were able to lay eggs.

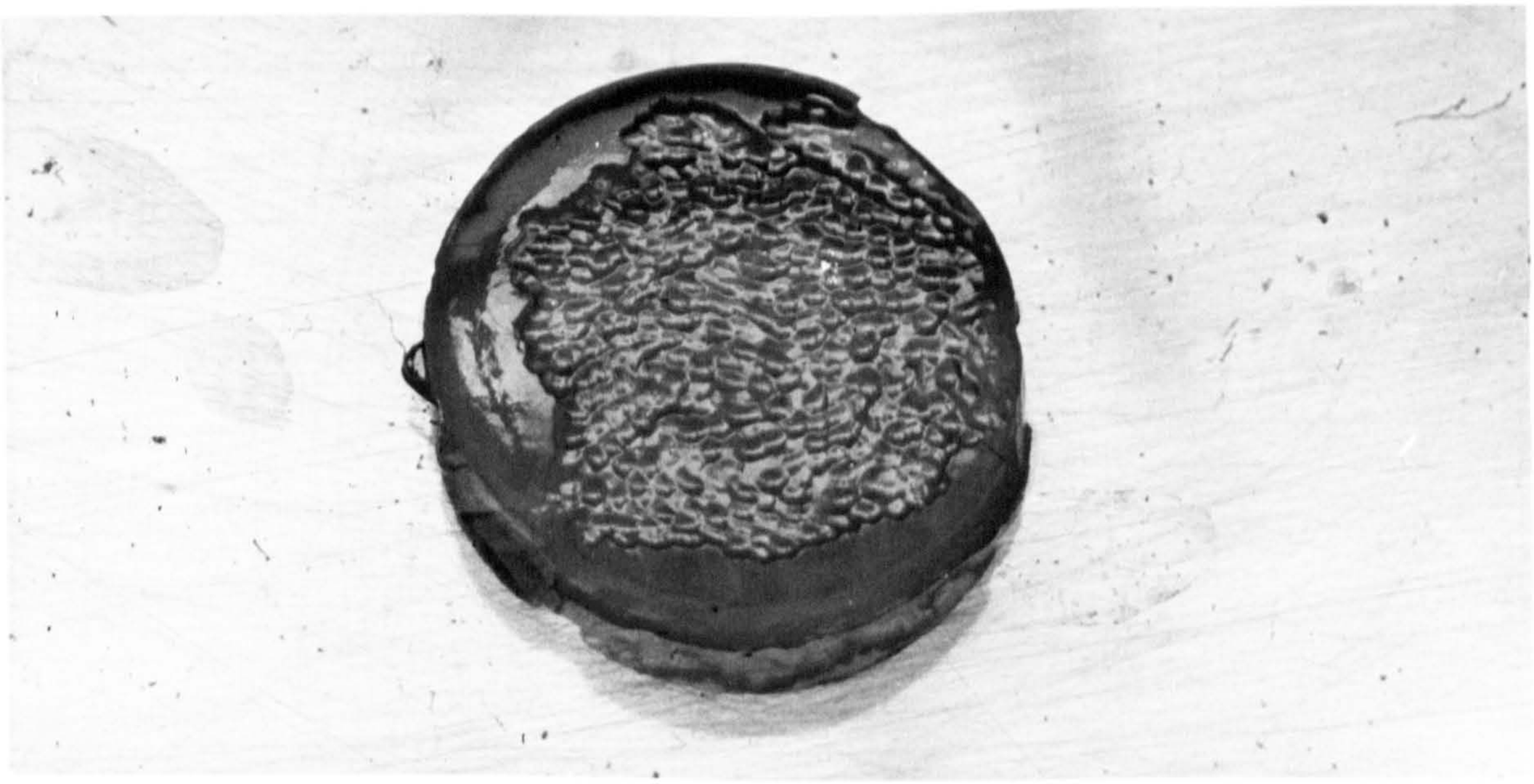
Previous attempts at monitoring the amount eaten by slugs had shown that the animals deposit mucus and faeces on their food as they feed, so determinations of weight loss are subject to inaccuracies. Other problems associated with evaporative loss from the food affected the palatability and treatment of the material also deterred slug feeding. Preliminary tests with agar showed that the amount of water lost from the agar discs due to evaporation was fairly small (7.83 - 16.37%) but there was an occasional weight gain suggesting that the discs may have absorbed water from the moist paper lining the feeding boxes. To overcome the problem of weight variations, a rating system was devised using visual determination of the amount of agar remaining as an index of the extent of feeding. Each disc was therefore scored from 0 - 5 so that 0 was equivalent to no feeding on the disc and 5 indicated that the disc was completely eaten (See Fig 12c). These values were subsequently converted to weight losses using a series of reference weights which had been compiled by artificially reducing 50 agar discs to correspond to the scores 0 - 5. These discs had been weighed at each stage in the reduction

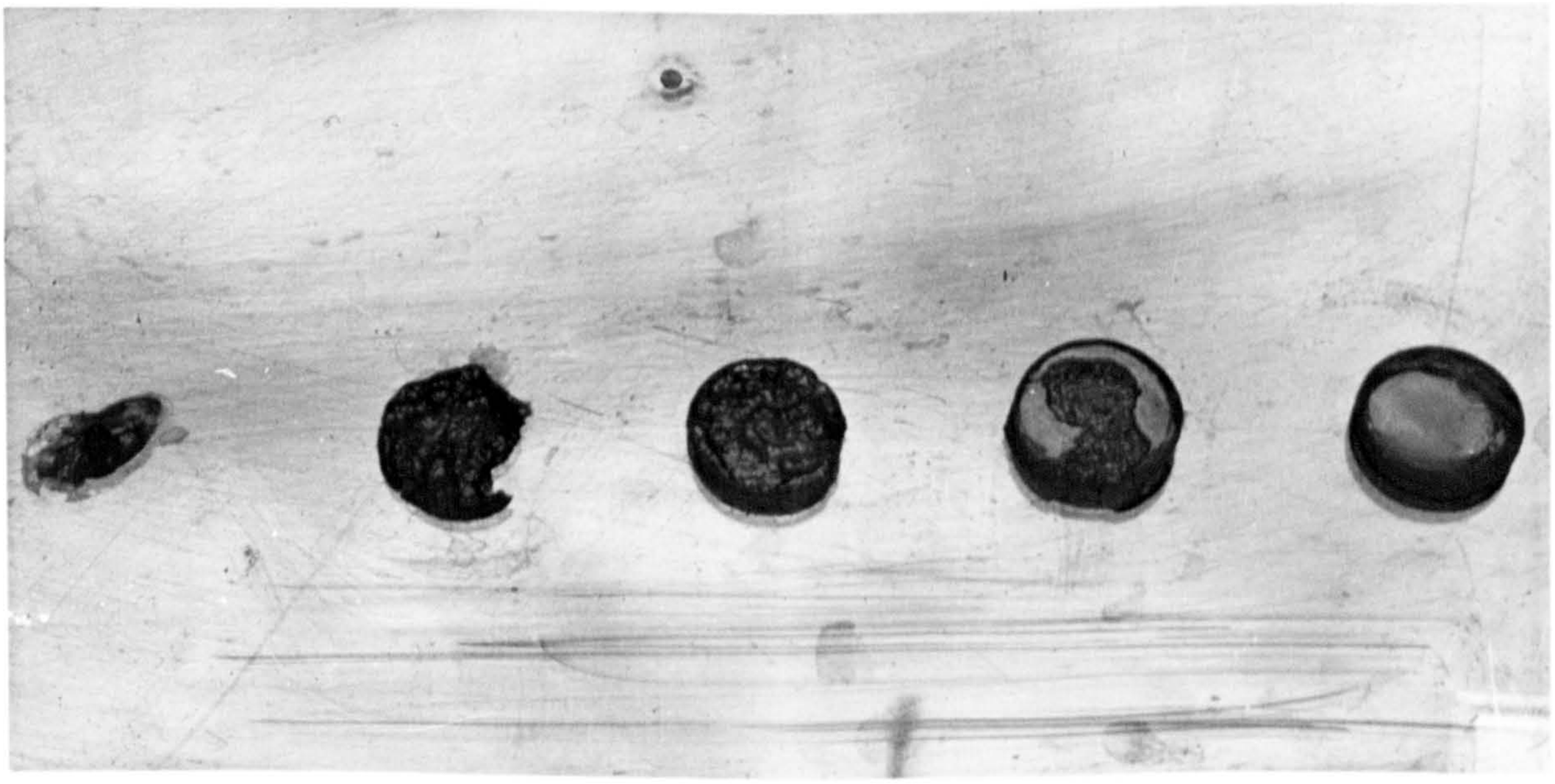
FIG. I2a POSITION OF SLUG AND AGAR DISCS IN FEEDING TEST

FIG I2b RADULA MARKS ON AGAR DISC WHICH INDICATE FEEDING ACTIVITY

FIG I2c AGAR DISCS REPRESENTING FEEDING ACTIVITY







process and an average value for the weight of a disc equivalent to each score obtained :

score 0 -	0.00mg.	(disc uneaten)
1	34.45mg.	
2	79.39mg.	
3	146.09mg.	
4	271.20mg.	
5	370.69mg.	(disc completely eaten)

At the end of a 24 hour feeding test therefore, the weight of disc eaten by each slug could be calculated to determine the attractiveness of various food additives. The main advantage of this feeding bioassay is that the slugs readily eat the material and feeding activity is easily assessed since the radula marks are clearly visible in the agar (See Fig.I2b).

Test animals

Feeding tests were made with adult Deroceras reticulatum when these were available. The animals were collected from several areas in the south of England and were grouped so that individuals of 300 - 600mg. weight were used. Attempts were made to rear slugs in the laboratory from eggs collected in the boxes housing the animals and although a high percentage of eggs hatched, the survival rate was always low. The young slugs were easily parasitised as infection appeared to spread rapidly through a population. During December 1978, feeding tests were made with D. caruanae collected from greenhouses since the cold weather caused D. reticulatum to move underground and they

could not be collected in sufficient quantity to be used in feeding tests. Fig I2a shows the position of food items in a feeding box with an active slug (Deroceras) moving over the moist surface. The photograph was taken at the end of a feeding test so the agar discs have been fed on.

Each test involved ten slugs, housed individually and offered a choice of two food items. In some situations, these foods were the same to determine whether feeding activity was biased as a result of any inherent characteristic of the test. Orientation, for example, was assessed to indicate whether the animals moved preferentially towards one end of the feeding box and therefore ate the food item in that end.

Both the amount of food eaten by each animal and the number of animals exhibiting a feeding response were considered to be important factors since any feeding attractant for use in commercial bait must not only encourage the animals to move towards the bait and feed but also to ingest a large quantity so that a lethal dose of toxicant may be consumed. The amount of food eaten by each slug was obtained by converting the feeding scores to a value which represented weight loss of each disc and the results averaged for the group of ten animals to indicate the extent of feeding on each of the two food items. The term "percent response" was used to record the number of animals which had fed on a particular food - a 70% response would therefore indicate that seven out of the ten animals had fed upon a food. As two food items were offered in each test, food A and food B, a value for the amount of each food eaten and for the percent response of each is included in the results.

The total meal size, food A plus food B, is also indicated.

The contribution of each food to the overall meal size is most easily shown in the ratio A/B, where A is the amount of food item A eaten and B is the amount of food item B eaten.

Where both foods have been eaten to the same extent, a value of 1.0 is obtained - this could be expected where both foods are the same. A highly attractive food A will produce a value in excess of 1.0 and an unpalatable food A or a more attractive food B will produce a value of less than 1.0.

The results from the feeding tests therefore provide three indications of feeding activity:

feeding score - the average amount of food consumed by the ten slugs in each group

percent response - the percentage of slugs which have fed on a disc of this food

A/B value - a ratio to compare consumption of one food item with the other

A small feeding score and a large percent response indicates that most of the animals have sampled the food but the feeding stimulus has not been maintained to encourage the animals to consume a large meal. A small feeding score with a small percent response indicates that the slugs were not motivated to feed and that the diet is unattractive or possible repellent. A large feeding score with a high percent response suggests that the diet is attractive to most or all of the test animals and that a large meal has been consumed indicating high palatability. A similarly

large feeding score with a small response indicates that although the diet is attractive, the animals are not being motivated to feed. This is discussed in the section on food memory (See P I22).

3.3 FEEDING TESTS WITH AN ALTERNATIVE GEL CARRIER

Since the agar feeding test involves heating the solution, volatile and heat degradable materials are difficult to incorporate into the conventional agar mixture. A cold setting agar was therefore sought to enable flammable and inflammable compounds to be formulated into agar sheets and to investigate whether the attraction of food materials was reduced by the normal process of preparation. A sodium alginate based gel - Manugel DLM (Alginate Industries Ltd., Girvan, Ayrshire) - was prepared according to manufacturer's instructions using calcium salts in neutral conditions. Samples were prepared with a bran extract in place of water, as a comparison with the normal bran/agar mixture. Samples of pressed yeast and metaldehyde were also prepared with the cold setting gel - it was hoped that the attraction of metaldehyde could be assessed, using a test where the animals were voluntarily ingesting the toxicant.

The results presented in Table 2 , indicate that this cold setting agar gel is unattractive to slugs, even when combined with a known attractant, bran. Feeding activity was low in the feeding tests where the normal bran/agar mixture was offered as one of the food choices but no feeding occurred in the other tests where both choices

TABLE 2 FEEDING TESTS WITH COLD SETTING AGAR

	FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
	Manugel/Bran		Agar/Bran			
	6.9	20	37.4	50	0.18	44.3
	0.0	0	18.3	40	-	18.3
	0.0	0	45.3	60	-	45.3
$\bar{x}$	2.3	6.67	33.67	50	0.07	35.97
	Manugel/Metaldehyde		Manugel/Bran			
	0.0	0	0.0	0	-	0.0
	0.0	0	0.0	0	-	0.0
	Manugel/Metaldehyde		Manugel/F9			
	0.0	0	0.0	0	-	0.0
	0.0	0	0.0	0	-	0.0

TABLE 3 FEEDING TESTS WITH DIFFERENT BRANS

	FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
	2.5g wheatbran		2.5g bran flakes			
	17.2	50	81.7	100	0.21	98.9
	10.3	30	61.2	80	0.17	71.5
$\bar{x}$	13.75	40	71.45	90	0.19	85.2
S.D.	4.88		14.50			
	5.0g wheatbran		10.0g wheatbran			
	85.9	100	100.6	70	0.78	196.5
	30.7	50	63.4	80	0.48	94.1
$\bar{x}$	58.3	75	82.0	75	0.67	140.3
S.D.	39.03		26.30			
	5.0g wheatbran		5.0g bran flakes			
	104.8	90	181.5	90	0.58	286.3
	28.6	70	131.9	100	0.22	160.5
	10.3	30	163.6	100	0.06	173.9
$\bar{x}$	47.9	63.33	159.0	96.67	0.30	206.9
S.D.	50.12		25.12			

were formulated with the cold setting Manugel. This suggests that the Manugel is repellent to the animals and deters feeding. It was therefore unsuitable for use in a feeding bioassay with Deroceras reticulatum.

3.4 FEEDING TESTS WITH BRAN

The ultimate aim of the feeding tests was to highlight any compounds which could be added to, or replace, the bran commonly used as an attractant in baits. A high quality standard food was necessary to equate with the attractiveness of commercial baits. Bran therefore seemed a suitable food for use in feeding trials but variations between different types of bran were found to occur.

The bran/agar discs used as a standard food in the feeding bioassay were initially formulated by adding 5.0g wheatbran to 100ml water and combining the filtered solution with 1.0g agar powder (P.I02). The set agar sheets could be stored in a constant temperature room (at $4^{o}C$) for approximately seven days without apparent loss of structure or attractiveness to the slugs. Fungal growth on the agar gradually occurred however, as the sheets became contaminated while discs were being cut. Fresh agar sheets were made if deterioration was observed.

Although these wheatbran discs were readily eaten by Deroceras, bran discs formulated from a filtered solution of bran flakes were found to be more palatable. Feeding tests to compare these two types of bran were made. The results are presented in Table 3 and Fig. I3 .

A comparison of a weak extract of wheatbran (2.5g/100ml water) and a similar extract of flakes produced

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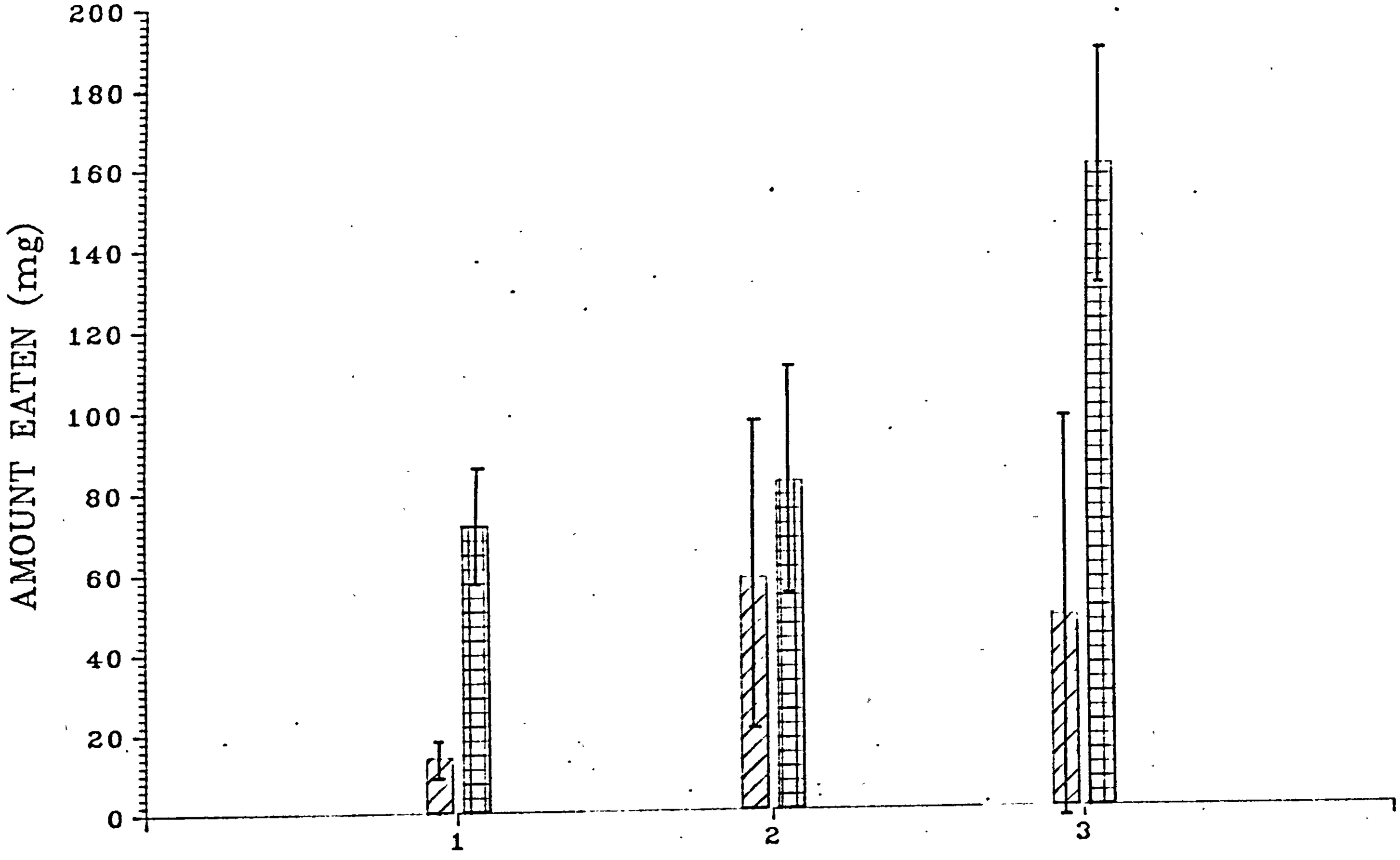
FIG. I3 CONSUMPTION OF FOOD BY Deroceras
TESTS WITH DIFFERENT BRANS

KEY	hatching	squares
x axis	I 2.5g wheatbran	2.5g bran flakes
	2 5.0g wheatbran	IO.Og wheatbran
	3 5.0g wheatbran	5.0g bran flakes

CONSUMPTION OF FOOD BY DEROCERAS
TESTS WITH DIFFERENT BRANS



an average total meal size of 85.2mg and an A/B ratio of 0.19 indicating that the latter food was far more attractive. A similar comparison of these two types of bran at a stronger concentration (5.0g/100ml water) produced an A/B value of 0.30 but the meal size was more than doubled. This large meal size was mainly due to the increase in the amount consumed by each animal since the percent response was already high at the weaker concentration of bran extract. A fairly direct concentration effect was therefore observed with an increase in stimulant concentration producing a corresponding increase in meal size. Comparison of a 5.0g/100ml water and a 10.0g/100ml water extract of wheatbran offered in the same diet did not however show such a direct correlation.

These feeding tests with bran illustrate that an extract of bran flakes (5.0g/100ml water) produces consistently large meal sizes and is highly attractive to Deroceras. Most of the feeding tests to compare attractants, which are presented in Section 4, were made with the original bran standard of 5.0g wheatbran. Since a standard food of the highest quality was sought, later tests were made with an extract of the more attractive bran flakes as a standard. The type of bran used is clearly stated in each test.

The difference between the two types of bran reported here may account for the variability between different commercial bait formulations which is reported in Section 5. Differences in the bran used as a carrier for the toxicant could be responsible for the range in

efficiency estimates recorded in the field since slugs appear to consume smaller amounts of a less attractive food and may therefore ingest a sub-lethal does of baits containing poor attractants.

3.5 FEEDING AND MOLLUSCICIDE FORMULATION

The two alternatives to improving bait efficiency are either to increase the concentration of toxicant or to try to increase the amount of bait eaten by each animal. The former approach may be limited by environmental and legislative controls as toxicity values for non-target organisms are continually reassessed. Increased toxicant concentration also increases production costs so attempts to improve the attractive qualities of the bait have been made. The objective of the feeding tests with Deroceras was to manipulate the amount eaten by the addition of suitable attractants. The variations in meal size can be related to the amount of bait eaten by slugs and the quantity of toxicant consumed in each meal correspondingly altered to improve the effectiveness of the bait.

A large surplus or margin of safety is allowed in the toxicant concentration of each pellet as a slug is unlikely to ingest the entire pellet. The time-lapse recordings have shown that a slug does not feed continuously for a long period, but rather feeds for several minutes on one food item and moves on until it encounters another. The slug therefore may ingest a sub-lethal does each time it feeds. Table 4 illustrates the amount of toxicant ingested when increasing weights of a carrier containing a known percentage of

TABLE 4 AMOUNT OF TOXICANT CONSUMED WITH INCREASING INTAKE OF FOOD

PERCENT CONCENTRATION OF TOXICANT	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200
1	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0
2	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0
3	0.3	0.6	0.9	1.2	1.5	1.8	2.1	2.4	2.7	3.0	3.3	3.6	3.9	4.2	4.5	4.8	5.1	5.4	5.7	6.0
4	0.4	0.8	1.2	1.6	2.0	2.4	2.8	3.2	3.6	4.0	4.4	4.8	5.2	5.6	6.0	6.4	6.8	7.2	7.6	8.0
5	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
6	0.6	1.2	1.8	2.4	3.0	3.6	4.2	4.8	5.4	6.0	6.6	7.2	7.8	8.6	9.0	9.6	10.2	10.8	11.4	12.0
7	0.7	1.4	2.1	2.8	3.5	4.2	4.9	5.6	6.3	7.0	7.7	8.4	9.1	9.8	10.5	11.2	11.9	12.6	13.3	14.0
8	0.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8.0	8.8	9.6	10.4	11.2	12.0	12.8	13.6	14.4	15.2	16.0
9	0.9	1.8	2.7	3.6	4.5	5.4	6.3	7.2	8.1	9.0	9.9	10.8	11.7	12.6	13.5	14.4	15.3	16.2	17.1	18.0
10	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.0	15.0	16.0	17.0	18.0	19.0	20.0
11	1.1	2.2	3.3	4.4	5.5	6.6	7.7	8.8	9.9	11.0	12.1	13.2	14.3	15.4	16.5	17.6	18.7	19.8	20.9	21.0
12	1.2	2.4	3.6	4.8	6.0	7.2	8.4	9.6	10.8	12.0	13.2	14.4	15.6	16.8	18.0	19.2	20.4	21.6	22.8	24.0

WEIGHT OF FOOD EATEN mg.

BOLD AREA REPRESENTS APPROXIMATELY 100 TIMES THE M.L.D. BAIT FORMULATION SHOULD AIM TO ACHIEVE INGESTION OF THIS AMOUNT OF TOXICANT TO ENSURE MAXIMUM EFFICIENCY.

metaldehyde are eaten. Increasing the amount of food consumed will correspondingly increase the amount of toxicant consumed with the food. A slug eating 40mg bait would therefore take in 2.0mg of toxicant. Using Henderson's estimate (1968) of 8.53µg (0.085mg) as the median lethal dose (M.L.D.) of metaldehyde, a slug would ingest 23.5 times the M.L.D. in this 40g of food. As the concentration of toxicant increases, the margin of safety is also increased so that a slug ingesting 100mg of this 5% bait would take in nearly 60 times the M.L.D.

The marked area in Table 4 represents approximately 8.5mg toxicant, which is 100 times the M.L.D. of metaldehyde. It is apparent that at higher concentrations of toxicant, a smaller meal size is sufficient to kill the animals.

Commercial bait pellets weigh between 20 and 30mg - these are the mini pellets now marketed by most manufacturers. Each pellet of a standard 5 - 6% metaldehyde formulation therefore provides 15 - 18 times the M.L.D.. The weaker (2.0 - 2.5%) baits used in some parts of the U.S.A. provide only 6 - 7 times the M.L.D. in each pellet. The safety margin is justified by the fact that several slugs will hopefully be poisoned by each pellet. Slugs are known to have an aggregated distribution and some may follow slime trails which can lead them to feed at the same site. The excess metaldehyde in each bait over and above that necessary to kill one slug can thus be reinterpreted as the number of slugs capable of being killed by one pellet.

Application rates can be increased with large slug pest populations, but the main objective remains:to ensure that the slugs ingest sufficient bait to die.

Williams (1972) examined bait consumption in three species of slug - Deroceras reticulatum, Milax budapestensis and Arion hortensis - using a chromic oxide food marker. He found that the addition of any amount of molluscicide to food caused a decrease in the amount eaten. The concentration of toxicant could be varied between 1% and 6% without causing any marked effect on bait consumption though there was a slight tendency for the consumption to increase at higher concentrations of molluscicide. Williams therefore recommended that a 4 - 6% bait be used to compensate for any reduction in bait consumption caused by the presence of the toxicant.

3.6 MEAL SIZE

Several factors may influence meal size in molluscs. Gelperin (1977) has suggested that gut stretch receptors are the most critical element determining meal size. He offered Limax maximus two different diets simultaneously and found that the volume of food ingested in one hour was approximately the same regardless of whether they ate all of one food or some of each. Neurophysiological recordings of feeding in this species indicated that a typical meal contains 552 ± 240 bites, with increased bite frequency in the first 100 bites.

Richter (1979) found that the large American slug, Ariolimax columbianus, had an average laboratory

consumption of 165mg oven dry weight per day on leaves of the fern Pteridium equilinum and Red Alder, Alnus rubra, with a preference for older leaves. Meal size of Deroceras was investigated by Pallant (1970a) who fed slugs on one of their natural foods - the creeping buttercup, Ranunculus repens,- and calculated that an average individual of 254.6mg live weight eats 60.9mg wet weight of food in 24 hours. Williams (1972), using a dry flour based diet, obtained a value of 33 - 35mg per day as the meal size for Deroceras but the difference in water content in the two foods may account for the discrepancy.

Senseman (1977a) indicated in laboratory tests with Ariolimax californicus that food hardness is important in determining meal duration but that meal size is relatively constant and limited by post-ingestional feedback from the gut stretch receptors. Increasing meal hardness causes a decrease in consumption rate and restricts meal size. Under these conditions, the concentration of feeding stimulant determines meal size. Slugs can discriminate between diets containing different concentrations of starch (Senseman 1977b) and apparently prefer pure starch to natural potato - Senseman suggests that this may be due to antifeedants in the potato. His investigations with the snail Helisoma (1977c) have indicated that short term cues such as meal size and long term cues such as nutritional adequacy control satiety and that learning aversions can modify the palatability of a food. The concept of a snail being capable of assessing the nutritional adequacy of its food is perhaps less easy to prove than the ability to

regulate food intake on a short term basis.

Although slugs can distinguish and select different food materials on the basis of odour, it is unclear whether they can differentiate individual components of their food. If this were possible, the animals may, as Senseman suggests, be able to select a nutritiously balanced diet but there is no evidence to support this hypothesis and it is unlikely that the nervous system of slugs is sufficiently well developed to select food items on the basis of nutritional content. It is more probable that slugs are able to learn food preferences so that they may feed at a later date on a food material which has proved palatable in the past. The work on food memory which follows indicates that slugs do need a number of different stimuli as they adapt to a food if offered to them on consecutive days and this acclimatisation to one food may be the means by which the animals ensure that they consume a balanced diet. In the field the animals have a wide choice of food and an inadequately balanced diet is improbable though adaptation to one food could encourage the animals to feed on all the available plants.

3.7 LEARNING IN MOLLUSCS

Molluscs appear to be capable of behavioural patterns often associated with higher animals. The freshwater pulmonate, Physa, was shown to be sensitive to a series of electric shocks but seemed incapable of conditioned responses involving association of a stimulus (Wells & Wells 1971). The gill-withdrawal response of

Aplysia has similarly been used to examine the learning process (Davis 1976) though any response which is persistently shown in reply to a standard stimulation is suitable. Davis suggests that molluscs are capable of behavioural plasticity - the ability to modify their behaviour according to experience acquired during a lifetime - and he outlines his strategy for the neuronal analysis of plasticity.

The gastropod nervous system incorporates a peripheral nerve net into the central nervous system (CNS) which is capable of mediating responses, often without the influence of the CNS - isolated gastropod tissue still has the capacity to respond to stimuli. Willows (1973) has indicated that this peripheral net may be the neuroanatomical site for habituation but neuro-physiological studies on gastropods are not yet capable of assigning any definite functions to identified cells. Behavioural studies do, however, indicate that these animals are able to respond to particular situations in a manner which suggests that they can utilise previous experience to their advantage. They are capable of sensitisation and habituation - the balance between these two must be tuned to prevent unnecessary arousal to unimportant stimuli.

Much work has recently been done on the behavioural neurophysiology of molluscs. In particular, the large Californian banana slug, Ariolimax californicus and Limax maximus have provided the basis for studies of neural activities associated with feeding. Learning and conditioning in molluscs has generally been investigated by the administration of aversive stimuli. Limax maximus, for example, can learn

to avoid a new palatable food if CO_2 poisoning is paired with this food (Gelperin 1975). This has been extended (Gelperin & Forsythe 1976) to involve yoked controls where the aversive stimulus is given in conjunction with a palatable food to only one of a pair of animals. The other animal receives the CO_2 poisoning after a time lapse of three hours so that there is no association with the food and thus provides a control to indicate the normal meal size. Intake of a palatable food (mushroom) was depressed from the second day in the group receiving food and poisoning at the same time and remained at this low level for ten days. Gelperin & Forsythe suggest an adaptive value in learning taste aversions and acceptable foods, especially in a species such as Limax which may live for 3 - 4 years and would benefit by the increased fitness to be obtained from learned modifications in food preferences.

Stephens & McGaugh (1972) examined learning and forgeting in the snail Helix aspersa by designing a behavioural assay in which the animals were trained to climb glass rods moistened with quinine (aversive stimulus) or water (neutral stimulus). The gravitational pull of the shell motivated the snails to climb and the latency in the climbing times provided a comparison between the two groups and between successive tests. Both long and short term memory were shown for the aversive stimulus and habituation to the neutral stimulus occurred, though the authors indicated that factors such as the length of laboratory housing and the reproductive and nutritional state of the animals appeared to influence the results. This experiment was replicated by Siegel & Jarvik (1974) who also found that

the snails could learn to avoid quinine but that the avoidance response persisted longer when an electric shock was used as the stimulus. Suzuki (1968) working with Ezohelix flexibilis, paired celery with punishment so that celery became repellent to the snails, and recorded that the avoidance reaction lasted 50 days.

It has been established that newly hatched Achatina fulica fed on a monophagous diet will subsequently orientate to this food in preference to a novel food. Preliminary experiments (Croll & Chase 1977a) suggested that a 48 hour exposure to food leads to a 20 day retention for the odour preference. Further work (Croll & Chase 1977b) indicated that snails fed on either carrot or cucumber for 86 days and then exposed to an alternating cycle of starvation and lettuce feeding, retained a preference for the original food for 120 days. The authors suggest that the snails are capable of a high degree of plasticity in their food finding behaviour and that there is an adaptive value in staying on a diet which has proved palatable in the past.

A food memory therefore seems to be a possible feature of gastropod nervous system. Experiments to condition the animals have relied almost exclusively on the administration of aversive stimuli and punishment. Feeding tests with Deroceras had indicated the possibility of a food memory so a controlled test was devised to investigate learning in the context of feeding. This provided an opportunity to examine the faculty of learning in a gastropod species without the complication of a shock treatment.

3.8 FOOD MEMORY EXPERIMENTS

Feeding tests in which agar discs were offered to Deroceras, Section 4, had indicated several discrepancies since a diet which was normally attractive could seemingly lose its potential to stimulate feeding. This could either be due to the fact that the animal habituated to the diet or to the fact that the attractive properties of the food declined over a number of tests. Although there was some evidence that a compound formulated into an agar sheet became less attractive after a few days, the original feeding activity should be capable of being restored by offering freshly prepared samples to the animals. Attempts to do this were unsuccessful indicating that the slugs had habituated to the diet and that feeding had declined after repeated exposure to the food. Some slugs had been used in more than one feeding test suggesting that a food memory may be involved but there had always been a rest period of at least 24 hours between tests.

A trial was therefore devised to compare feeding activity in adult Deroceras maintained under constant conditions on a laboratory prepared diet which was known to be attractive and nutritionally adequate and which could be easily assayed for feeding activity. Two agar diets were prepared, one with a bran extract and the other with a solution of pressed yeast obtained from a brewery - these two foods had been shown (see Section 4) to provide a suitable food material in that the slugs readily ate the discs and since egg laying was not inhibited it can be assumed that the diet is nutritionally adequate for periods of several weeks.

The feeding protocol was similar to other feeding tests - thirty freshly collected Deroceras were divided into three groups and placed in individual feeding boxes with the appropriate diet for 24 hours. The food was changed daily and the boxes cleaned at the same time. Feeding discs which had been in the boxes were scored for attractiveness by assigning to one of the six categories which represented the volume of food remaining after 24 hours and the results converted to a weight loss. The experiment was terminated on day 31 as two dead slugs were found and the determination of a food memory depended on the continued use of the same animals.

It was necessary to use two different batches of pressed yeast-F9 (from Fuller's Brewery) and W8 (from Watney-Mann Brewery) – since the first batch began to deteriorate after one week. A portion of the W8 sample was freeze dried for use in the rest of the trial. The standard wheatbran discs used for the first ten days were replaced by the more attractive bran flake discs on day 11. There was therefore some variation in the basic foods.

Feeding tests to compare the batches of pressed yeast had indicated that W8 was possibly more attractive than F9 - in a trial where slugs were offered a choice of these two foods, the average weight eaten was 50.45mg of the F9 and 84.65mg of the W8. The difference between the two brans was more marked with an average meal size of 47.9mg for the wheatbran and 159.0mg for the bran flakes when they were offered in combination.

TABLE 5 FOOD MEMORY EXPERIMENTS - GROUP A

Consumption of agar based diet by ten Deroceras over a 31 day period

Day	Amount eaten mg	Percent response	Amount eaten mg	Percent response	Total eaten mg	A/B
	FOOD A - Bran		FOOD B - F9 Yeast			
1	120.8	90	72.4	80	193.2	1.67
2	67.7	50	30.7	50	98.4	2.21
3	80.3	90	13.8	40	94.1	5.82
4	29.7	60	14.8	30	44.5	2.02
5	11.4	20	14.6	10	26.0	0.78
6	34.2	60	14.8	30	49.0	2.31
7	37.6	70	23.8	30	61.4	1.58
	FOOD A - Bran		FOOD B - W8 Yeast			
8	11.4	20	30.5	30	41.9	0.37
9	29.4	40	59.7	50	89.1	0.49
10	7.9	10	59.7	50	67.6	0.13
11	180.4	100	82.2	50	262.6	2.19
12	163.2	90	39.7	50	202.9	4.11
13	133.2	80	45.1	40	178.3	2.95
14	87.0	80	53.0	50	140.0	1.64
	FOOD A - Bran		FOOD B - W8 Yeast			
15	88.4	80	67.6	60	156.0	1.31
16	80.2	50	54.3	60	134.5	1.48
17	46.4	60	49.8	50	96.2	0.93
18	47.6	60	74.6	70	122.2	0.64
19	74.4	60	99.3	60	173.7	0.75
20	57.6	40	47.6	60	105.2	1.21
21	38.6	60	43.1	60	81.7	0.90
DAYS 22 - 28 - Starvation period						
	FOOD A - Bran		FOOD B - W8 Yeast			
29	34.2	60	42.1	70	76.3	0.81
30	18.3	40	54.5	80	72.8	0.58
31	45.1	40	50.0	80	95.1	0.90

TABLE 6 FOOD MEMORY EXPERIMENTS - GROUP B

Consumption of agar based diet by ten Deroceras over a 31 day period

Day	Amount eaten mg	Percent response	Amount eaten mg	Percent response	Total eaten mg	A/B
	FOOD A - Bran		FOOD B - Bran			
1	29.7	60	91.5	80	121.2	0.32
2	10.3	30	29.7	60	40.0	0.35
3	21.7	50	26.2	50	47.9	0.83
4	10.3	30	7.9	10	18.2	1.30
5	6.9	20	6.9	20	13.8	1.00
6	3.4	10	3.4	10	6.8	1.00
7	6.9	20	0.0	0	6.9	-
	FOOD A - Bran		FOOD B - W8 Yeast			
8	0.0	0	205.3	100	205.3	-
9	0.0	0	121.9	100	121.9	-
10	0.0	0	128.5	100	128.5	-
11	59.9	70	189.4	100	249.3	0.32
12	19.3	30	73.6	90	92.9	0.26
13	15.9	20	98.3.	90	114.2	0.16
14	19.3	30	73.6	90	92.9	0.26
	FOOD A - Bran		FOOD B - Bran			
15	27.3	40	49.8	60	77.1	0.55
16	41.9	50	26.2	50	68.1	1.60
17	41.9	40	18.3	40	60.2	2.29
18	19.3	30	45.4	50	64.7	0.43
19	10.3	30	33.9	40	44.2	0.30
20	45.3	60	37.4	50	82.7	1.21
21	30.7	50	15.9	20	46.6	1.93
DAYS 22 - 28 - Starvation period						
	FOOD A - Bran		FOOD B - W8 Yeast			
29	11.4	20	65.9	100	77.3	0.17
30	14.8	30	62.5	90	77.3	0.24
31	11.4	20	58.0	90	69.4	0.20

TABLE 7 FOOD MEMORY EXPERIMENTS - GROUP C

Consumption of agar based diet by ten Deroceras over a 31 day period

Day	Amount eaten mg	Percent response	Amount eaten mg	Percent response	Total eaten mg	A/B
	FOOD A - F9 Yeast		FOOD B - F9 Yeast			
1	95.8	90	74.5	80	170.3	1.29
2	18.3	40	21.7	50	40.0	0.84
3	37.6	70	37.6	70	75.2	1.00
4	21.7	50	14.8	30	36.5	0.12
5	11.4	20	25.2	60	36.6	0.45
6	26.2	50	14.8	30	41.0	1.77
7	30.7	50	22.8	40	53.5	1.35
	FOOD A - Bran		FOOD B - W8 Yeast			
8	71.1	70	19.3	30	90.4	3.68
9	90.3	70	27.3	40	117.6	3.31
10	52.0	60	29.7	60	81.7	1.75
11	153.6	80	71.1	40	224.7	2.16
12	75.6	70	22.8	40	98.4	3.32
13	133.3	90	14.8	30	148.1	9.01
14	96.0	80	17.2	50	113.2	5.58
	FOOD A - W8 Yeast		FOOD B - W8 Yeast			
15	18.3	40	18.3	50	36.6	1.00
16	56.5	60	38.6	60	95.1	1.46
17	27.3	40	41.9	50	69.2	0.65
18	77.8	70	30.7	50	108.5	2.53
19	18.3	40	71.3	90	89.6	0.26
20	62.5	90	53.3	70	115.8	1.17
21	41.0	80	22.8	40	63.8	1.80
DAYS 22 - 28 - Starvation period						
	FOOD A - Bran		FOOD B - W8 Yeast			
29	20.7	60	56.7	80	77.4	0.37
30	17.2	50	21.7	50	38.9	0.79
31	22.8	40	29.7	50	52.5	0.77

FIG I4 TOTAL AMOUNT OF AGAR DIET EATEN BY SLUGS ON CONTROLLED DIETS

CHOICE OF FOOD OFFERED TO EACH GROUP:

DAY	GROUP A	GROUP B	GROUP C
I - 7	wheatbran + pressed yeast	wheatbran + wheatbran	pressed yeast pressed yeast
8 - IO	wheatbran + pressed yeast	wheatbran + pressed yeast	wheatbran + pressed yeast
II - I4	bran flakes + pressed yeast	bran flakes + pressed yeast	bran flakes + pressed yeast
I5 - 2I	bran flakes + pressed yeast	bran flakes + bran flakes	pressed yeast + pressed yeast
22 - 28	starved	starved	starved
29 - 3I	bran flakes + pressed yeast	bran flakes + pressed yeast	bran flakes + pressed yeast

TOTAL AMOUNT OF AGAR DIET EATEN PER 24 HOURS
AVERAGE FOR TEN SLUGS

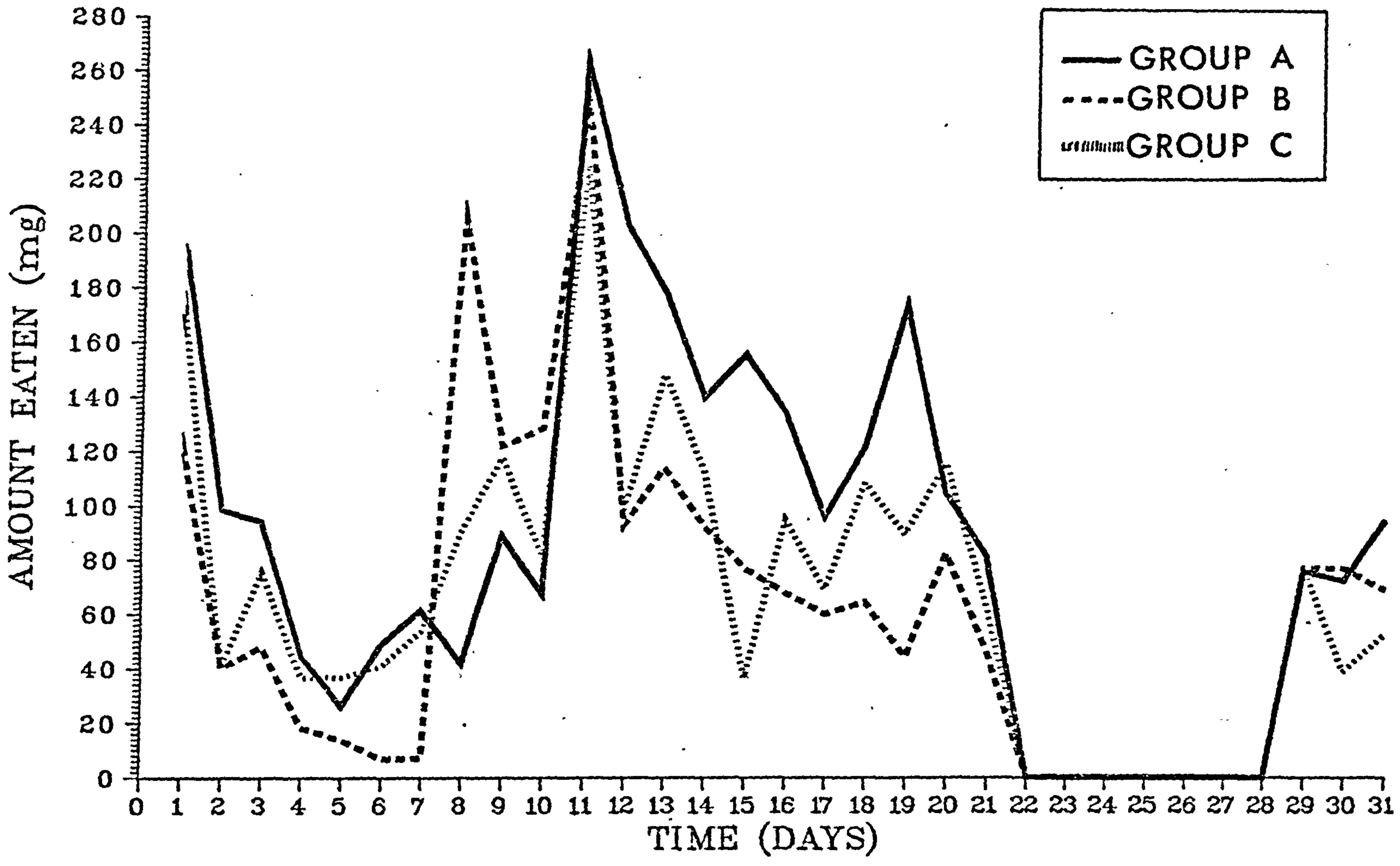


FIG. I5 TOTAL AMOUNT OF AGAR DIET EATEN BY SLUGS IN GROUP A

DAY	FOOD A	FOOD B
I - 7	wheatbran	F9 yeast
8 - IO	wheatbran	W8 yeast
II - I4	bran flakes	W8 yeast
I5 - 2I	bran flakes	W8 yeast
22 - 28	starved	
29 - 3I	bran flakes	W8 yeast

GROUP A TOTAL AMOUNT OF AGAR DIET EATEN PER 24 HOUR AVERAGE FOR 10 SLUGS.

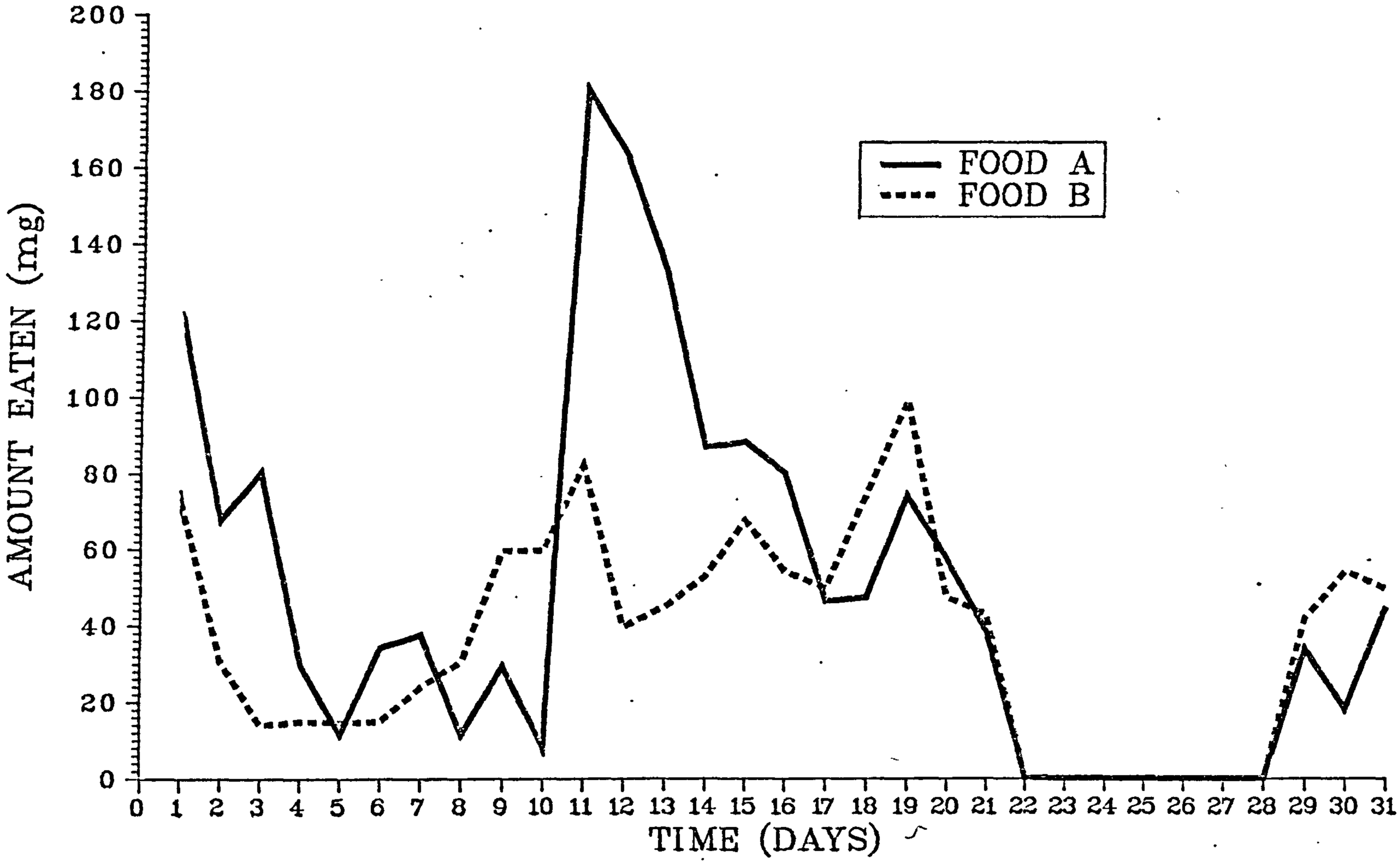


FIG. I6 TOTAL AMOUNT OF AGAR DIET EATEN BY SLUGS IN GROUP B

DAY	FOOD A	FOOD B
I - 7	wheatbran	wheatbran
8 - IO	wheatbran	W8 yeast
II - I4	bran flakes	W8 yeast
I5 - 2I	bran flakes	bran flakes
22 - 28	starved	
29 - 3I	bran flakes	W8 yeast

GROUP B TOTAL AMOUNT OF AGAR DIET EATEN PER 24 HOUR AVERAGE FOR 10 SLUGS

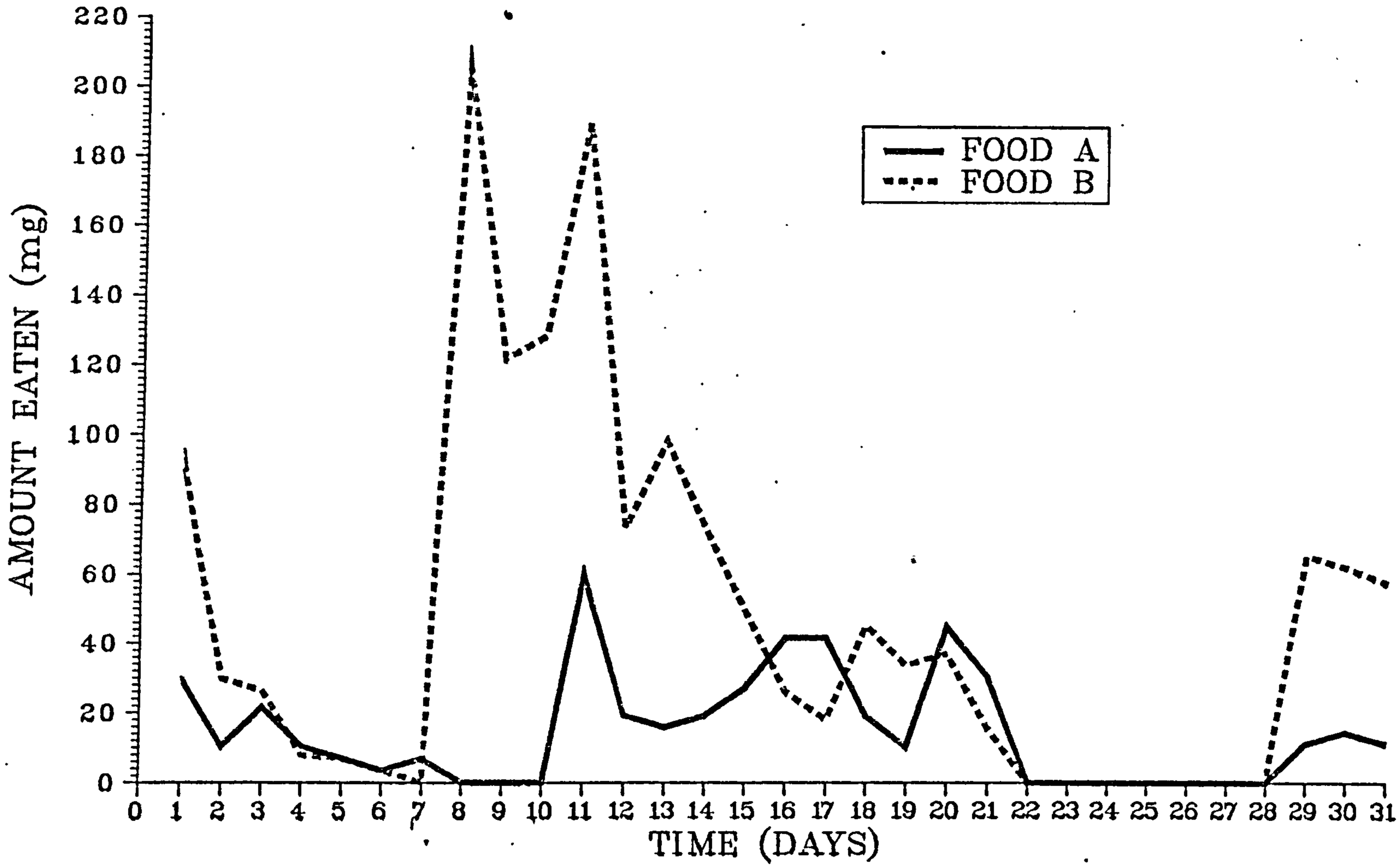


FIG. I7 TOTAL AMOUNT OF FOOD EATEN BY SLUGS IN GROUP C

DAY	FOOD A	FOOD B
I - 7	F9 yeast	F9 yeast
8 - IO	wheatbran	W8 yeast
II - I4	bran flakes	W8 yeast
I5 - 2I	W8 yeast	W8 yeast
22 - 28	starved	
29 - 3I	bran flakes	W8 yeast

GROUP C TOTAL AMOUNT OF AGAR DIET EATEN PER 24 HOUR AVERAGE FOR 10 SLUGS

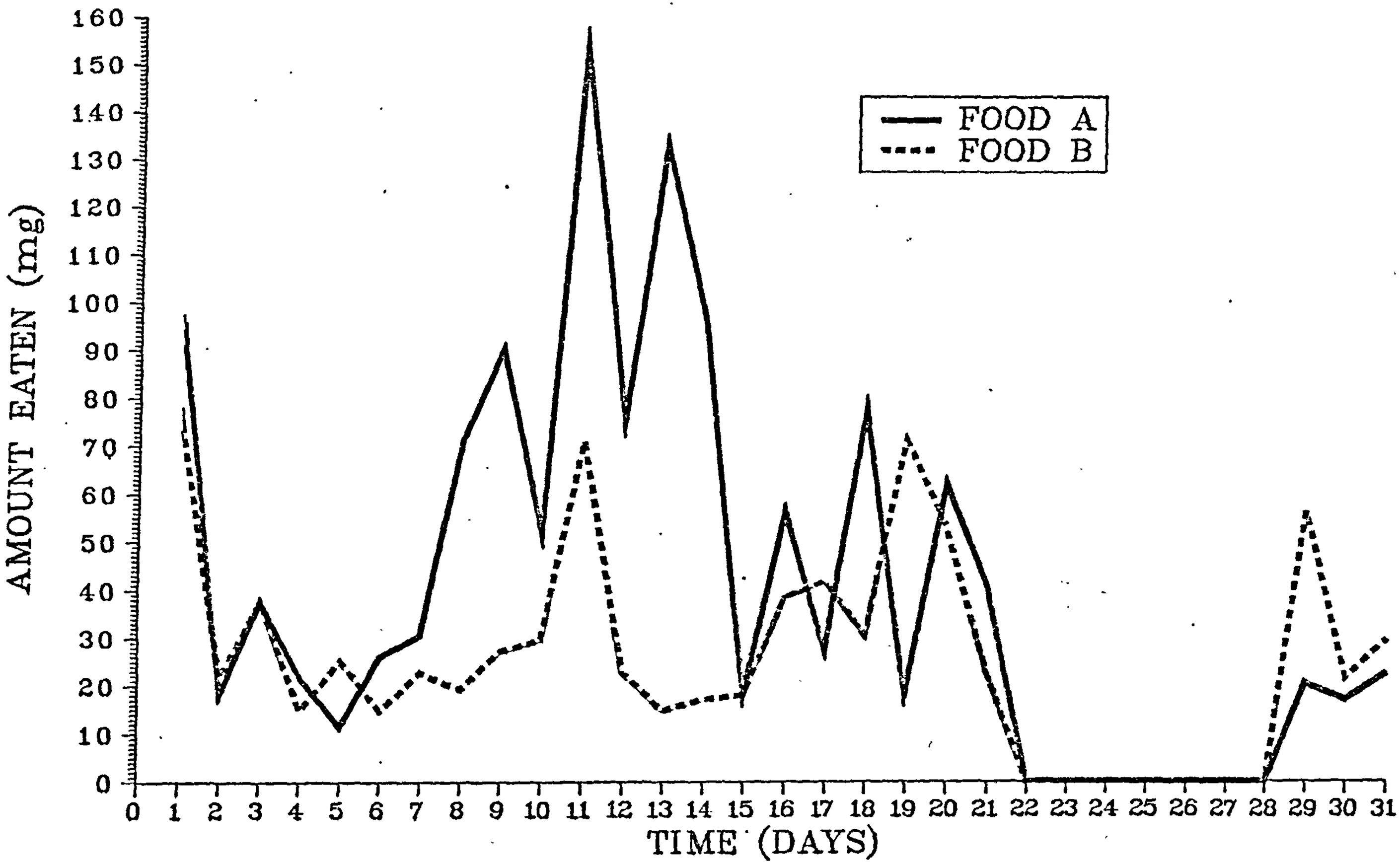


FIG. I8 PERCENTAGE RESPONSE OF SLUGS TO EACH FOOD - GROUP A (control group)

DAY	FOOD A	FOOD B
I - 7	wheatbran	F9 yeast
8 - IO	wheatbran	W8 yeast
II - I4	bran flakes	W8 yeast
I5 - 2I	bran flakes	W8 yeast
22 - 28	starved	
29 - 3I	bran flakes	W8 yeast

GROUP A – PERCENTAGE RESPONSE OF SLUGS TO EACH FOOD

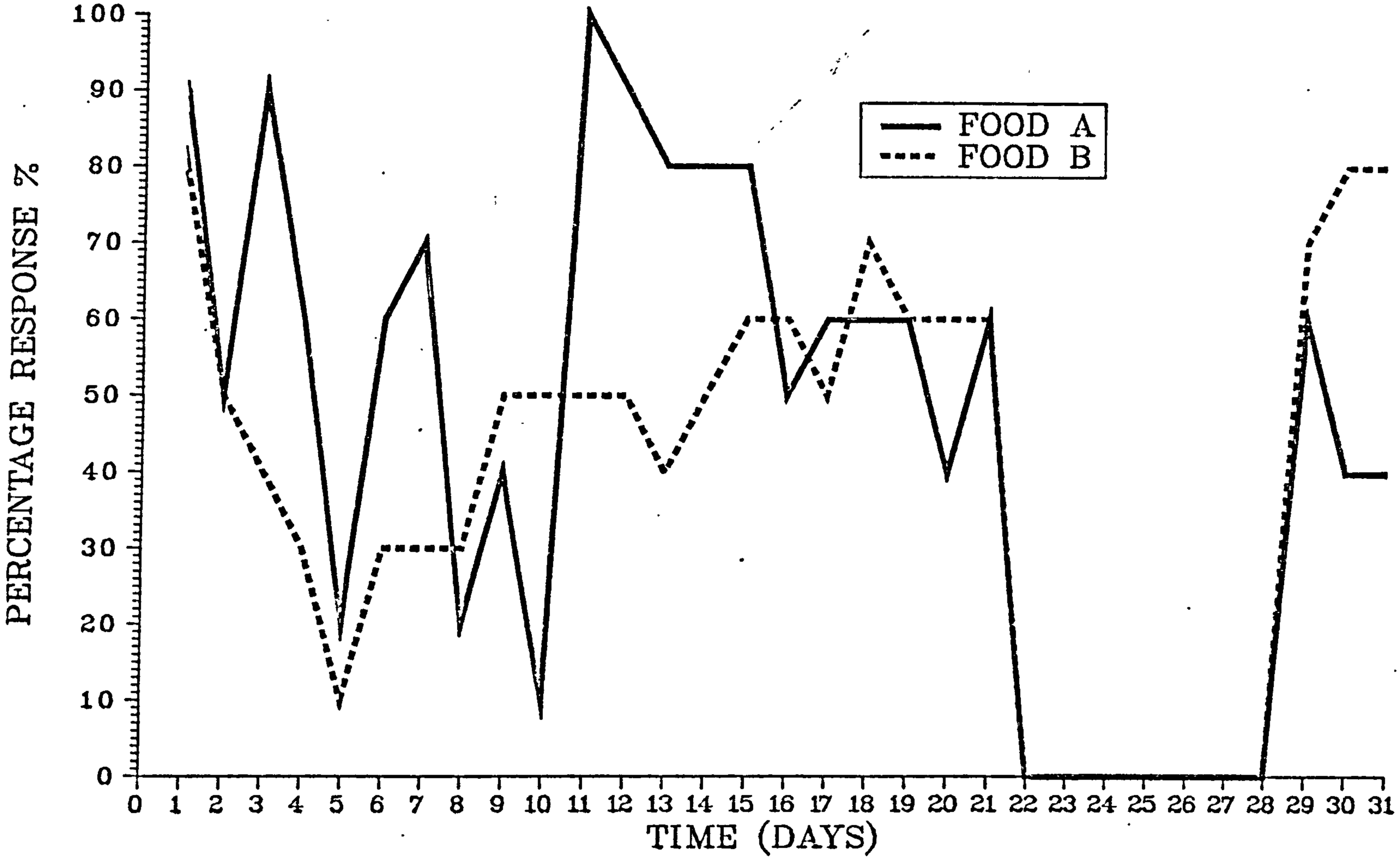


FIG. I9 PERCENTAGE RESPONSE OF SLUGS TO EACH FOOD - GROUP B ('bran' group)

DAY	FOOD A	FOOD B
I - 7	wheatbran	wheatbran
8 - IO	wheatbran	W8 yeast
II - I4	bran flakes	W8 yeast
I5 - 2I	bran flakes	bran flakes
22 - 28	starved	
29 - 3I	bran flakes	W8 yeast

GROUP B – PERCENTAGE RESPONSE OF SLUGS TO EACH FOOD

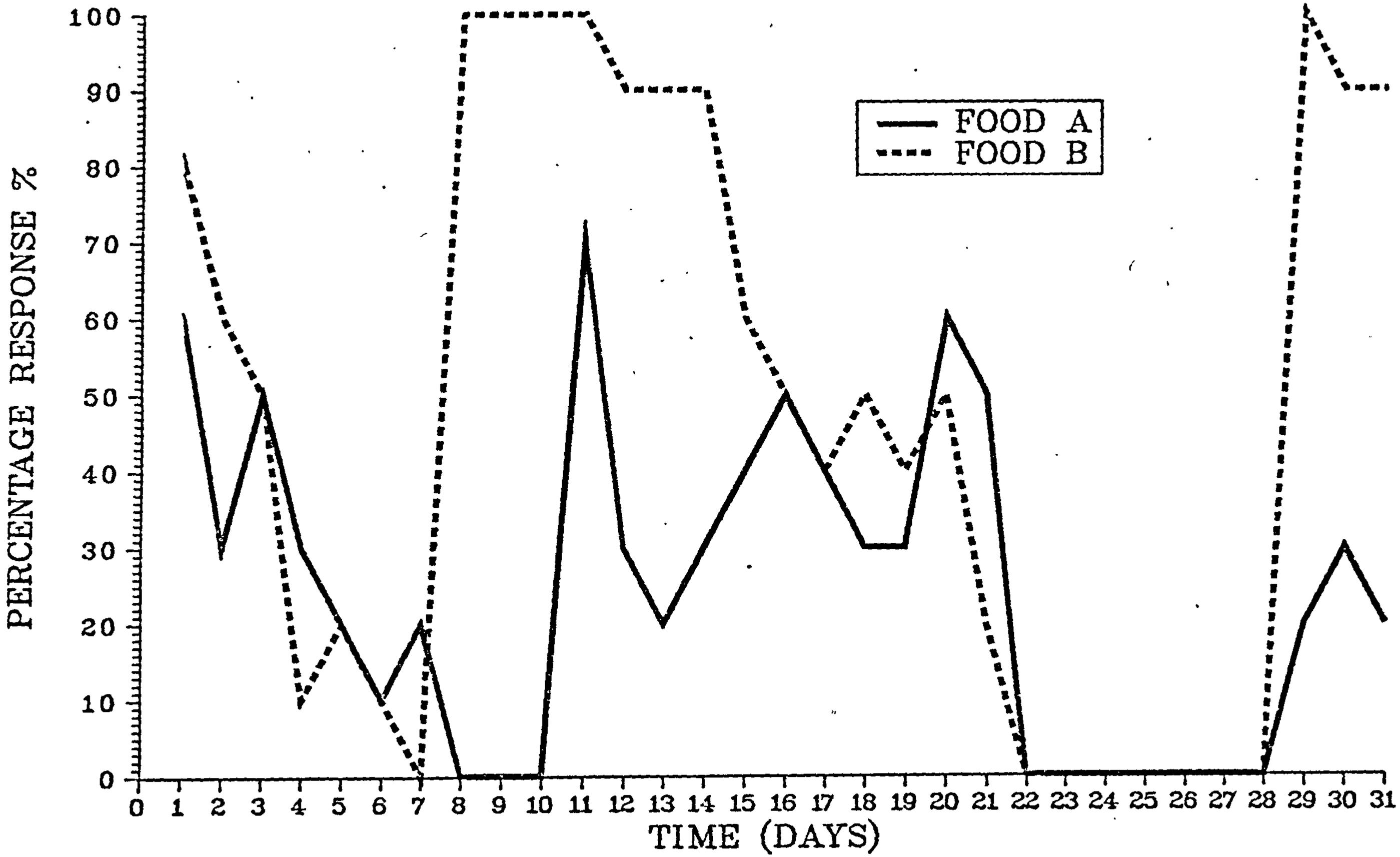
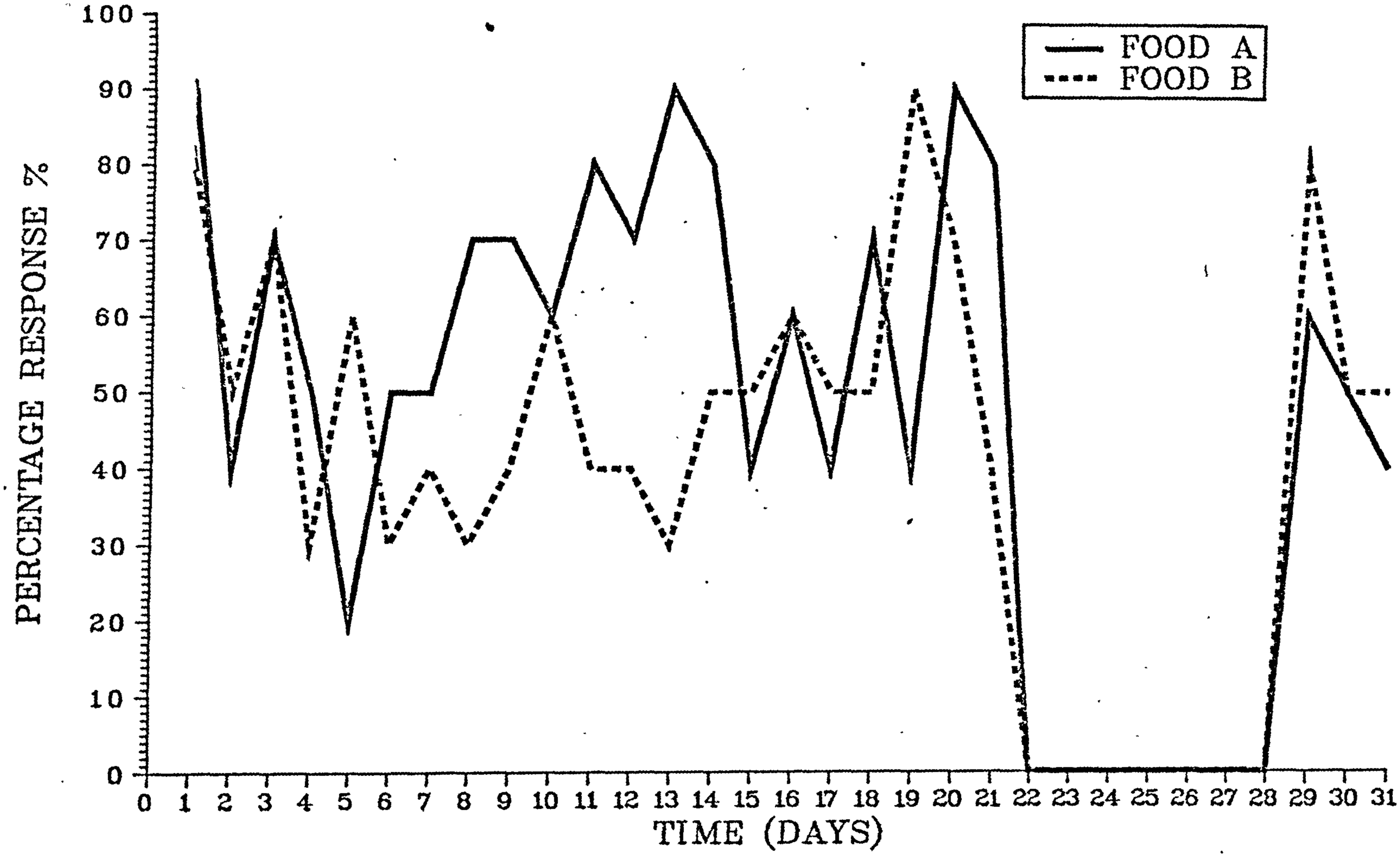


FIG. 20 PERCENTAGE RESPONSE OF SLUGS TO EACH FOOD - GROUP C ('yeast' group)

DAY	FOOD A	FOOD B
I - 7	F9 yeast	F9 yeast
8 - IO	wheatbran	W8 yeast
II - I4	bran flakes	W8 yeast
I5 - 2I	W8 yeast	W8 yeast
22 - 28	starved	
29 - 3I	bran flakes	W8 yeast

GROUP C – PERCENTAGE RESPONSE OF SLUGS TO EACH FOOD



The three groups of animals were designated A, B, and C. Group A animals received the same choice of food throughout the experiment and thus provided a control series of meal sizes. The diet of groups B and C was altered at intervals of seven days to assess the effect of introducing a novel food. All three groups of slugs were starved from days 22 - 28 to compare the effects of food deprivation on the control group, A, and the two groups, B and C, for which the diet was altered.

Table 5 shows the amount of each food eaten by the ten animals in group A over the 31 day test period, together with the percent response and the A/B value for each day. Tables 6 and 7 present the same information for groups B and C respectively.

Fig. I4 shows the total meal size for each group per day and indicates a marked decline in consumption over each seven day period. The introduction of a novel food at the start of a new seven day period (or on day 11 in the case of the 'new' type of bran) caused an increase in the total amount eaten but this was not maintained and gradually declined.

Figs. I5, I6 and I7 show the consumption of Food A and Food B for the groups A, B and C respectively.

Figs. I8, I9 and 20 show the percentage response of each group to both foods i.e. the number of animals which fed on Food A and Food B each day.

All these groups showed a high initial consumption, with meal size on the first day 193.2mg for the "choice" group, A, 121.2mg for the "bran" group, B, and

170.3mg for the "pressed yeast" group, C. These values fell considerably over the next few days with group A averaging 80.94mg on the total meal size for the first week, group B averaging 34.4mg and group C averaging 64.73mg. Feeding totals for the "choice group", A, were consistently much higher than the "bran" group, B, and generally higher than the "pressed yeast" group, C.

The introduction of a new pressed yeast sample, W8, on day 8 did not markedly alter the quantity eaten by groups A or C which had previously been offered pressed yeast. Group B responded to the introduction of this novel food with an average increase in total meal size from 6.9mg on day 7 to 205.3mg on day 8. On days 8, 9 and 10 none of the animals in this group ate the bran discs so both the feeding scores and the percent response were zero for this food and the pressed yeast discs constituted the entire diet. The introduction of bran discs to group C on day 8, a novel food for these animals, also provided an increase in the consumption of the new food though this was less marked than that of group B. The new batch of yeast, W8, caused a slight increase in the consumption of this food B by group A though this was reversed on day 11 when the new type of bran was introduced.

All animals received the same diet in the second week, but from day 11 the more attractive extract of bran flakes was offered to all three groups as the bran feeding stimulant in place of the normal wheatbran. Meal size was maximal for all groups on day 11, the initial exposure to this bran, with an average value of 262.6mg for group A,

249.3mg for group B and 224.7mg for group C. Group A continued to receive the same diet - bran flake discs and batch W8 pressed yeast - for the rest of the trial and showed a steady decline in consumption. A starvation period (days 22 - 29) did not increase the amount eaten when the slugs were subsequently offered these foods again.

On day I5, groups B and C reverted to their original diets. In both groups, consumption fell when the animals were once again receiving a single type of food. On many days the ratio A/B varied considerably from the expected value of I.O. The reasons for this are not clear since other feeding tests had indicated that there was no preference for a particular side of the feeding box. The values for the seven day period were closer to I.O, however, so that group C for example,in which the ratio varied from 0.26 to 2.53 on days I5 - 22, had an average value of I.27 for this period. Therefore, not only did the total meal size fall when the animals were offered only a single type of food, but the contribution of each food disc was more equal than the previous week when the novel food discs had been preferentially eaten.

A starvation period (days 22 - 29) allowed seven days for the animals to use up food reserves formed during the continuous 2I day feeding period and should have made the slugs more responsive to food when they were offered a choice of bran and yeast on day 29. Total meal size in all three groups however,was not improved on the pre-starvation levels.

During this three day post-starvation period, the feeding responses of the three groups were more similar than

on days 8 - 14 when all 30 slugs had received the same diet. Comparison of food consumption showed that in groups A and C, pressed yeast was slightly more attractive than the bran discs. Group B showed a similar response though the difference was more marked. This would suggest that the starvation period had eliminated much of the preferential response shown by groups B and C. It appears that, for long term feeding, the pressed yeast/agar discs were more readily eaten than the bran discs (this is confirmed with feeding tests in section 4) although the introduction of a new type of bran on day 11 had produced an initial rise in consumption.

Group B initially fed on two bran discs, maintained some preference for the pressed yeast food, but this was less marked than their initial exposure to this diet on day 8. If habituation to a diet were retained over a starvation period, group C might be expected to show a preference for the bran discs offered on days 29 - 31. Since this did not happen it is suggested that the habituation deteriorates and the two foods, bran and pressed yeast, are treated on their attractive merits alone. The two food choices were thus accepted without the complications of previous exposure to either food.

Had the animals survived longer, a third food could have been introduced to compare the consumption of this with the two toxic components of the diet. The experiments do indicate, however, that Deroceras will adapt to a diet and that meal size can be manipulated by offering a novel food to the animals. There are few reports of molluscs

adapting to a diet and showing a decrease in the amount eaten over a period of time.

Yamashita et al (1979) compared the amount of food eaten by laboratory housed and newly collected Deroceras of 100mg weight. The animals were given leaf discs cut from Kenya white clover (Trifolium sempilosum) and the area and dry weight of the discs recorded after 48 hours feeding. The newly collected slugs ate an average of 0.92mg dry matter per slug per day, while the value for the laboratory housed slugs was much reduced at 0.22mg.

Senseman (1976) also showed a decrease in the response to monotonous diets in Ariolimax californicus and suggested three possible reasons for this:

1. The slugs build up metabolic reserves during the experiment so that the long term elevation in feeding threshold causes a decreased responsiveness.
2. The animals are unresponsive because of the test conditions.
3. There is a long term elevation in the feeding threshold due to previous experience with the food item.

He found that slugs fed on potato, normally an attractive food, rejected it after a while even when no other food was available. Starved slugs fed on stale beer incorporated into 4% agar ate well at first but by the 6th day, this food was rejected. When these animals were divided into two groups and offered two different foods, the slugs which subsequently fed on apple juice/agar consumed a large meal while the group maintained on the beer/agar continued to reject the food, The introduction of a novel food, apple juice/agar, indicated

that the slugs were still capable of feeding but that continuous exposure to a single food had caused the feeding stimulus to decline.

3.9 CONCLUSIONS

These tests using agar discs as a carrier for feeding stimulants such as bran, have provided a suitable bioassay for Deroceras and overcame many of the problems encountered with other food substrates. Maximum meal size is quite variable but the experiments on food memory have indicated that the amount consumed by laboratory housed Deroceras will decline over a period of time if the animals are continuously offered the same diet. It is possible this may be caused by a nutritionally inadequate diet but many eggs were found in the boxes housing the slugs suggesting that reproductive success was not impaired and some animals were reared in the laboratory on such agar based diets.

Williams (1972) showed that meal size declined when a toxicant was added to the diet of slugs, but there is little published work relating food consumption of molluscs to bait formulation. Fisher & Orth (1975) suggested that snails from regularly baited areas may lose their susceptibility to metaldehyde but did not attempt to explain how this could happen. The work presented here suggests that previous exposure to a particular food can deter Deroceras from eating the same food at a later date and indicates that some form of habituation must occur. The metaldehyde resistance could therefore be due to habituation

to the carrier or the toxicant itself. This further emphasises the need for good attractants in commercial baits since, if the initial feeding stimulation is low, meal size may be too small to supply a lethal dose and subsequent exposure to this bait will result in an even smaller consumption of food.

The importance of maintaining a high feeding stimulation must therefore be considered in bait formulation. Another major implication of these results is in feeding tests where laboratory housed slugs are reared on a culture. Animals maintained on a monotonous diet show a decline in feeding activity and may show a similar decline in other behavioural responses.

The results presented here suggest that feeding tests with Deroceras are subject to long term variables such as previous exposure to food items. These factors must be considered in the analysis of the results of these tests.

SECTION 4 ATTRACTANTS AND REPELLANTS

ATTRACTANTS

INTRODUCTION

Several cases of slugs and snails being attracted to beer have been reported in the literature (Northern 1970, Selim 1973, Smith & Boswell 1970, Stout 1968) and it is well known to gardeners that a tray of beer placed amongst susceptible plants will cause these pests to move towards the dish and drown in the beer. Smith & Boswell compared several fermented and unfermented liquids for their attractancy to slugs and found that beer - either fresh or stale - was significantly more attractive than the others. They also compared baits containing either metaldehyde or Bay 37344 (methiocarb) which were moistened with either water or beer and found that beer-moistened baits killed more Deroceras reticulatum than the water-moistened baits.

Selim (1973) used a similar, simple bioassay to estimate beer attractiveness - he placed 50ml. beer in 100 ml. beakers and left these near slug infested ivy. He did however, attempt to isolate and identify the attractive components of beer by fractionation and suggested three possible attractive substances - acetoin, diacetyl and dihydroxyacetone. The number of slugs trapped with each of these compounds was however, much lower than the number trapped with beer.

A more precise determination of the attractive qualities of beer is required so the feeding bioassay using

agar discs was adapted to quantify the response. The beer samples were compared initially to a reference substance, bran, which is known to be a feeding stimulant for slugs.

Each feeding test with Deroceras involved offering two foods to a group of ten animals. Over 250 tests were made with various potentially attractive substances incorporated into agar and offered in combination with one another. Since this necessitated the use of a large number of slugs, some were used in more than one feeding test and the differences in consumption between these 'old' slugs and the 'new' animals which were receiving a diet for the first or second time became apparent only after a large number of feeding tests had been carried out. The literature on molluscan diets gives no definite indication that the animals habituate to a diet after repeated exposure to the same food and the work of Croll & Chase (1977a, 1977b) - see P121 - indicates that Achatina fulica preferentially orientates towards the odour of a food which it has previously eaten. This would therefore suggest that previous exposure to a diet encourages the animal to feed on this food at a later date.

The results presented here suggest that the slugs habituate to a diet so that it gradually becomes less attractive. This then casts doubts on the validity of the results of some of the feeding tests presented in this chapter since a factor other than food palatability is influencing the meal size of laboratory prepared diets offered to the animals. The work on food memory (Section 3) was initiated as a result of these feeding tests and subsequently confirmed

that Deroceras does indeed show a decline in response to a monotonous diet. The results presented here have therefore been interpreted both on the basis of the relative attractiveness of each component in the diet (food A v. food B) and as a comparison between 'old' and 'new' Deroceras since the work on food memory suggested that length of laboratory housing could influence meal size.

4.I THE BREWING PROCESS

Beer is produced from the alcoholic fermentation of brewers hopped malt extract (brewers wort) using one of two major groups of yeast - Saccharomyces carlsbergensis and S.cerevisiae. Strains of S. cerevisiae accumulate as a yeasty head at the surface of the fermenting mixture and produce the ale or beer traditional in Great Britain. Strains of S. carlsbergensis are bottom yeasts which settle to the base of the fermenting vessel and produce lager beers. An outline of the various stages in the brewing process may provide a simple guide to the products of each stage and clarify the terminology referred to later.

Malt is prepared from barley grains which are germinated, dried to preserve the enzymes formed in the grain, and then crushed. Proteolytic activity produces amino acids which are essential for yeast nutrition. The malt and adjuncts are then 'mashed' with large volumes of water and the soluble extract or wort filtered off from the spent grain. The ionic content of the water used in the mashing process is important as calcium and bicarbonate ions influence the pH of the wort. Mashing causes

hydrolysis of the starch stored in the malt and produces sugars - the temperature is then increased to inactivate the enzymes and the wort is washed and boiled with hops (Humulus lupulus) which provide the bitter flavour. This hopped wort is filtered through the hop residue and the cooled mixture is inoculated with yeast. The metabolism of the yeast converts the sugars to alcohol and carbon dioxide, absorbing amino acids in the process but liberating a large number of by-products also, including fusel alcohols, glycerol, acetic acid, acetaldehyde and many other compounds. The yeast is later removed by centrifugation and filtration. The beer is allowed to mature and condition to regulate flavour and the semi-solid pressed yeast, which is rich in protein, is sold as a foodstuff.

The natural flavour of beer represents a complex balance between the individual constituents and the ratio of each component depends on minor modifications of the process in each brewery. There is no scale of concentration, however, which determines how much of an influence each component has on the final flavour of the beer and a component present in minute quantities (less than 0.1 ppm) can have an important effect. Flavour analysis has traditionally relied upon organoleptic evaluation though gas chromatography has enabled the presence and concentration of the major components to be identified.

4.2 PRELIMINARY FEEDING TESTS TO COMPARE ATTRACTIVENESS OF BREWING BY-PRODUCTS

A local brewery, Watney-Mann, Mortlake, London

was selected for ease of access and samples of representative stages of the brewing process taken to ascertain whether they were attractive to slugs. The samples collected were:

1. Malt dust - the finely ground waste from germinated barley.
2. Spent grains - reclaimed from the mashing process.
3. Pressed yeast - a mixture of yeast and wort which is pressed out of the vats after fermentation and which contains large amounts of water, depending on the efficiency of the presses.
4. Yeast slurry - the liquid discharged into drains after washing the feremented yeast mixture.
5. Yeast & finings - the liquid run out from the bottom of the settling tanks which therefore contains a high percentage of finings or Isinglass.
6. Breakings - discharge from the tankers which have delivered to retailers. This sample contains a large amount of sediment but is occasionally added to other beers.

Samples 1 and 2 are solid by-products, obtained in the early stages of the brewing process before either hops or yeasts are added to the sweet wort. The other samples are all obtained from later stages when all the additives have been incorporated into the fermenting mixture. Variations in these four samples are due to the formation of chemical compounds during metabolism of the yeast. Most of the by-products are sold as fertilisers and feedstuffs.

Each sample was subjected to preliminary behavioural tests with three species of slug -

Deroceras reticulatum, D.caruanae and Limax maximus - to highlight the more attractive samples. The behaviour of the animals was observed for a ten minute period in the presence of each sample, tested in combination with a bran extract - a positive score was recorded each time a slug reached one of the food sources.

The more promising samples were then combined with agar and offered to individual Deroceras reticulatum with a wheatgerm/agar disc as described in the feeding bioassay (section 3). The brewing by-product samples were prepared at a concentration of 5.0g wet weight of the semi-solid sample 3 and IOml of samples 4, 5 and 6. All these samples were dissolved in lOOml water and added to lg agar powder. Discs of 1.2cm diameter were cut from the agar sheets, offered to Deroceras for 24 hours and the feeding activity assessed at the end of this period by assigning a score to each disc and converting these scores to weight losses from the discs.

The discs used in these initial feeding tests are shown in Fig 2I . Each group of 20 discs is the remains of the food offered to the animals in the feeding test - each of the ten animals received two discs, one from the top and one from the bottom row. The top row in the five pairs of agar discs shown in the photograph is the bran component of each diet and for the fifth feeding test, the bottom row is also bran discs. For the first four feeding tests, the bottom row is the brewing by-product - either yeast plus finings, breakings, pressed yeast or yeast slurry. It is apparent that feeding on the pressed

FIG. 2I CONSUMPTION OF FOOD BY Deroceras

PRELIMINARY FEEDING TESTS WITH BREWING

BY-PRODUCTS

Agar discs showing feeding activity of slugs. The photograph indicates that the pressed yeast discs (bottom row, centre group) were preferentially eaten

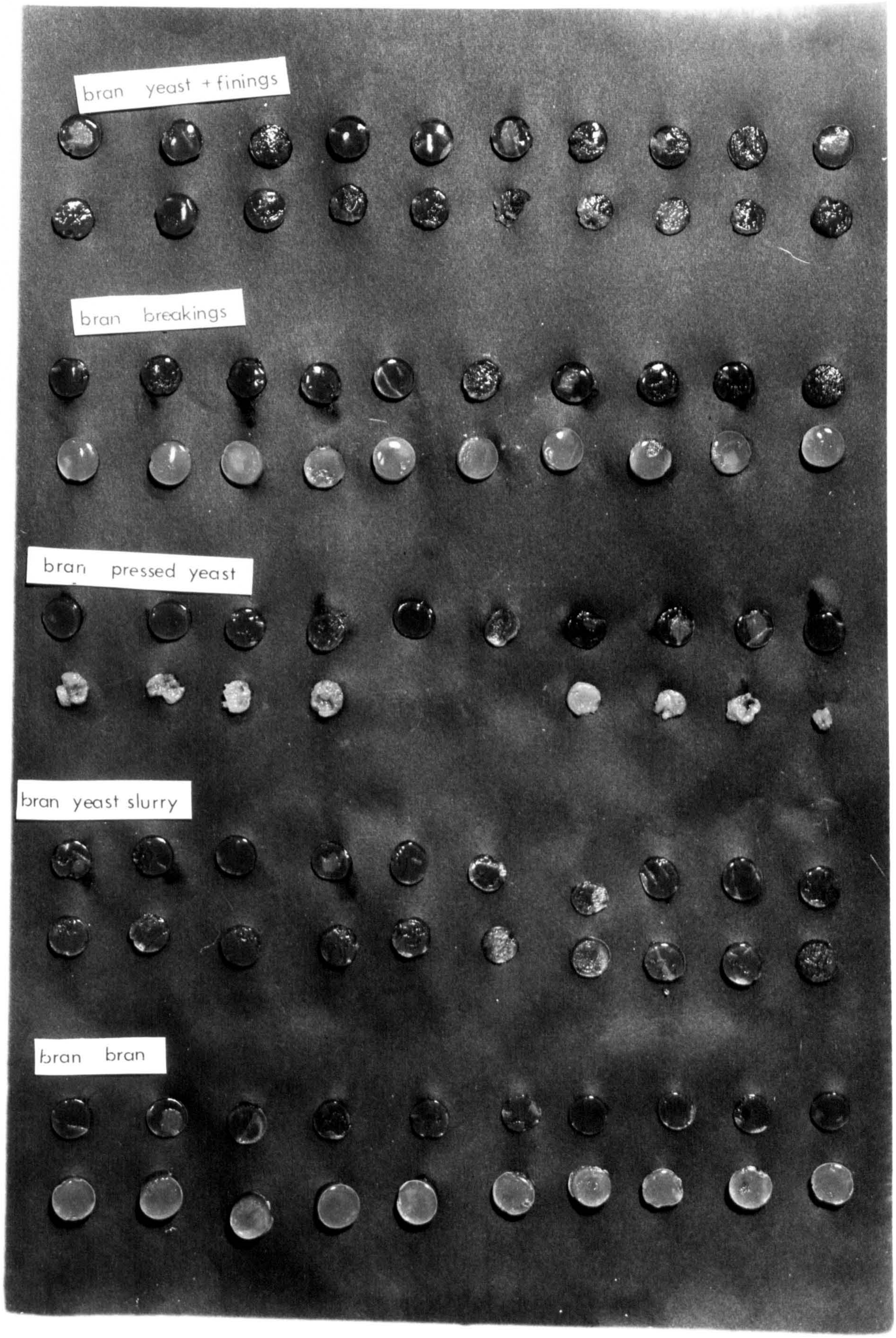

bran yeast + finings
bran breakings
bran pressed yeast
bran yeast slurry
bran bran

TABLE 8 PRELIMINARY FEEDING TESTS TO COMPARE ATTRACTIVENESS OF BREWING BY-PRODUCTS

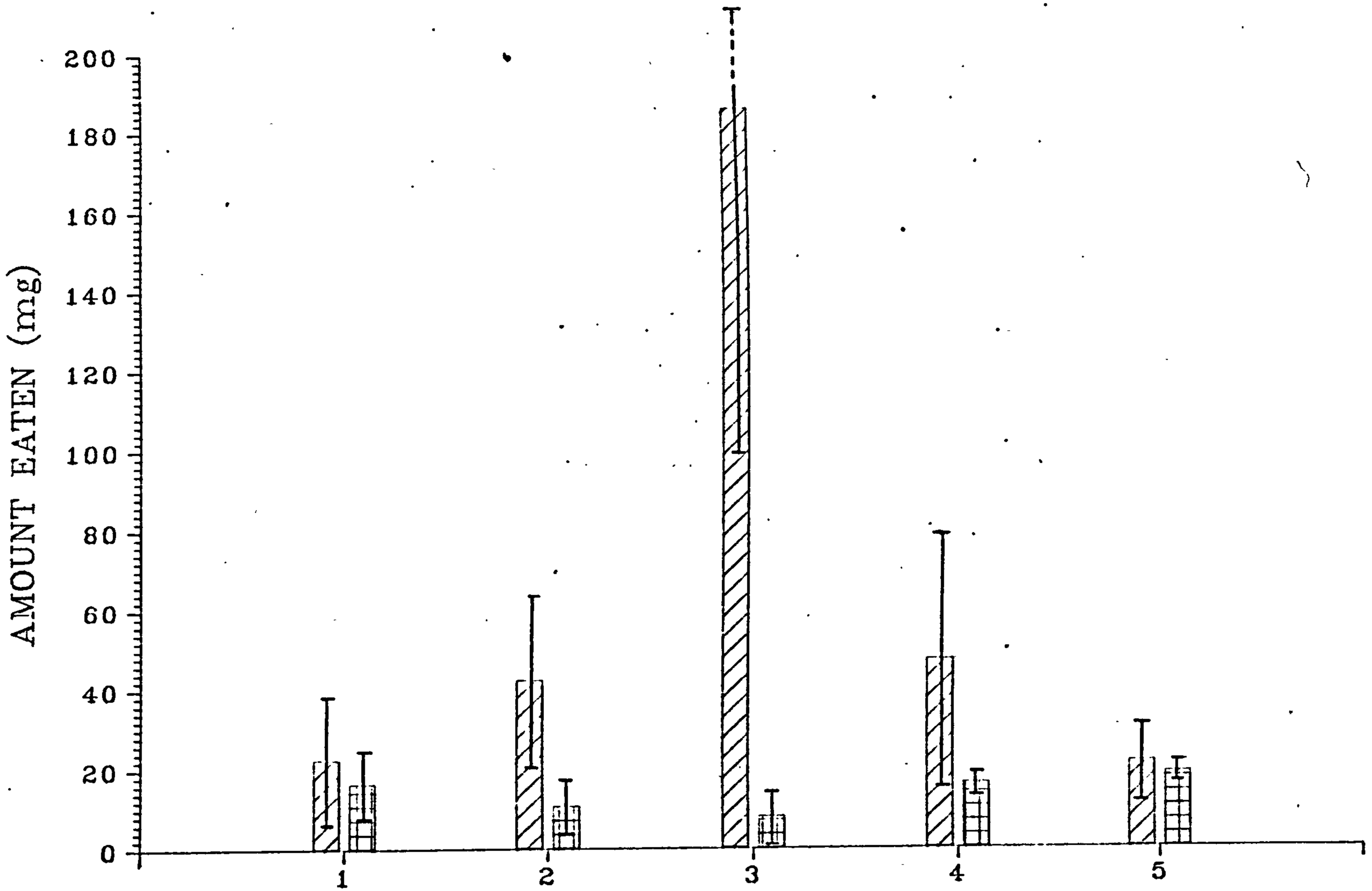
FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
YEAST + FININGS		BRAN			
36.5	80	6.9	20	5.29	43.4
27.6	80	24.1	70	1.15	51.7
3.4	10	18.3	40	0.19	21.7
$\bar{x}$ 22.50	56.7	16.43	43.3	1.37	38.93
S.D. 17.3		8.75			
BREAKINGS		BRAN			
65.8	100	18.3	40	3.60	84.1
40.0	90	6.9	20	5.80	46.9
21.7	50	6.9	20	3.14	28.6
$\bar{x}$ 42.50	80	10.70	26.67	3.97	53.2
S.D. 22.16		6.58			
PRESSED YEAST		BRAN			
89.2	80	3.4	10	26.24	92.6
256.8	100	17.2	50	14.93	274.0
210.6	80	3.4	10	61,94	214.0
$\bar{x}$ 185.53	86.67	8.0	23.33	23.19	193.53
S.D. 86.57		7.97			
YEAST SLURRY		BRAN			
37.4	50	17.2	50	2.17	54.6
84.6	90	18.3	40	4.62	102.9
20.7	60	13.8	40	1.50	34.5
$\bar{x}$ 47.57	66.67	16.43	43.33	2.90	64.0
S.D. 33.14		2.35			
BRAN		BRAN			
29.7	60	20.7	60	1.43	50.4
13.8	40	17.2	50	0.80	31.0
$\bar{x}$ 21.75	50	18.95	55	1.15	40.7
S.D. 11.24		2.47			

FIG 22 CONSUMPTION OF FOOD BY Deroceras

PRELIMINARY TESTS WITH BEER BY-PRODUCTS

KEY	hatching	squares
x axis	I yeast + finings	bran
	2 Breakings	bran
	3 pressed yeast	bran
	4 yeast slurry	bran
	5 bran	bran

CONSUMPTION OF FOOD BY DEROCERAS
BEER BY–PRODUCTS (FOOD A) COMPARED WITH BRAN (FOOD B)



yeast discs was the most pronounced with two of the ten discs being completely eaten during the 24 hour feeding test.

The values for the weight of food consumed are shown in Table 8. The tests were replicated three times (twice for the test in which both foods offered to the slugs were bran discs). The average amount of food consumed in each of the feeding tests in Fig 22, together with the standard error for each of the replicated tests.

The wide variation in the amount consumed in each test suggests that the meal size can be manipulated by the addition of attractive components to the diet. Each test is compared with a bran control by the ratio A/B and in all but one of the tests this value is more than 1.0 indicating that a greater amount of the beer product/ agar disc, food 4, was consumed. The results also indicate that the pressed yeast sample was signifcantly more attractive than the other by-products with an average value of 158.18mg of the yeast/agar disc consumed by the 30 animals tested, compared with an average value of 7.43mg of bran/agar disc eaten in the same test. The number of slugs feeding on the discs - the percent response - was also high in this group and the highest total meal size, 274.0mg, was obtained in animals feeding on this diet.

Tests run concurrently offered two bran/agar discs to each animal and provided a standard value for the amount of food each slug was likely to consume in 24 hours. The value obtained suggested that slugs consume a total meal size of approximately 50mg when fed on bran/agar discs and

that 50% of the animals can be expected to feed on the discs.

These values indicated that the pressed yeast by-product from the brewing industry was the most promising candidate for an attractive supplement to slug baits, so further tests were made on this product only. Subsequent visits to the brewery were made over a period of 18 months to compare different batches of yeast. Another local brewery, Fuller , Smith & Turner Ltd (Chiswick, London) was also included in the feeding tests since the brewing process here is more traditional. Copper vessels are used at Fuller's instead of the stainless steel vessels in modern breweries and it is quite possible that the attractive nature of the yeast could be enhanced or reduced by minor variations in the brewing process since the composition of the beer varies from one brewery to another. Samples were coded for identification and comparison purposes:

W = Watney Mann Brewery, F= Fuller's Brewery, with a sequential numbering system relating to the date of collection.

W1 collected	8th November 1978	F1 collected	30th March 1979
W2 collected	11th November 1978	F2 collected	14th May 1979
W3 collected	29th November 1978	F3 collected	22nd May 1979
W4 collected	14th December 1978	F4 collected	20th June 1979
W5 collected	15th January 1979	F5 collected	4th July 1979
W6 collected	30th January 1979	F6 collected	19th July 1979
W7 collected	23rd May 1979	F7 collected	16th August 1979
W8 collected	23rd May 1980	F8 collected	11th January 1980

4.3 FEEDING TESTS TO COMPARE WATNEY'S YEAST WITH BRAN

The different batches of pressed yeast were formulated into agar sheets on the day of collection and offered to Deroceras reticulatum as food A in combination with a bran/agar disc as food B. The results of the tests with the Watney's yeast are presented in Table 9 as an average meal size for each group of ten animals. A different type of bran was used in the feeding tests with batch W8 so these results have been excluded from the combined analysis of the 19 feeding tests with batches W1, W2, W5, W6 and W7.

Wheatbran, a standard attractive food for slugs, was found to be relatively unattractive when offered to Deroceras with a choice of pressed yeast/agar. The ratio A/B compares the two foods directly and the average value for the 19 feeding tests indicates that the yeast, food A, was eaten in preference to the bran discs, food B. The average total meal size consumed over the 24 hour period is significantly more than the 40 - 50mg eaten when two bran discs were offered to Deroceras. The discrepancies between each test were large however, with the total meal size ranging from 53.5mg to 274.0mg and the A/B value ranging from 0.76 where the bran was the most attractive food to 61.94mg where the yeast was the most important component of the diet - this latter value was however, a single inexplicably high figure. The average consumption of each food is presented in Fig.23 for the six batches, together with the standard error of the replicates.

Meal sizes for the tests with batches W1 and

TABLE 9 FEEDING TESTS TO COMPARE WATNEYS PRESSED YEAST WITH BRAN

	FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
	WI		BRAN			
	89.2	80	3.4	IO	26.24	92.6
	256.8	IOO	I7.2	50	I4.93	274.O
	2IO.6	80	3.4	IO	6I.94	2I4.O
$\bar{x}$	I85.53	86.67	8.O	23.33	23.I9	I95.53
S.D.	86.57		7.97			
	W2		BRAN			
	253.I	IOO	IO.3	30	24.57	263.4
	IO2.9	IOO	3.4	IO	30.26	IO6.3
$\bar{x}$	I78.O	IOO	6.85	20	25.99	I84.85
S.D.	IO6.2I		4.88			
	W5		BRAN			
	76.9	80	O.O	O	-	76.9
	I3I.9	IOO	3.4	IO	38.79	I35.3
	79.2	IOO	28.6	70	2.77	IO7.8
	69.8	60	24.I	70	2.90	93.9
	59,I	IOO	3I.O	90	I.9I	90.I
$\bar{x}$	83.38	88	I7.42	48	4.79	IOO.8
S.D.	28.23		I3.07			
	W6		BRAN			
	73.7	80	26.2	50	2.8I	99.9
	93.6	90	22.8	40	4.II	II6.4
	59.O	80	40.O	90	I.48	99.O
	27.6	80	36.5	80	O.76	64.I
$\bar{x}$	63.48	82.5	3I.38	65	2.O2	94.86
S.D.	27.80		8.I8			
	W7		BRAN			
	80.3	90	3I.8	60	2.53	II2.I
	II3.6	90	24.I	70	4.7I	I37.7
	35.2	50	I8.3	40	I.92	53.5
	49.O	90	36.5	80	I.34	85.5
	57.7	70	57.7	90	I.O	II5.4
$\bar{x}$	67.I6	78	33.68	68	I.99	IOO.84
S.D.	37.7O		I3.54			
	W8		BRAN			
	II9.4	IOO	5O.O	80	2.39	I69.4
	83.6	70	98.I	90	O.85	I8I.7
$\bar{x}$	IOI.5	85	74.O5	85	I.37	I75.55
S.D.	25.3I		34.OI			

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FIG. 23 CONSUMPTION OF FOOD BY Deroceras
TESTS WITH WATNEY'S YEAST

KEY	hatching	squares
x axis	I WI	wheatbran
	2 W2	wheatbran
	3 W5	wheatbran
	4 W6	wheatbran
	5 W7	wheatbran
	6 W8	bran flakes

CONSUMPTION OF FOOD BY DEROCERAS
WATNEYS YEAST (FOOD A) COMPARED WITH BRAN (FOOD B)

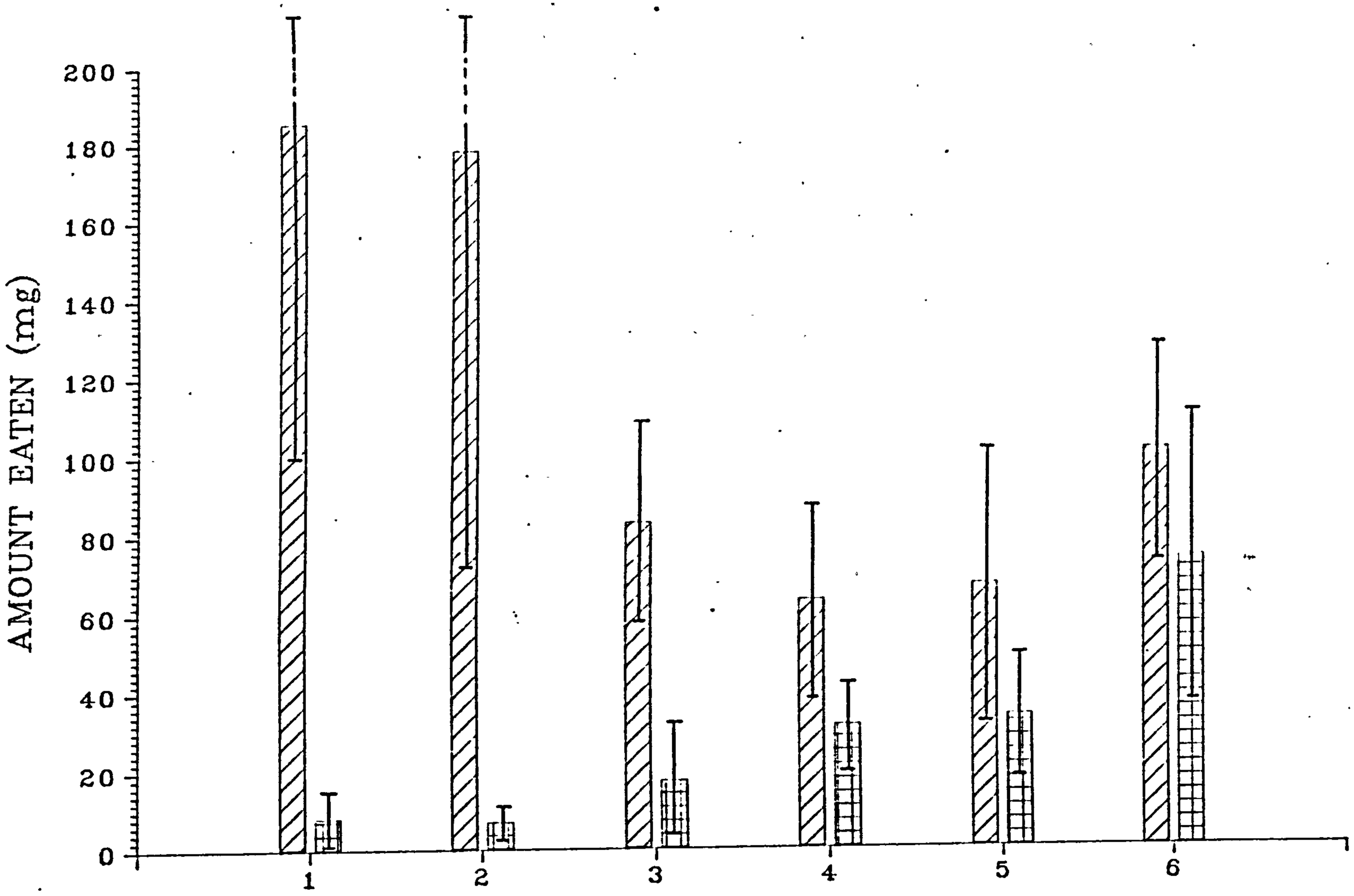


TABLE IO CONSUMPTION OF AGAR DIETS CONTAINING WATNEY'S YEAST, FOOD A, OR BRAN, FOOD B. - NEW ANIMALS

FOOD A		FOOD B			
Amount eaten mg	Percent response	Amount eaten mg	Percent response	A/B	Total eaten mg
89.2	80	3.4	10	26.24	92.6
256.8	100	17.2	50	14.93	274.0
253.1	100	10.3	30	24.57	263.4
102.9	100	3.4	10	30.26	106.3
210.6	80	3.4	10	61.94	214.0
93.6	90	22.8	40	4.11	116.4
113.6	90	24.1	70	4.71	137.7
80.3	90	31.8	60	2.53	112.1
76.9	80	0.0	0	76.90	76.9
131.9	100	3.4	10	38.79	135.3
79.2	100	28.6	70	2.77	107.8
$\bar{x}$ 135.28	91.82	13.49	32.73	10.03	148.77
S.D. 70.21		11.73			

TABLE II CONSUMPTION OF AGAR DIETS CONTAINING WATNEY'S YEAST, FOOD A, OR BRAN, FOOD B - OLD ANIMALS

FOOD A		FOOD B			
Amount eaten mg	Percent response	Amount eaten mg	Percent response	A/B	Total eaten mg
73.7	80	26.2	50	2.81	99.9
27.6	80	36.5	80	0.76	64.1
69.8	60	24.1	70	2.90	93.9
59.1	100	31.0	90	1.91	90.1
59.0	80	40.0	90	1.48	99.0
35.2	50	18.3	40	1.92	53.5
57.7	70	57.7	90	1.00	115.4
49.0	90	36.5	80	1.34	85.5
$\bar{x}$ 54.59	74.29	33.40	72.86	1.63	87.99
S.D. 17.08		13.01			

W2 were higher than subsequent tests with other batches suggesting that batch variability may exist. The percent response was more constant - an average of 85.26% for food A and 50% for food B indicating that a large number of slugs ate the pressed yeast disc but the volume they ingested was more variable. Comparison of the Watney's sample was done by analysis of variance (Appendix 1) which contrasted the mean amount of food A consumed for each batch. The difference between batches was found to be significant ($P = 0.05$). The amount of food B consumed in each of these tests was similarly compared (Appendix 1) and the difference between the consumption of bran for the corresponding groups of yeast is also significant ($P = 0.05$). Unlike the food A, the bran/agar discs were made from the same wheatbran for all 19 tests so the volume of this food eaten should have been relatively constant.

The feeding results have been rearranged in Tables IO and II into two groups - tests in which the animals have been housed in the laboratory for more than 4 days and have received this diet more than once before and those tests in which the slugs have been housed for less than 4 days and received this diet for the first or second time. The former group have been designated 'old' and the latter group 'new'. If the meal sizes for each of these groups are investigated separately, it appears that the 'new' group consumed a larger total meal size - average 148.77mg - and that food A was over ten times as attractive as food B - average A/B value 10.03. The total meal size for the 'old' group was 87.99mg, over 60mg less than the

corresponding value in the 'new' group. The A/B value was also much lower - 1.63 - indicating that the contribution of food A was much reduced. Tests with fresh yeast batches suggested that this was not due to ageing of the yeast.

The percent response indicates that the number of animals consuming food A, the pressed yeast/agar discs, did not fall considerably in the 'old' group but the amount of this food consumed by each animal fell more noticeably. The improvement in the meal size of food B, the bran/agar discs, was due to both an increase in the number of animals responding and to an increase in the volume of food consumed by each animal.

The introduction of a novel, attractive food into the diet of Deroceras caused a large increase in the total meal consumption and animals which had received this choice of diet in previous feeding tests no longer exhibited this high consumption. It appears that these slugs habituate to the diet and the two foods, A and B, become more similar in their attractiveness. A reexamination of meal sizes of the different batches of pressed yeast shows that the apparently more attractive batches, W1 and W2, involved 'new' groups of animals only and the three less attractive batches W5, W6 and W7, involved both 'old' and 'new' groups. It is thus possible that the batch variability was due to the difference between these 'old' and 'new' groups rather than any difference in the attractiveness of the pressed yeast itself.

4.4 FEEDING TESTS TO COMPARE FULLER'S YEAST WITH BRAN

Six different batches of Fuller's pressed yeast collected over a period of three months and formulated into agar sheets on the day of collection, were offered to Deroceras as food A in combination with wheatbran/agar discs as food B. A total of 28 feeding tests (Table I2), each involving 10 slugs, were made with batches F2, F3, F4, F5 and F6. Since the test with batch F7 involved a different type of bran, the results from this have been excluded from the combined calculations. The total average meal size of 110.24mg was slightly less than the similar tests with the Watney's samples and the overall A/B value of 1.05 indicates that the two foods, Fullers pressed yeast and bran, were equally attractive to Deroceras. Again meal size varied considerably, though as there were no extraordinarily large meal sizes, the range was less marked than the Watney samples had indicated - 40.0mg to 180.8mg. The average consumption of each food is presented in Fig 24 for the six batches, together with the standard error of the replicates.

Analysis of variance (Appendix 1) to compare the means of the amount of food A consumed for each of the five batches of Fuller's pressed yeast indicated a significant difference. (P - 0.05). The corresponding consumption of food B in each of these batches was also significantly different (P = 0.05). This again suggested a batch variability in the attractive nature of food A and since food B was the same in all tests, the amount of this food consumed should be fairly constant.

The 27 tests were divided into 'old' and 'new'

TABLE I2 FEEDING TESTS TO COMPARE FULLERS PRESSED YEAST WITH BRAN

	FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
	F2		BRAN			
	29.7	60	I20.8	90	0.25	I50.5
	F3		BRAN			
	94.9	I00	50.I	I00	I.89	I45.0
	9I.5	90	29.7	60	3.08	I2I.2
	43.I	60	25.2	60	I.7I	68.3
	38.6	60	82.5	90	0.47	I2I.I
	34.2	60	24.9	40	I.37	59.I
	40.0	90	6.9	20	5.80	46.9
$\bar{x}$	57.05	76.67	36.55	6I.67	I.56	93.6
S.D.	28.I7		26.40			
	F4		BRAN			
	43.I	60	I8.3	40	2.36	6I.4
	68.9	70	2I.7	50	3.I8	90.6
	26.2	50	I3.8	40	I.90	40.0
	74.5	80	49.0	90	I.52	I23.5
	53.0	50	33.I	70	I.60	86.I
	72.4	80	30.7	50	2.36	I03.I
	40.8	60	43.2	80	0.94	84.0
	I38.6	I00	20.7	60	6.70	I59.3
	74.5	80	49.0	90	I.52	I23.5
	33.I	70	87.0	90	0.38	I20.I
	67.9	80	44.3	70	I.53	II2.2
$\bar{x}$	63.0	70.9	37.35	66.36	I.69	I00.35
S.D.	30.6		20.79			
	F5		BRAN			
	I04.8	90	88.2	I00	I.I9	I93.0
	44.3	70	I04.3	I00	0.42	I48.6
	68.I	I00	52.2	80	I.30	I20.3
	25.2	60	62.3	90	0.40	87.5
$\bar{x}$	60.6	80	76.75	92.5	0.79	I37.35
S.D.	34.3		23.82			
	F6		BRAN			
	35.2	50	I05.I	I00	0.33	I40.3
	83.7	I00	97.I	I00	0.86	I80.8
	48.8	70	74.8	I00	0.65	I23.6
	34.2	60	52.0	60	0.66	86.2
	40.0	90	32.I	80	I.25	72.I
$\bar{x}$	48.38	74	72.22	88	0.67	I20.6
S.D.	20.57		30.52			
	F7		BRAN			
	96.8	60	I02.7	80	0.94	I99.5

FIG. 24 CONSUMPTION OF FOOD BY Deroceras
TESTS WITH FULLER'S YEAST

KEY	hatching	squares
x axis	I F2	wheatbran
	2 F3	wheatbran
	3 F4	wheatbran
	4 F5	wheatbran
	5 F6	wheatbran
	6 F7	bran flakes

CONSUMPTION OF FOOD BY DEROCERAS
FULLERS YEAST (FOOD A) COMPARED WITH BRAN (FOOD B)

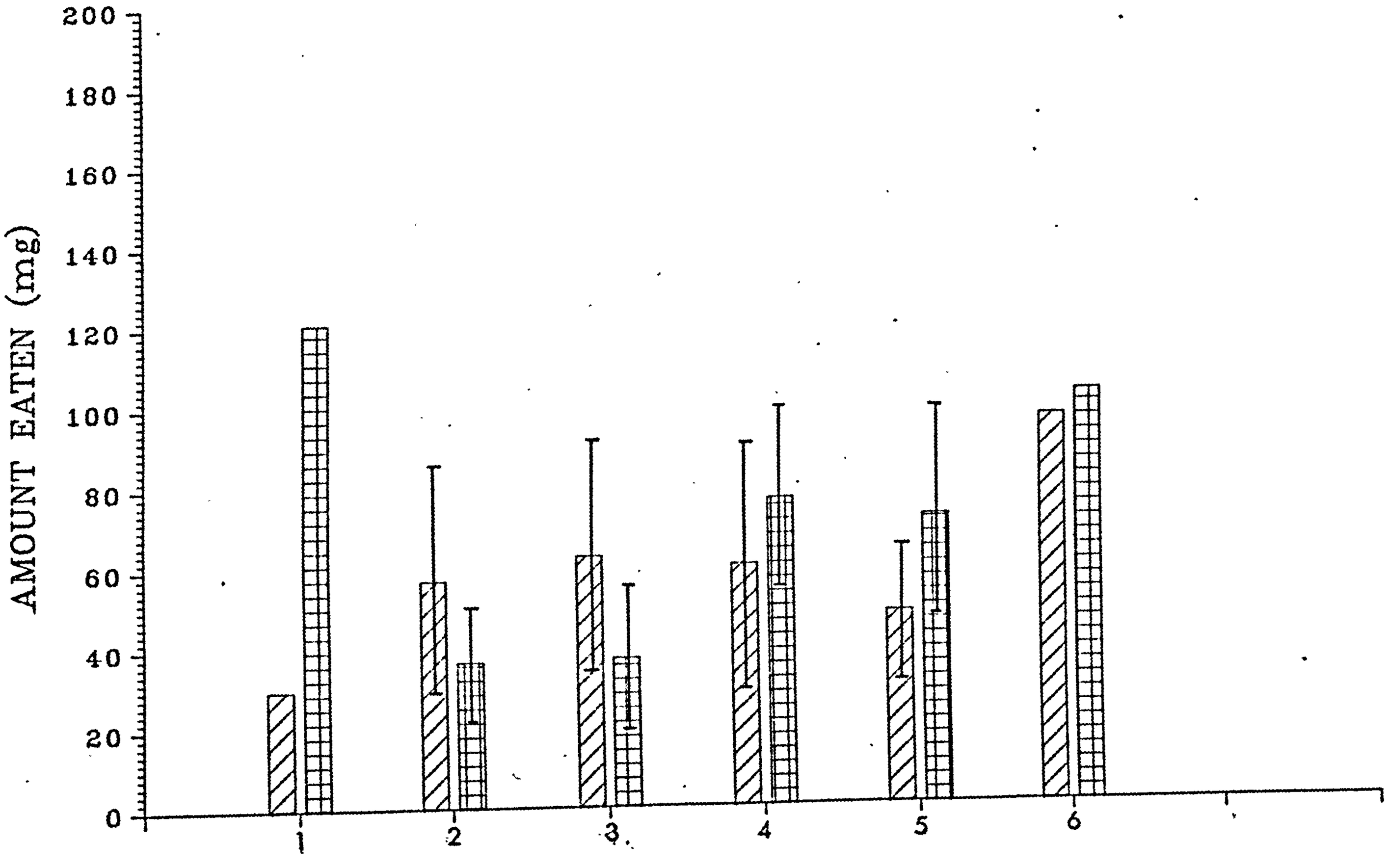


TABLE I3 CONSUMPTION OF AGAR DIETS CONTAINING FULLER'S YEAST (FOOD A) AND BRAN (FOOD B)- NEW ANIMALS

FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
94.9	I00	50.I	I00	I.89	I45.0
9I.5	90	29.7	60	3.08	I2I.2
I38.6	I00	20.7	60	6.70	I59.3
I04.8	90	88.2	I00	I.I9	I93.0
74.5	80	80.3	90	0.94	I54.8
33.I	70	87.0	90	0.38	I20.I
74.5	80	49.0	90	I.52	I23.5
53.0	50	33.I	70	I.60	86.I
72.4	80	30.7	50	2.36	I03.I
40.8	60	43.2	80	0.94	84.0
67.9	80	44.3	70	I.53	II2.2
44.3	70	I04.3	I00	0.42	I48.6
68.I	I00	52.2	80	I.30	I20.3
40.0	90	32.I	80	I.25	72.I
$\bar{x}$ 7I.3I	8I.43	53.2I	80.00	I.34	I24.52
S.D. 29.2I		26.I4			

TABLE I4 CONSUMPTION OF AGAR DIETS CONTAINING FULLER'S YEAST (FOOD A) AND BRAN (FOOD B) - OLD ANIMALS

29.7	60	I20.8	90	0.25	I50.8
43.I	60	25.2	60	I.7I	68.3
38.6	60	82.5	90	0.47	I2I.I
43.I	60	I8.3	40	2.36	6I.4
68.9	70	2I.7	50	3.I8	90.6
26.2	50	I3.8	40	I.90	40.0
34.2	60	24.9	40	I.37	59.I
40.0	90	6.9	20	5.80	46.9
35.2	60	I05.I	90	0.33	I40.3
83.7	50	97.I	I00	0.86	I80.8
48.8	I00	74.8	I00	0.65	I23.6
25.2	60	62.3	90	0.40	87.5
34.2	60	52.0	60	0.66	86.2
$\bar{x}$ 42.38	65.38	54.26	67.69	0.78	96.64
S.D. I6.77		38.8I			

groups , Tables I3 & I4.Variation inmeal size is again apparent with the total meal size for the 'new' group averaging 124.52mg and the 'old' 96.93mg. The A/B ratios were 1.34 and 0.78 respectively. The amount of food A consumed therefore fell since it formed the major component of the diet in the 'new' group and food 'B', the bran discs, were the major component of the diet in the 'old' group.

Average meal sizes in these feeding tests were still sufficiently high to indicate that both foods are attractive to Deroceras. Approximately 80% of the slugs in the new group consumed some of each type of food and approx. 66% of the slugs in the 'old' group. The consistent amount of the bran discs eaten in both groups suggest that the slugs do not habituate to this food in the same manner that they appear to adapt to the pressed yeast diet. The reason for this is not clear.

4.5 FEEDING TESTS IN WHICH FOOD A AND FOOD B ARE BOTH YEASTS

Table I5 presents the results from a series of tests where slugs were offered two discs of the same food - in each case a yeast/agar mixture. A test is also shown where the sample W8 was combined with a bran extract in place of water to form W8 bran discs. The A/B ratio for all these tests approximated to 1.0 indicating that both food materials are similar in their ability to stimulate feeding in Deroceras. This confirms that the feeding bioassay is suitable for estimating the attractiveness of two food materials as one would expect two discs of the same food to be consumed to the same extent.

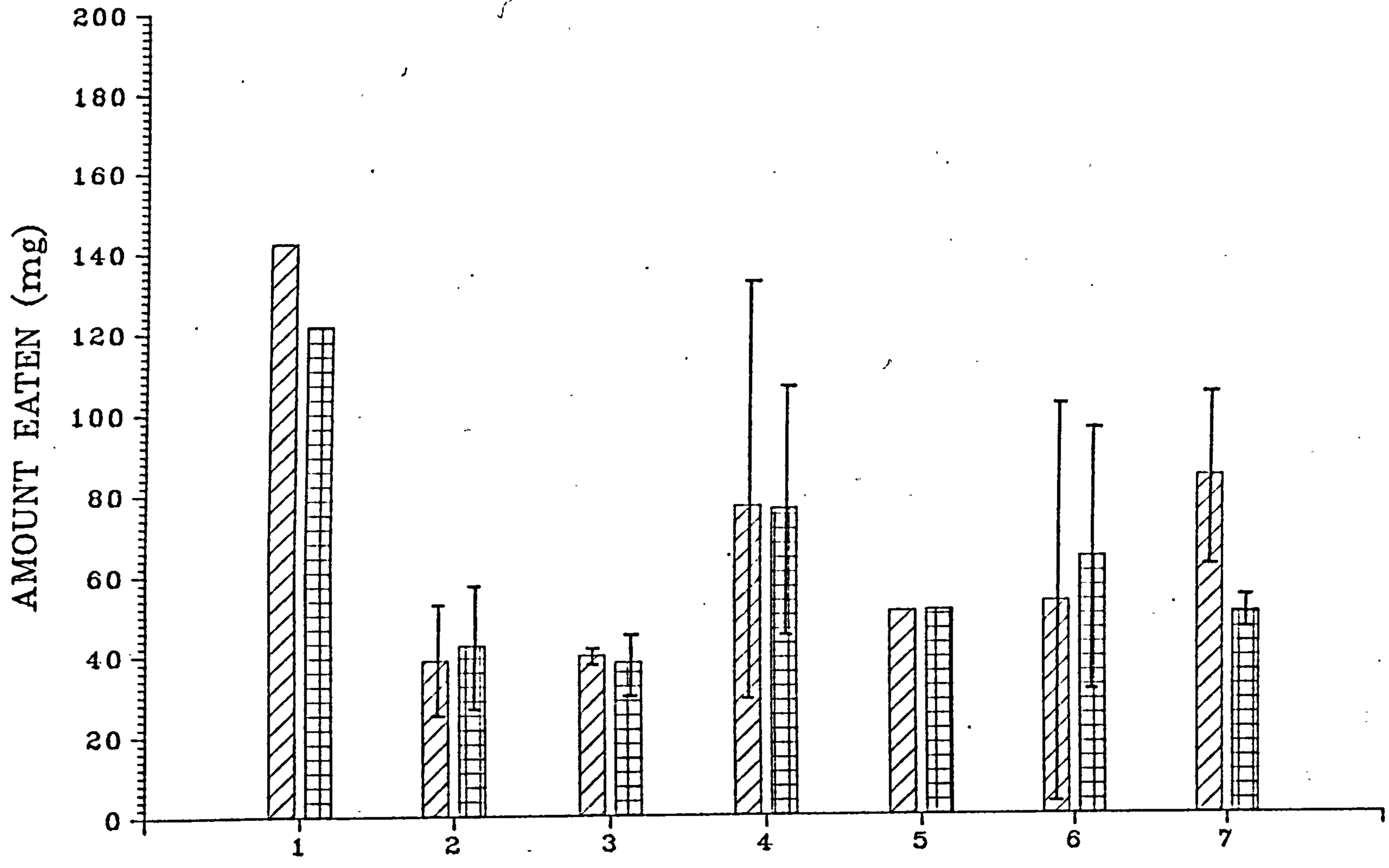
TABLE I5. CONSUMPTION OF FOOD BY Deroceras - TESTS WHERE BOTH FOOD A AND FOOD B ARE PRESSED YEAST

FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
W1		W1			
142.4	80	121.8	80	1.17	264.2
W5		W5			
38.6	60	49.8	60	0.78	88.4
52.2	80	52.2	80	1.00	104.4
25.2	60	25.2	60	1.00	50.4
$\bar{x}$ 38.76	66.67	42.4	66.67	0.91	81.07
S.D. 13.50		14.94			
W6		W6			
38.7	80	43.2	100	0.89	82.1
41.0	80	33.1	80	1.24	74.1
$\bar{x}$ 39.85	80	38.15	90	1.10	78.0
S.D. 1.63		7.14			
W8		W8			
133.1	70	92.4	70	1.44	225.5
61.0	60	96.1	70	0.63	157.1
37.6	70	41.0	80	0.92	78.6
$\bar{x}$ 77.23	66.67	76.50	73.33	1.01	153.73
S.D. 49.78		30.80			
W8Bran		W8Bran			
50.9	70	51.1	70	1.00	102.0
W7		F3			
61.2	80	59.9	70	1.02	121.1
132.7	100	55.7	90	2.38	188.4
19.3	30	115.0	90	0.17	134.3
50.0	80	55.4	70	0.90	105.4
3.4	10	35.5	90	0.10	38.9
$\bar{x}$ 53.32	60	64.3	82	0.83	117.62
S.D. 10.06		29.89			
W8		F9			
100.2	60	47.6	60	2.11	147.8
69.1	90	53.3	70	1.30	122.4
$\bar{x}$ 84.65	75	50.45	65	1.68	134.9
S.D. 21.99		4.03			

FIG. 25 CONSUMPTION OF FOOD BY Deroceras TESTS WHERE BOTH FOOD A AND FOOD B ARE PRESSED YEAST

KEY	hatching		squares
x axis	I	WI	WI
	2	W5	W5
	3	W6	W6
	4	W8	W8
	5	W8Bran	W8Bran
	6	W7	F3
	7	W8	F9

CONSUMPTION OF FOOD BY DEROCERAS
TESTS WITH FOOD(A) & FOOD(B) BOTH YEASTS



Pressed yeast from each brewery was compared by offering slugs a choice of each food in the same feeding test. The results of tests to compare W7 and F3 and W8 and F9 are also presented in Table I5 . Batches W7 and F3 were collected on consecutive days and when offered in a series of feeding tests produced an average A/B value of 0.83 and an average total meal size of 117.62mg. A 't' test to compare the differences between the means of the two sets of paired observations indicated that there was no significant difference ($P = 0.05$) (see Appendix 1). The two samples, W7 & F3, can therefore be considered to be equal in attractiveness. In four of the five tests, the total meal size averaged over 100mg - the fifth test involved 'old' animals which had received this diet on more than two occasions already, and the total meal size, at 38.9mg, was markedly reduced.

The tests with samples W8 and F9 indicated a greater difference with the former sample apparently more attractive. This yeast had been collected 16 days after the F9 sample however, and although the latter had been kept in cold storage, other tests - P_{I70} - had indicated that palatability declines as the sample ages. The percent response was more constant for the two different batches. The results of these feeding tests are presented in Fig 25 .

4.6 FEEDING TESTS TO COMPARE YEAST CONCENTRATION

Sample W2 was formulated into agar sheets at two concentrations - 2.5g wet weight per 100ml water and 5.0g wet weight per 100ml water. Three feeding tests to

TABLE I6 FEEDING TESTS TO COMPARE YEAST CONCENTRATION

FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
W2 2.5g/100ml water		Bran			
36.5	80	6.9	20	5.29	43.4
35.3	70	17.2	50	2.05	52.5
46.7	90	17.2	50	2.72	63.9
$\bar{x}$ 39.50	80	13.77	40	2.87	53.27
S.D. 6.26		5.95			
W2 2.5g/100ml water		W2 5.0g/100ml water			
24.9	40	164.5	80	0.15	189.4
26.2	50	111.5	80	0.23	137.7
36.5	80	82.3	70	0.44	118.8
$\bar{x}$ 29.20	56.67	119.43	76.67	0.24	148.63
S.D. 6.36		41.67			
F4 5.0g/100ml water		F4 5.0g/100ml water			
63.4	80	68.9	70	0.92	132.3
F4 10g/100ml water		F4 5.0g/100ml water			
67.9	80	18.3	40	3.71	86.2
F4 15g/100ml water		F4 5.0g/100ml water			
77.8	70	31.8	40	2.45	109.6
F4 20g/100ml water		F4 5.0g/100ml water			
112.8	90	39.7	50	2.84	152.5

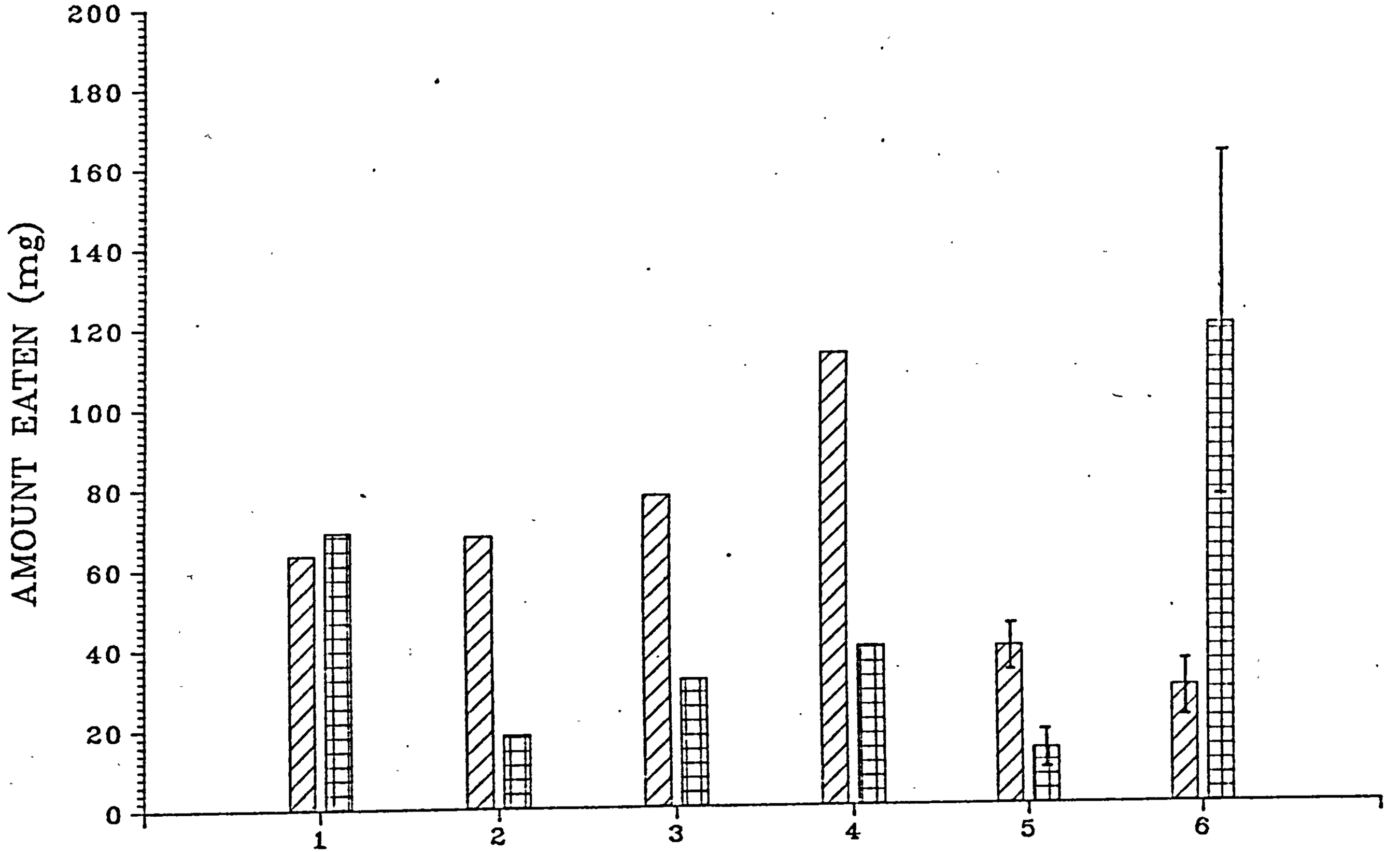
compare the attractiveness of the less concentrated W2 sample with wheatbran discs (Table I6) indicated an average meal size of 53.27mg in 24 hours with the bran forming only a minor constituent of the diet (13.77mg). Three further tests to compare the less concentrated and more concentrated samples of W2, indicated that meal size could be greatly improved by inclusion of the highly attractive stronger W2 sample. An average meal size of 119.43mg was obtained for this food with an A/B value of 0.24 indicating that this food, B, formed the major contribution of the diet, The amount of the weaker W2 eaten fell slightly when these discs were offered in combination with a more attractive food. The results suggest that the concentration of attractive components within the diet can be altered to increase meal size.

Four concentrations of an F4 yeast sample were formulated with water: 5.0g/100ml, 10g/100ml, 15g/100ml and 20g/100ml. Each concentration was offered to ten Deroceras in combination with the standard 5.0g/100ml concentration. The results (Table I6) show a gradual increase in the amount of the F4 discs eaten with increasing concentration so that the 20g concentration is eaten to twice the extent of the 5.0g concentration. This does not mean however that total meal size is correspondingly increased and although the 20g sample of F4 produced the largest meal size, the 10g and 15g concentrations had smaller meal sizes than the weakest 5g formulation. This is due to the variable size of the second component of the diet, in this case a 5.0g formulation of F4. Where the two choices of food were of

FIG. 26 CONSUMPTION OF FOOD BY Deroceras
TESTS TO COMPARE YEAST CONCENTRATION

KEY		hatching	squares
x axis	I	W2 2.5g/IOOml	wheatbran
	2	W2 2.5g/IOOml	W2 5.Og/IOOml
	3	F4 5.Og/IOOml	F4 5.Og/IOOml
	4	F4 IOg /IOOml	F4 5.Og/IOOml
	5	F4 I5g /IOOml	F4 5.Og/IOOml
	6	F4 20g /IOOml	F4 5.Og/IOOml

CONSUMPTION OF FOOD BY DEROCERAS
TESTS TO COMPARE YEAST CONCENTRATION



equal concentration, the slugs consumed approximately 65mg of each to give a total meal size of 132.3mg in 24 hours. With the stronger W4 formulations, the consumption of the standard 5.0g formulation fell but there was some evidence that improving the concentration of the feeding stimulant to 20g/100ml water encouraged the slugs to generally consume larger meals.

Maximum meal size may well be limited by stretch receptors in the slug gut but within this constraint there is much scope for manipulating the consumption of individual constituents of the diet. The results of these tests are presented graphically in Fig.26 .

4.7 DETERIORATION OF YEAST SAMPLE

The pressed yeast collected from a brewery is a highly perishable material with a water content of up to 80% but since most of the water is held within the yeast cells the structure of the material is fairly solid. The texture can vary from a sticky dough to a dry, crumbly consistency depending on the efficiency of the presses, (Acraman & Smith 1976). The life of the yeast cells is dependent on temperature and unless stored at low temperature the pressed yeast deteriorates in a number of days. When the yeast cells die, the membrane ceases to function and the osmotic pressure gradient falls. Liquids then diffuse through the cell wall, breaking down the structure of the material. The semi-liquid yeast evolves large amounts of CO_2 which causes foaming and hastens the breakdown of the structure. Flavour spoilage of the yeast

can then readily occur.

The importance of deterioration and the formation of possible spoilage ingredients necessitated that the agar sheets incorporating the yeast be made up on the day of collection. The shelf life could be prolonged by storing the pressed yeast in a cold room or freezer, but the attractiveness of the material appeared to decline with age which made it difficult to compare successive batches with one another. The most recently collected batch was always the more attractive component in a feeding test. Table I7 shows the results of three feeding tests where both food items are yeast samples collected from the same brewery.

TABLE I7. FEEDING TESTS ON DETERIORATION OF YEAST

Amount eaten mg	Percent response	Amount eaten mg	Percent response	A/B	Total eaten mg
W1		W2			
119.5	90	220.7	100	0.54	340.2
W5		W6			
20.7	60	94.9	100	0.22	115.6
W7 old		W7 new			
41.9	50	84.4	70	0.50	126.3

The test to compare W1 (food A) and W2 (food B) gave an A/B value of 0.54 although the total meal size was exceptionally high at 340.2mg. The interval between collection of the two samples was only 3 days and the high meal size of W1 indicated that this batch was still attractive but the more recently produced W2 sample was even more palatable. A comparison between batches W5 and W6 gave an A/B value of 0.22 and the small meal size of W5 indicates

that this 15 day old sample had lost much of its attractiveness. Although all ten animals ate the new W6 yeast discs the amount eaten was much lower than the corresponding meal size for the W1 - W2 feeding test. This would support the concept of batch variability. A further test to compare 'old' agar discs of sample W7 with 'new' discs, formulated eight days later from a portion of this same yeast sample stored in a freezer, produced an A/B value of 0.50. Incorporating the yeast into the agar sheets does not therefore appear to prevent the deterioriation of the attractive nature of the material.

It would seem that the yeast complex rapidly loses at least some of its attractive qualities. This may be due to the loss of volatiles, but since further feeding tests - P I83- indicated that the volatiles are not of major importance in conferring palatability, this is unlikely. It is more probable that spoilage factors form within the yeast and the rate of formation may depend on the amount of water initially present in the sample and the conditions under which it is stored. This may be the cause of inconsistencies between different batches since the yeast is continuously filtered off from the beer and the vats from which the samples were taken may have been awaiting collection for two or three days. The actual age of each sample was therefore unpredictable and the variable water content of each would have affected the rate of deterioration.

The feeding tests had however, indicated a definite increase in palatability of the agar discs when this yeast complex was incorporated into the solution, so

despite the shortcomings of standardisation with this attractant, further tests were made to attempt to define the feeding incentive contained within the brewers yeast.

Preliminary feeding tests indicated that the brewers yeast was the most attractive material produced during the fermentation process and could be readily incorporated into agar sheets for use in a feeding bioassay. The pressed yeast obtained from a brewery is a mass of yeast cells combined with a number of other components which have been produced during the fermentation process. At the stage in the process when the yeast is separated off from the wort (which is refined to produce the final product, beer) germinated barley, hops and yeast have all contributed to the flavour of the material. Chemicals formed during the metabolism of the yeast add to the flavour and the resultant complex of odorous substances is extremely difficult to analyse even with highly sophisticated apparatus.

The feeding tests have attempted to eliminate constituents of the yeast complex which are unattractive to Deroceras and to highlight those which appear to be responsible for the superior performance of the substance as a food material. A product such as brewers yeast is extremely suitable for use as an attractant in baits since it is a waste product and produced in large quantities and the feeding tests indicate that it increases meal size when compared with a conventional attractant such as bran. The problems that do exist with this material are associated with the rapid deterioration and possible variability between yeast produced at different sites and on different days.

These two factors may be linked however since different batches of yeast may have a variable water content and water present in the yeast accelerates its breakdown. It is suggested that freeze-drying the material to remove the water would preserve the attractive qualities and make the yeast suitable for incorporating into commercial baits. Breweries do freeze-dry some of the yeast they produce, particularly if the material is not to be collected for a number of days, so freeze-drying does appear to be an accepted method of prolonging the life of the yeast.

4.8 FEEDING TESTS WITH FREEZE-DRIED YEAST

A method was sought to extend the time within which the yeast sample could be used in feeding tests. Since the water content of the material accelerates spoilage, a sample of pressed yeast was freeze dried to remove the water and enable the yeast to be reconstituted at will. This was then compared with freshly collected yeast to determine whether the attractive qualities of the yeast are preserved in the freeze-drying process.

A small quantity of yeast was made into a slurry with distilled water and poured into a 250ml glass vessel. This was rotated so that the yeast mixture formed a thin film over the entire inner surface of the vessel which was then slipped into a bath of liquid nitrogen. This was left for approximately 12 hours so that the yeast solution was frozen into the inner surface and the vessel was then attached to a freeze drying machine (New Brunswick Scientific C. Cryoliser - Freeze Dryer) with a series of airtight joints.

TABLE I8 FEEDING TESTS WITH DIACETYL (Deroceras caruanae)

FOOD A amount eaten mg	response percent	FOOD B amount eaten mg	response percent	A/B	total eaten mg
Diacetyl A		Bran			
0.0	0	129.9	80	-	129.9
Diacetyl B		Bran			
0.0	0	124.4	80	-	124.4
Diacetyl C		Bran			
0.0	0	123.1	100	-	123.1
Diacetyl D		Bran			
6.9	20	125.2	100	0.06	132.1
Diacetyl E		Bran			
3.4	10	38.0	70	0.09	41.4
0.0	0	34.15	60	-	34.15
6.9	20	33.1	70	0.21	40.0
$\bar{x}$ 3.4	10	35.08	66.67	0.10	38.48

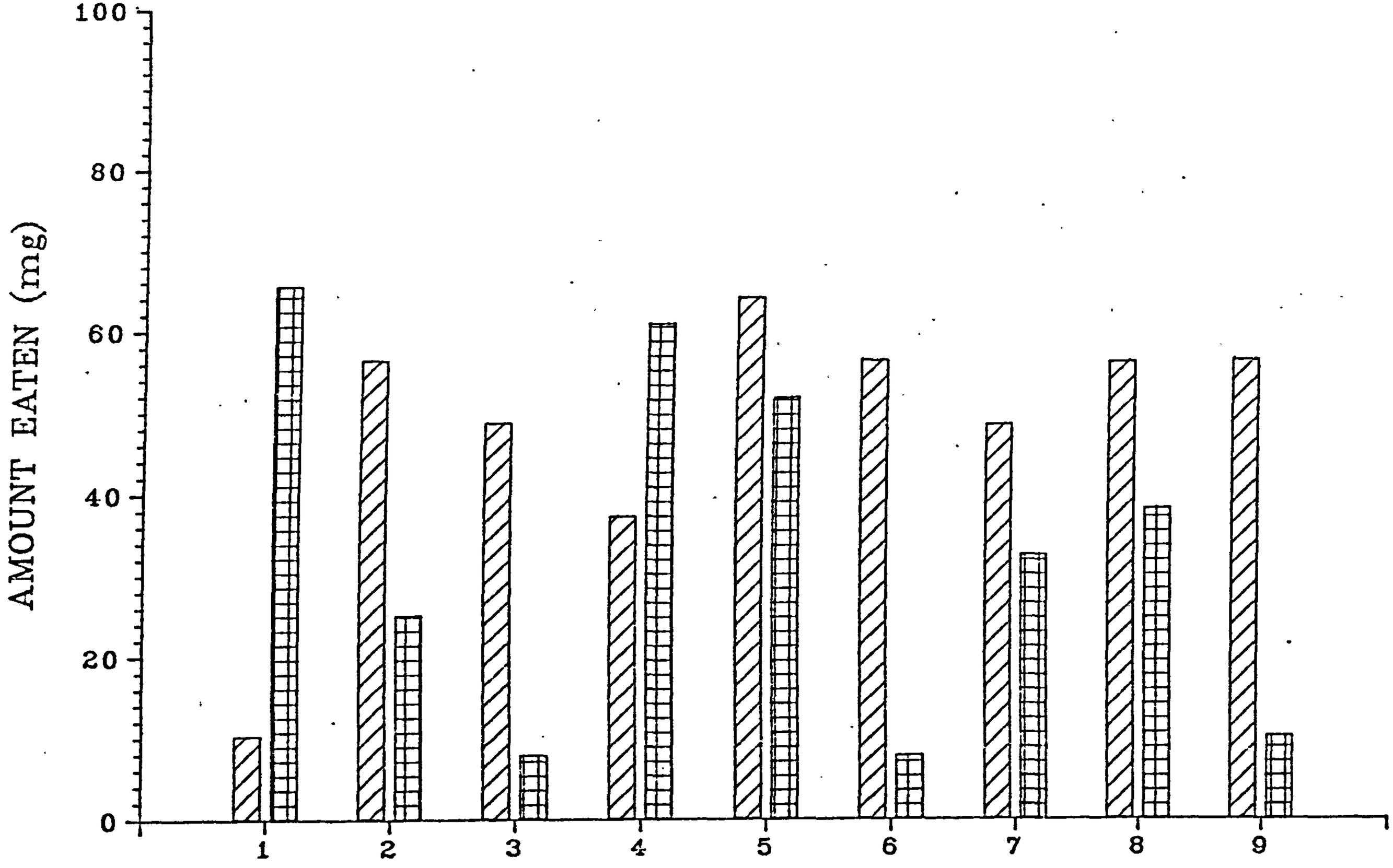
TABLE I9 FEEDING TESTS WITH FREEZE DRIED YEAST

FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
W7 A		W7			
10.3	30	65.7	80	0.16	76.0
W7 B		W7			
56.7	80	25.2	60	2.25	81.9
W7 C		W7			
49.0	90	7.9	10	6.20	56.9
W7 A		F3			
37.6	70	61.2	80	0.61	98.8
W7 B		F3			
64.4	70	52.2	80	1.23	116.6
W7 C		F3			
56.8	100	7.9	10	7.19	64.7
W7 A		Bran			
49.0	90	32.9	50	1.49	81.9
W7 B		Bran			
56.7	80	38.6	60	1.47	95.3
W7 C		Bran			
56.9	80	10.3	30	5.52	67.2

FIG. 27 CONSUMPTION OF FOOD BY Deroceras
TESTS WITH FREEZE-DRIED YEAST

KEY x axis	hatching	squares
I	W7 freeze dried 'A'	W7
2	W7 freeze dried 'B'	W7
3	W7 freeze dried 'C'	W7
4	W7 freeze dried 'A'	F3
5	W7 freeze dried 'B'	F3
6	W7 freeze dried 'C'	F3
7	W7 freeze dried 'A'	wheatbran
8	W7 freeze dried 'B'	wheatbran
9	W7 freeze dried 'C'	wheatbran

CONSUMPTION OF FOOD BY DEROCERAS
TESTS WITH FREEZE DRIED YEASTS



Water was removed from the sample at low temperature and pressure for several hours with the glass vessel sitting in a bath of acetone/dry ice to ensure the sample remained frozen. The yeast formed fine grains caking the inside of the glass - these were scraped off and collected to form approximately 1g. of the freeze dried yeast concentrate.

Freeze dried brewers yeast was compared with freshly collected yeast and with bran in a series of feeding tests. The freeze dried compound was reconstituted with either water or hopped wort:

0.5g in 100ml. water - A

1.0g in 100ml. water - B

1.0g in 100ml. hopped wort - C

Hopped wort, obtained from a brewery, is the solution which is filtered off from the hops. It is rich in sugars and has the bitter flavour from the hops but has not yet been inoculated with yeast.

1.0g of agar powder was added to each solution and the mixtures were then heated so that they would set and form solid sheets from which discs could be cut. Discs of each type, A, B and C were offered to slugs in combination with either standard (5g. wet weight in 100ml water) W7 yeast, standard F3 yeast or wheatbran. The results are shown in Table I9 and Fig 27.

All the animals used in these tests had also been used in earlier feeding tests to assess the attractive nature of brewers yeast, No new slugs were available as the weather was dry when these samples were prepared. The total meal size is therefore lower than could be expected

if the animals had been recently collected.

The W7 A sample - 0.5g freeze dried yeast in 100ml water - was less palatable than the standard 5g wet weight of both the W7 and the F3 agar discs, though the total meal size was relatively high as these latter foods were readily consumed. The W7 A was, however, more palatable than the bran control discs with an A/B value of 1.49.

The W7 B discs were rather more attractive than the standard W7 preparation with an A/B value of 2.25. Palatability was approximately equal to the F3 discs (A/B of 1.23) and improved when compared to the bran discs (A/B of 1.47). This concentration of 1.0g freeze dried yeast reconstituted in 100 ml water is perhaps the level that is most consistent with the standard preparation of fresh yeast.

The freeze dried samples reconstituted in wort, W7 C, showed improved palatability in all three tests with an A/B value of 6.2, 7.19 and 5.52 respectively for the standard W7, the standard F3 and the bran discs. The consumption of these control foods, food B in each test, was markedly reduced when compared with the previous tests and the number of slugs eating the discs was low. Total meal size was not therefore increased despite the attractiveness of the freeze dried yeast in hopped wort, food A in each case. This indicates the complexity of feeding in Deroceras since the slugs may consume a large volume of an attractive food in preference to another palatable food, but without reaching a total meal size that other feeding tests have indicated is possible. The reason for the fall in consumption of food B when presented to the slugs in

combination with the W7 C discs is not clear as these hopped wort discs are readily consumed themselves and are therefore not producing repellents.

4.9 YEAST ANALYSIS

Having established the attractive nature of the yeast, further analysis of the compound was necessary to determine precisely which constituents of it increased feeding activity of slugs. The pressed yeast obtained from a brewery is a mixture of yeast cells and the products of fermentation. The cell membrane regulates movement of compounds into and out of the cell so lipophilic solubility, molecular size and degree of branching of the molecule are important factors in determining both the uptake and retention of materials from the fermentation vat. The yeast used in the fermentation process can thus influence the nature of the products formed. Metabolism of the yeast removes most of the sugars in the wort but residual carbohydrates account for the sweetness of the beer. The bulk of the amino acids of the wort are also assimilated during fermentation so that beer contains only low levels of amino acids and the pressed yeast is rich in this nutritional component. As the yeast multiplies during the fermentation process to between three and eight times its initial volume, a large surplus is formed. This is then sold to a commercial food manufacturer and a savoury yeast extract is produced after the addition of flavourants. The crude analysis of pressed yeast obtained from the food company highlights the nutritional value of this:

A is a typical yeast extract analysis (Ellison J. 1974). (Approximately 98% of the yeast extract is pressed yeast).
B is from a table of by-products of the brewing process industry (Ingledew et al 1977)

	A	B
Moisture	27%	4.3
Protein	44%	50.0
Salt (as NaCl)	10%	-
Ash (excluding NaCl)	13%	10.0
Carbohydrate	6%	-
Fat	Trace	0.5
Crude fibre	-	0.5
N_2 free extract	-	34.7

This commercial utilisation of brewers yeast involves hydrolisation of yeast protein by autolysis - this is initiated by plasmolysis of the yeast cells with heat and sodium chloride. The yeast cell bodies are then removed by centrifugation. The isohumulones, which are responsible for the bitter flavour, must also be removed so the pH of the mixture is raised to dissociate the molecules into soluble salts which are easily extracted (Acraman 1966).

A sample of the freeze .ried yeast, batch F8, was analysed by B.P. Research Centre, Sunbury for volatile components, using gas chromatography techniques. Distilled water and pentan - 3 - one were added to a a weighed portion of the sample and eluted on a column - 2.15m x 4mm ID 20

per cent TCP ON chromosorb W. The temperature was maintained at 56°C and the sample analysed intermittently over a period of 18 hours after which there was no significant increase in the levels of the volatile constituents. The resultant analysis was:

COMPONENT	% BY MASS
unknown	0.0004
acetaldehyde	0.03
acetone	0.005
ethanol	2.8
diethyl acetal	0.01
unknown	0.005

The feeding bioassay used to assess the feeding activity of Deroceras on various foods was limited by the fact that the agar mixture required heating to incorporate the test chemicals into the solution. Inflammable materials were therefore unsuitable for use in such a test and the volatile materials described above could not, by virtue of their volatility, be combined into these agar sheets.

The volatile components of the yeast are therefore of more incidental interest. The alcohol content of most beers is 3 - 5% by volume and since most of this appears to be lost as volatiles, it cannot be an important component of the attractant present in the yeast/agar discs.

Evidence from the literature suggests that other fermented compounds are unattractive to slugs so that it is more likely to be flavour components of beer which are responsible for its palatability to slugs.

Selim (1973) used steam distillation and solvent extraction to fractionate beer and compare each extract for its ability to attract slugs. The simple bioassay he employed involved placing 50ml beer or test solution amid slug infested ivy and the results are reported as the ratio of drowned slugs in the beer control to those in the test solution. The animals found in his test were almost exclusively Limax marginatus. He suggests that three chemicals could be involved in the attractive nature of beer. Diacetyl (butane - 2, 3 - dione) is an important flavour constituent of beer but produces an off flavour if the concentration rises. Watney-Mann believe that the concentration of diacetyl in their beer is 50 - 100 p.p.b. (threshold level of detection is 100p.p.b.) and that of acetoin is 2 p.p.m. (threshold level of detection is 50 p.p.m.) Dihydroxyacetone is not known to them. It may be that American beers - Selim's work was carried out using canned American beer - have a higher level of these chemicals, as the diacetyl is at the limit of its detection in British beers and acetoin is generally present in high gravity beer only.

Diacetyl was selected as a test chemical for the feeding bioassay - since it is miscible with water, it presented no problem in formulation. Slugs are reportedly attracted to stale beer in which the concentration of this

chemical may have risen, so a representative range of diacetyl concentrations was examined. The lower concentrations of diacetyl (1 - 100 ppb.) are similar to the level found in beer, yet there was no evidence of this chemical stimulating feeding activity. The results suggest that diacetyl may, in fact, be exerting an inhibitory effect on feeding in D. reticulatum. All the values for diacetyl compared unfavourably with the wheatbran control which was eaten in large quantities by the D. caruanae. Since Selim's figures for the number of slugs attracted to diacetyl were low (one slug compared with 25 attracted to a beer sample) and diacetyl was the most promising of his three candidate attractants, it was decided, on the basis of the above results not to continue with feeding tests on the chemicals he proposed as attractants. (See Table I8).

In a report on beer quality, Hough et al (1971) have reviewed the major chemical constituents of beer. Although ethanol and the higher, fusel, alcohols are quantitively the most important volatile components, they are not necessarily the most potent flavouring agents. Esters, acids and aldehydes, notably acetaldehyde, can be detected at a lower threshold than the corresponding alcohols. Bishop (1971) has also reported that although the most important single flavour characteristic of beer is bitterness, only 15 - 60 ppm. of bitter substances are detected.

The principal higher alcohols in beer are 3-methylbutanol (isoamyl alchohol), 2-methylbutanol (active amyl alcohol), 2-methylpropanol (isobutyl alcohol), propanol and

phenethyl alcohol (B-phenylethanol). Engan (1974) reports that the concentration of these fusel alcohols changes throughout the fermentation process and is dependant on the yeast strain, the composition of the wort and many other factors. The levels of the amyl alcohols, the most important influencers of flavour, reportedly decline with age emphasising the difficulties of producing a standard flavour profile.

Other flavours are common to commercial beers, Clapperton (1978) found that the caprylic flavour was due to an additive effect of several carboxylic acids : C_6 - hexanoic acid, C_8 - octanoic acid, C_{10} - decanoic acid and C_{12} - dudecanoic acid. Flavour analysis of beer generally combines a detailed chemical analysis with sensory evaluation by a panel of testers. These results have indicated that many synergistic and antagonistic interactions occur and that threshold values may not be directly interpreted as the level which will initiate a response.

As beer contains many thousand chemicals, a systematic approach was adopted in an attempt to provide a general hypothesis for the type of attractants found in beer, rather than the examination of beer components on an ad hoc basis.

4.10 FEEDING TESTS TO COMPARE VOLATILE AND NON-VOLATILE COMPONENTS OF YEAST

A pressed yeast sample, W6, was analysed for the attractive nature of the volatiles using vacuum distillation to separate off the volatile chemicals. A small amount of pressed yeast was predried in a weighed

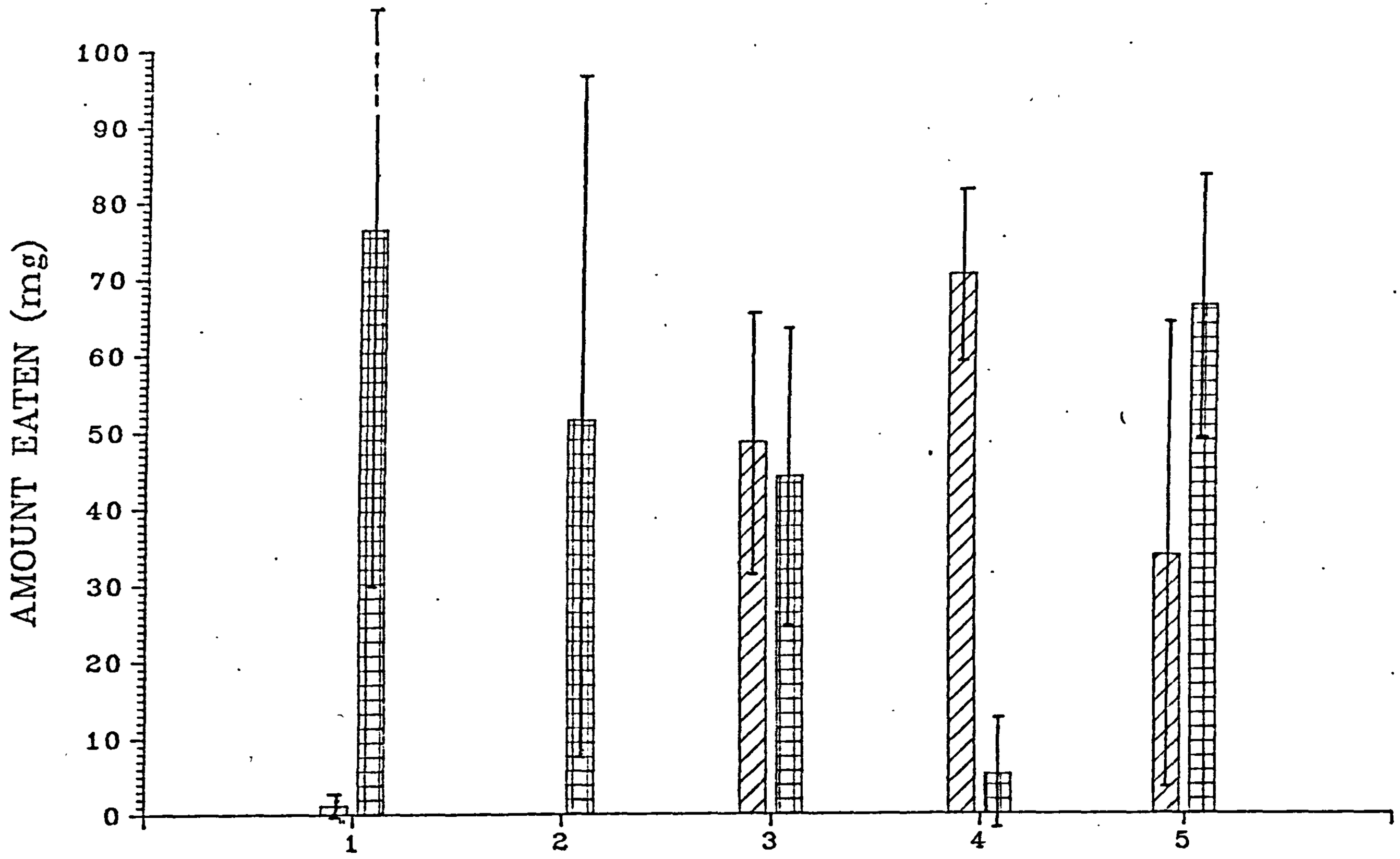
TABLE 20 FEEDING TESTS TO COMPARE VOLATILE AND NON-VOLATILE COMPONENTS OF YEAST

FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
Sample I (W6)		Bran			
0.0	0	125.3	100	-	125.3
3.4	10	71.3	90	0.48	74.7
0.0	0	32.9	50	-	32.9
$\bar{x}$ 1.13	3.33	76.5	80	0.01	77.63
S.D. 1.96		46.42			
Sample I (W6)		W5			
0.0	0	21.7	50	-	21.7
0.0	0	81.6	100	-	81.6
$\bar{x}$ 0.0	0	51.65	75	-	51.65
S.D. 0.0		42.36			
Sample 2 (W6)		Bran			
65.7	80	44.3	70	1.48	110.0
34.2	60	63.4	80	0.54	97.6
46.6	70	25.2	60	1.85	71.8
$\bar{x}$ 48.83	70	44.3	70	1.10	93.13
S.D. 15.87		19.10			
Sample 2 (W6)		W5			
78.0	90	10.3	30	7.57	88.3
63.6	100	0.0	0	-	63.6
$\bar{x}$ 70.8	95	5.15	15	13.75	75.95
S.D. 10.18		7.29			
Sample 2 (W6)		W6			
7.9	10	74.3	60	0.11	82.2
67.9	80	46.6	70	1.46	114.5
26.2	50	79.0	80	0.33	105.2
$\bar{x}$ 34.0	46.67	66.63	70	0.51	100.63
S.D. 30.75		17.51			

FIG 28 CONSUMPTION OF FOOD BY Deroceras
TESTS TO COMPARE VOLATILE AND NON-VOLATILE COMPONENTS OF YEAST

KEY	hatching	squares
x axis	I W6 sample I	wheatbran
	2 W6 sample I	W5
	3 W6 sample 2	wheatbran
	4 W6 sample 2	W5
	5 W6 sample 2	W6

CONSUMPTION OF FOOD BY DEROCERAS TESTS TO COMPARE VOLATILE & NON-VOLATILE COMPONENTS OF YEAST



vessel at water pump vacuum over concentrated hydrochloric acid. This was then placed in Quickfit apparatus and evacuated to 0.1mm pressure using a vacuum pump. The volatiles were collected on a molecular sieve in a bath of liquid nitrogen and the system subsequently reversed to collect the volatiles from the molecular sieve. This distillation crudely separated the yeast sample into two components - the volatiles (Sample 1) and the more stable fraction (sample 2). These two components were analysed for their attraction to slugs by combining into agar sheets and feeding to Deroceras. The results are presented in Table 20 and Fig.28 .

The volatiles collected in the distillation of a sample of pressed yeast appeared to be unattractive when compared with agar discs containing wheatbran. This may however, be due to the fact that the volatile chemicals were lost in the preparation of the agar discs and these results must therefore be treated with caution. It is apparent though, that the non-volatile material, sample 2, is attractive to Deroceras when offered in combination with either bran or pressed yeast discs of agar. The sample 2 and the bran discs were of similar attractiveness, with an A/B ratio of 1.10 for three feeding tests. The sample 2 proved less attractive than a freshly collected yeast complex, W6, but more attractive than an older yeast sample, W5, which had been maintained in a cold room for three weeks. This further supports the hypothesis that the attractive nature of the pressed yeast declines with age.

The amount of sample 2 agar discs consumed by the

slugs indicates that the major attractiveness of the yeast resides in the non-volatile chemicals rather than those which are readily vapourised - this was expected since the preparation of the agar sheets involves heating the solution and volatile chemicals would be lost at this stage. Some volatiles may contribute to the attractiveness of the yeast but their loss by distillation does not diminish the amount eaten by slugs to any great extent.

4.11 FEEDING TESTS WITH CENTRIFUGED YEAST

The yeast sample F2 was centrifuged to compare the yeast cells and the intracellular material, for their ability to stimulate feeding activity in Deroceras, and to determine whether either component could individually prove as attractive as the combined product. The sample was therefore centrifuged so that the yeast cells sank to the bottom and the supernatant could be separated. Both components were made into agar sheets and offered to the slugs in combination with either wheatbran or standard preparations of samples F2 and F3. The results are presented in Table 2I and Fig. 29 .

The F2 supernatant, as food A, gave an A/B value of 0.60 with the standard 5g wet weight formulation of F2 as food B and a value of 0.21 with F3 as food B. The newer yeast, F3, produced a much higher meal size of 129.8mg which is consistent with other results showing that the freshest yeast is most attractive.

Meal sizes with the yeast sediment were, however, regularly high. Comparing F2 sediment, food A, with bran, food B, produced an average A/B of 2.52 - this included one

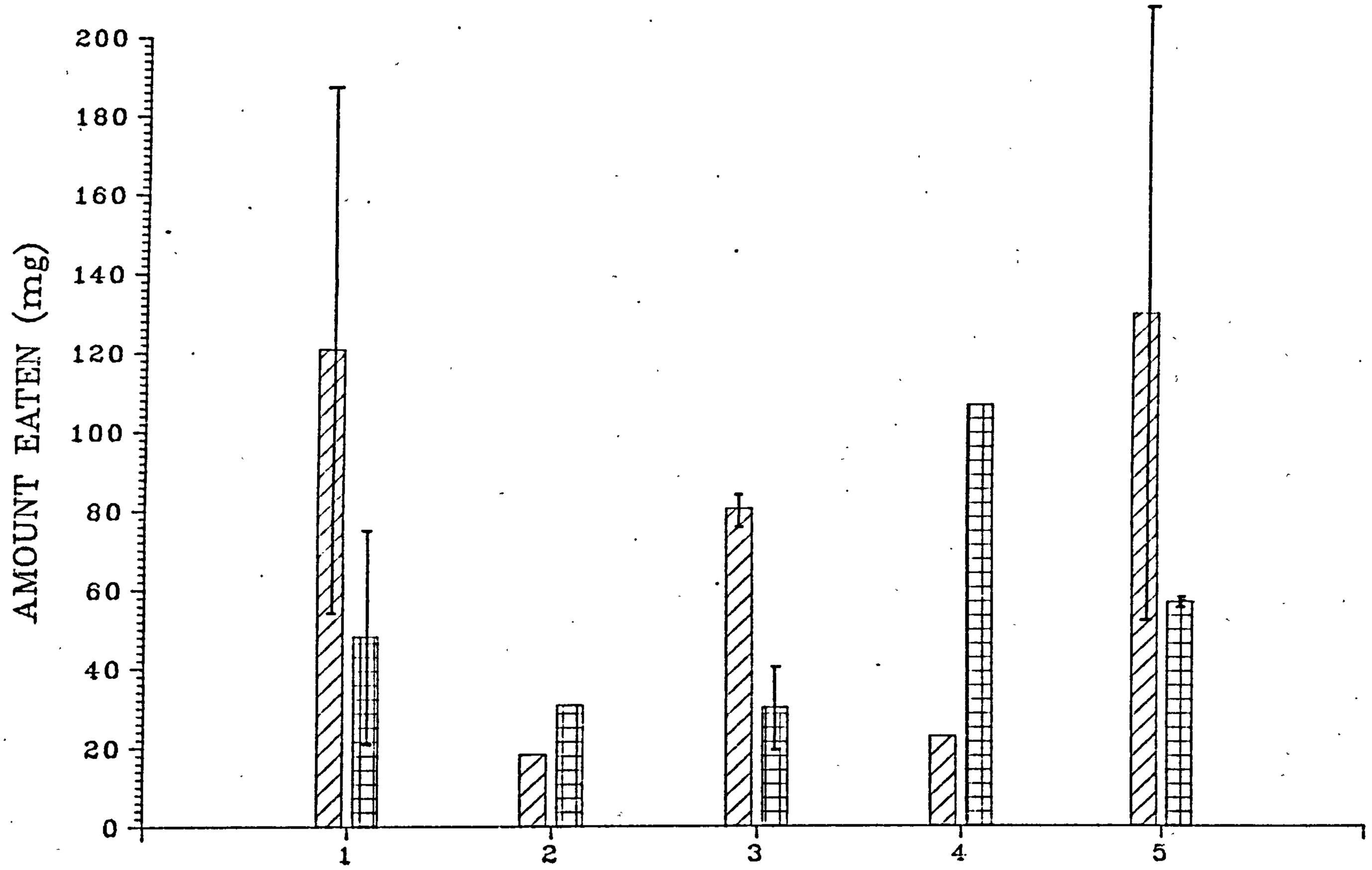
TABLE 2I FEEDING TESTS WITH CENTRIFUGED YEAST

FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
F2 sediment		Bran			
75.7	60	94.9	80	0.80	170.6
223.1	90	20.7	60	10.78	243.8
189.4	80	33.1	70	5.72	222.5
77.2	90	63.2	60	1.22	140.4
66.6	70	27.6	80	2.41	94.2
93.6	90	49.0	90	1.91	142.6
$\bar{x}$ 120.93	80	48.08	73.33	2.52	194.26
S.D. 67.50		27.62			
F2 supernatant		F2			
18.3	40	30.7	50	0.60	48.0
F2 sediment		F2			
76.7	80	23.8	30	3.22	100.5
88.2.	60	26.2	50	3.37	114.4
75.7	60	22.8	40	3.32	98.5
82.6	80	47.7	80	1.73	130.3
$\bar{x}$ 80.80	75	30.13	50	2.68	110.93
S.D. 5.80		11.80			
F2 supernatant		F3			
22.8	40	107.0	90	0.21	129.8
F2 sediment		F3			
180.3	90	56.5	60	3.19	236.8
80.2	50	57.6	40	1.39	137.8
$\bar{x}$ 130.25	70	57.05	50	2.28	187.3
S.D. 70.78		0.78			

FIG 29 CONSUMPTION OF FOOD BY Deroceras
TESTS WITH CENTRIFUGED YEAST

KEY	hatching	squares
xaxis	I F2 sediment	wheatbran
	2 F2 supernatant	F2
	3 F2 sediment	F2
	4 F2 supernatant	F3
	5 F2 sediment	F3

CONSUMPTION OF FOOD BY DEROCERAS
TESTS WITH CENTRIFUGED YEAST



test where the ratio was less than 1.0 as 'old' animals were used and the feeding response had apparently declined. The high palatability of the yeast sediment was shown to persist when it was compared with the standard F2 yeast as food B. The average A/B value of 2.68 and the total meal size of 110.93mg eaten in 24 hours indicated a preference for the yeast sediment rather than the whole yeast sample and suggested that the attractiveness resides largely in the sediment. Comparing the F2 sediment with a newer yeast sample F3 as food B showed that the sediment preference was still sustained, even when a newer yeast sample was offered. The amount of food B eaten was improved but the number of animals eating the whole yeast sample was not improved.

4.12 FEEDING TESTS WITH BUFFERED AND DEBITTERED YEAST

Four different samples of pressed yeast in agar were prepared using a phosphate buffer (pH 7) in place of water. These agar discs were compared with standard preparations of either pressed yeast, wheatbran or a combination of these two foods (W8 Bran).

A similar preparation of debittered yeast was prepared by washing a yeast sample (F2) with sodium bicarbonate and formulating this into agar sheets. A preparation was also made using a commercially debittered yeast, Yesta, which is used as a flavourant in the food industry. 1g of Yesta powder was dissolved in 100ml water and made up into agar sheets in the standard way. The results from these feeding tests are presented in Table 22

TABLE 22 FEEDING TESTS WITH BUFFERED AND DEBITTERED YEAST

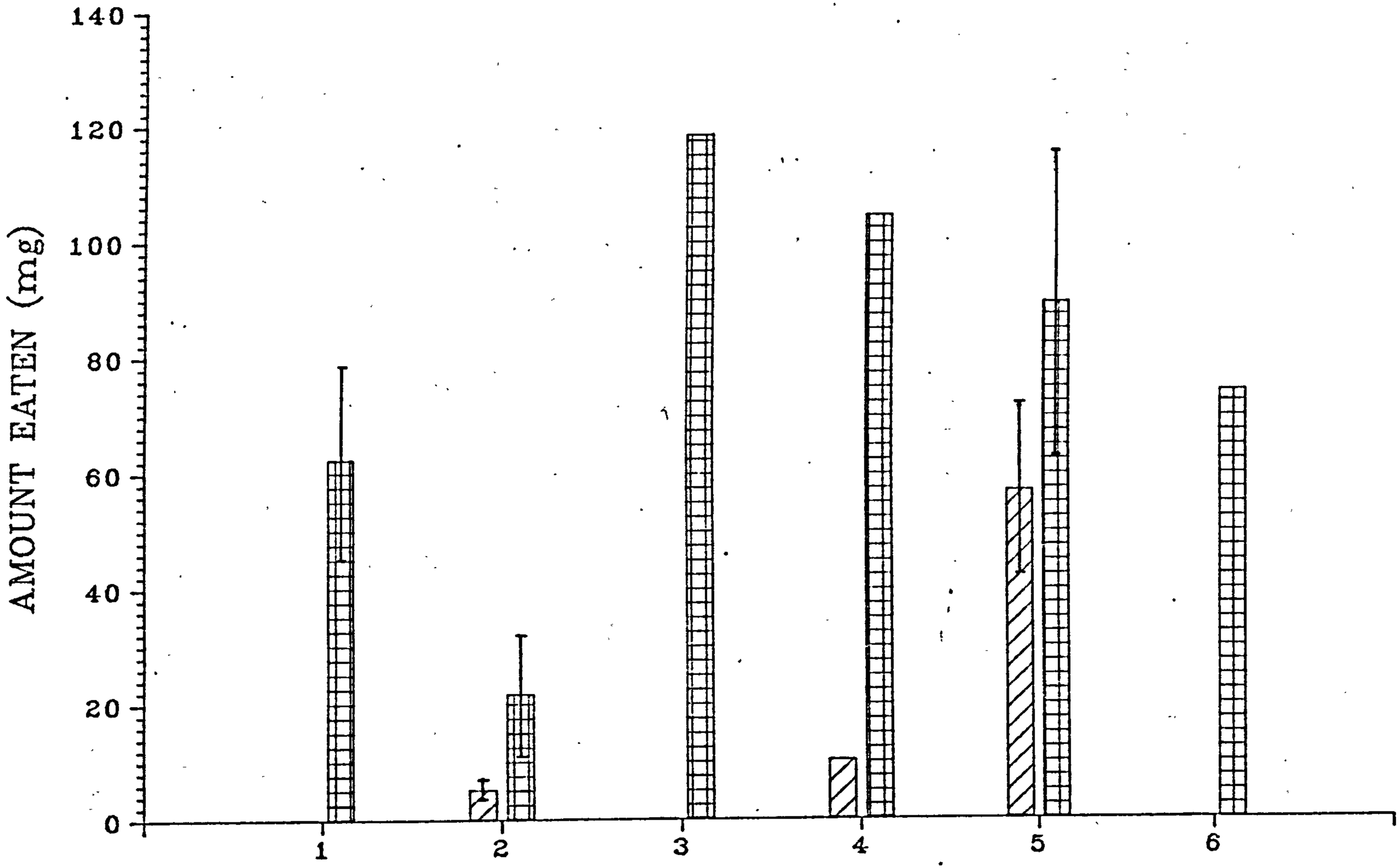
FOOD A amount eaten mg	FOOD A percent response	FOOD B amount eaten mg	FOOD B percent response	A/B	total eaten mg
F2 Buffered		F2			
0.0	0	77.8	70	-	77.8
0.0	0	65.7	80	-	65.7
0.0	0	43.8	70	-	43.8
$\bar{x}$ 0.00	0	62.43	73.33	-	62.42
S.D. 0.0		17.23			
F6 Buffered		F6			
6.9	20	29.7	60	0.23	36.6
3.4	10	13.8	40	0.25	17.2
$\bar{x}$ 5.15	15	21.75	50	0.24	26.90
S.D. 2.47		11.24			
W8 Buffered		W8Bran			
0.0	0	118.6	100	-	118.6
F7 Buffered		Bran			
10.3	30	104.7	70	0.10	115.0
Yesta - debittered		Bran			
58.7	60	90.3	70	0.65	149.0
75.9	80	71.1	70	1.07	147.0
36.3	60	99.5	90	0.36	135.8
44.0	50	90.3	70	0.49	134.3
74.6	70	53.3	70	1.40	127.9
53.3	70	134.0	80	0.40	187.3
$\bar{x}$ 57.13	65	89.75	75	0.64	146.88
S.D. 16.01		27.31			
F2 plus $NaHCO_3$		Bran			
0.0	0	74.5	80	-	74.5

FIG 30 CONSUMPTION OF FOOD BY Deroceras

TESTS WITH BUFFERED AND DEBITTERED YEAST

KEY	hatching	squares
x axis	I F2 buffered	F2
	2 F6 buffered	F6
	3 W8 buffered	W8Bran
	4 F7 buffered	wheatbran
	5 Yesta - debittered	wheatbran
	6 F2 + $NaHCO_3$	wheatbran

CONSUMPTION OF FOOD BY DEROCERAS
TESTS WITH BUFFERED & DEBITTERED YEASTS



and Fig. 30 .

The tests with buffered yeast samples all indicated that pH is important in maintaining the attractiveness of the yeast discs. Feeding activity was minimal on all discs formulated with the phosphate buffer and as the total amount eaten in each was reasonably high, the A/B values are all extremely low. The importance of pH may have contributed to the lack of palatability of the yeast sample debittered by the addition of $NaHCO_3$ since this also altered the pH of the mixture. The normal pH of the wort/yeast mixture is approximately pH4 and although this difference appears to affect the palatability of the yeast discs, the cause of this loss of attraction is not easy to define.

Yesta, a commercial debittered yeast obtained from Bovril Ltd, produced consistently high meal sizes when offered in combination with bran discs. The A/B value of 0.64 indicates that the bran was more palatable but the number of slugs consuming the discs was approximately equivalent. Although the Yesta product is debittered, it does have a strong flavour of its own which may be responsible for the rather high feeding activity on this food. In the commercial debittering process, substances which have been absorbed onto the yeast surface during the fermentation process are removed. These are mainly proteins originating from the barley and substances from the hops.

The tests to compare a sample of F2 which has been debittered by the addition of sodium bicarbonate, with standard bran discs indicated that the debittered sample

loses all its attractiveness. It is possible that the addition of the $NaHCO_3$ produced antifeedants but other tests have indicated that the hops cause an increase in the palatability and their removal is therefore likely to decrease consumption.

4.13 FEEDING TESTS WITH YEAST CULTURES

Samples of the two yeasts normally used in the brewing industry, Saccharomyces cerevisiae (SCV) and S. carlsbergensis (SCB) were obtained from the Commonwealth Mycological Institute, Kew, and cultured on sterile agar plates. Agar discs were initially prepared by scraping yeast filaments from the surface of the culture, dissolving in 100ml water and combining with powdered agar in the usual manner. Further feeding tests were then carried out using discs cut directly from the agar culture. Results from these tests are shown in Table 23 and Fig. 3I.

A solution of yeast filaments incorporated into agar sheets produced only small meal sizes averaging 28.7mg for the SCB - wheatbran tests and 22.4mg for the SCV - wheatbran tests. The contribution of the bran was approximately equal to that of the SCB but less than that of the SCV. A comparison of the two yeasts, S. carlsbergensis and S. cerevisiae, suggested that the former was the more attractive with both a larger meal size and a greater number of animals eating the discs.

Both yeasts were compared to the pressed yeast sample W5 to examine the contribution of the yeasts to the overall fermentation by-product. The latter was significantly more attractive so that in four of the tests this formed

TABLE 23 FEEDING TESTS WITH YEAST CULTURES

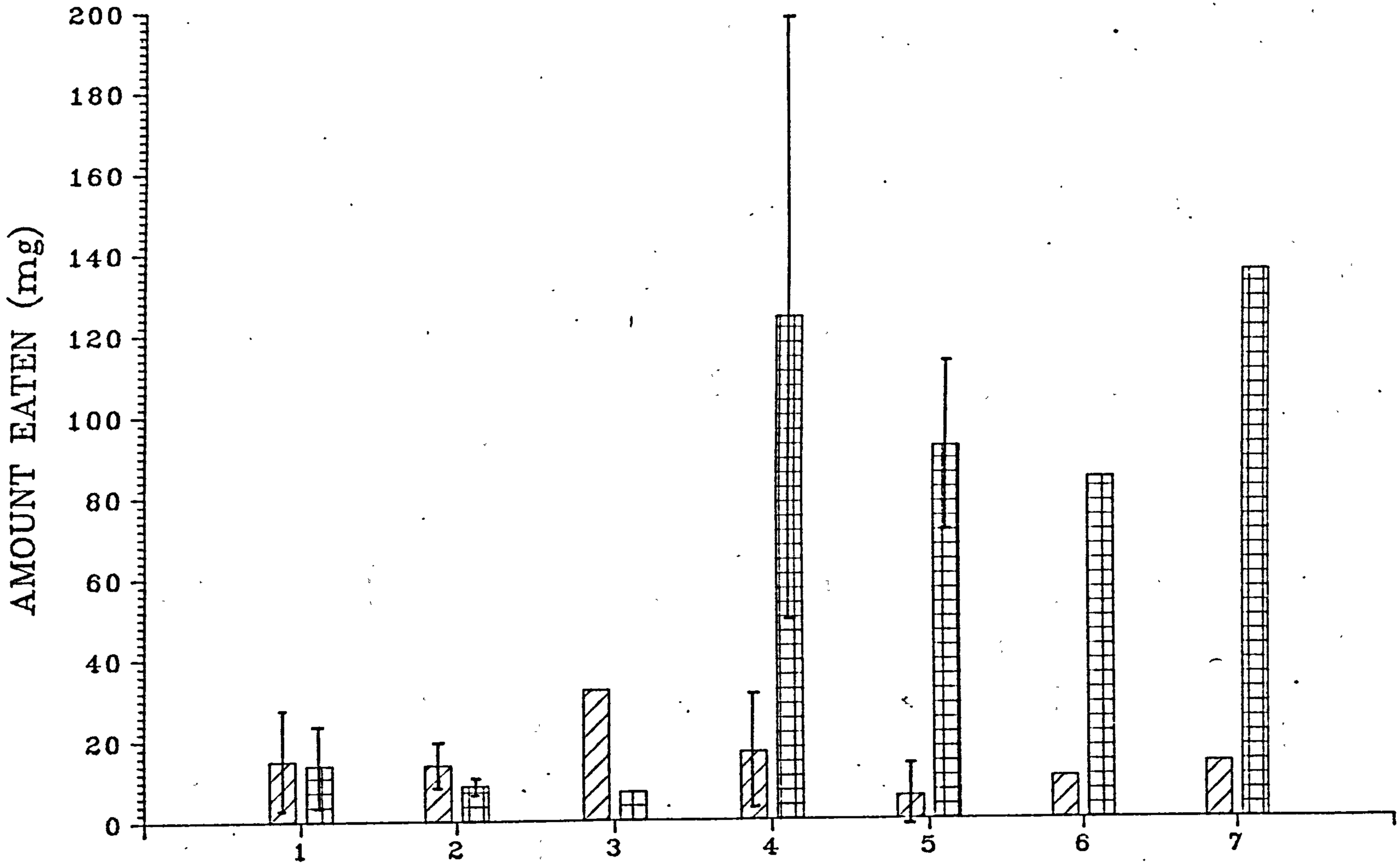
Saccharomyces carlsbergensis = SCB, Saccharomyces cerevisiae = SCV

FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
SCB		Bran			
10.3	30	6.9	20	1.49	17.2
27.6	80	24.1	70	1.15	51.7
6.9	20	10.3	30	0.67	17.2
$\bar{x}$ 14.93	43.33	13.77	40	1.08	28.70
S.D. 11.10		9.11			
SCV		Bran			
6.9	20	10.3	30	0.67	17.2
20.7	60	6.9	20	3.00	27.6
$\bar{x}$ 13.80	40	8.6	25	1.60	22.4
S.D. 9.76		2.40			
SCB		SCV			
32.1	80	6.9	20	4.65	39.0
SCB		W5			
0.0	0	202.0	100	-	202.0
26.2	50	115.0	90	0.23	141.2
24.1	70	55.2	50	0.44	79.3
$\bar{x}$ 16.77	40	124.07	80	0.14	140.84
S.D. 14.59		73.82			
SCV		W5			
0.0	0	104.8	90	-	-104.8
0.0	0	115.2	90	-	115.2
0.0	0	82.5	90	-	82.5
10.3	30	63.2	60	0.16	73.5
18.3	40	95.8	90	0.19	114.1
$\bar{x}$ 5.72	14	92.3	84	0.06	98.02
S.D. 8.33		20.22			
SCV		Beer sediment			
10.3	30	84.4	70	0.12	94.7
SCB		Beer sediment			
13.8	40	135.2	100	0.10	149.0

FIG. 3I CONSUMPTION OF FOOD BY Deroceras
TESTS WITH YEAST CULTURES

KEY	hatching	squares
x axis	I SCB	wheatbran
	2 SCV	wheatbran
	3 SCB	SCV
	4 SCB	W5
	5 SCV	W5
	6 SCV	Beer sediment
	7 SCB	Beer sediment

CONSUMPTION OF FOOD BY DEROCERAS
TESTS WITH YEASTS CULTURES



the entire diet and the discs cut from the yeast cultures were not eaten at all. S. carlsbergensis yeast again proved to be more attractive than S. cerevisiae although the latter is commonly used in the manufacture of British beers.

The beer sediment discs were compared with the two yeasts, SCB and SCV. The A/B ratio of 9.80 and 8.I9 respectively indicates that the sediment was more palatable though some feeding on the yeast discs was observed so the yeasts must have some attraction for the slugs. The metabolites of the yeast may be more important in determining the attractiveness of yeast than the cultures themselves.

4.I4 FEEDING TESTS WITH HOPS & HOPPED WORT

The contribution of hops to the attractiveness of beer and pressed yeast was assayed by examining the feeding responses of slugs to solutions of dried hops and to hopped wort. Dried hops were soaked in distilled water for 24 hours, filtered through No. I Whatman filter paper and the filtrate incorporated into the agar solutions. A sample of hopped wort, the mixture which is refined to produce beer, was obtained from the Brewing Research Foundation, Nutfield, Surrey. IOOml of this was similarly combined with powdered agar to form hopped wort discs for feeding experiments. The results of these feeding tests are presented in Table 24 and Fig. 32.

The hopped wort compared favourably with both bran and the yeast samples F2, F3, and W7. The A/B value of the hopped wort discs, food A was 3.03 with the wheatbran and ranged from 4.09 to I4.4I with the

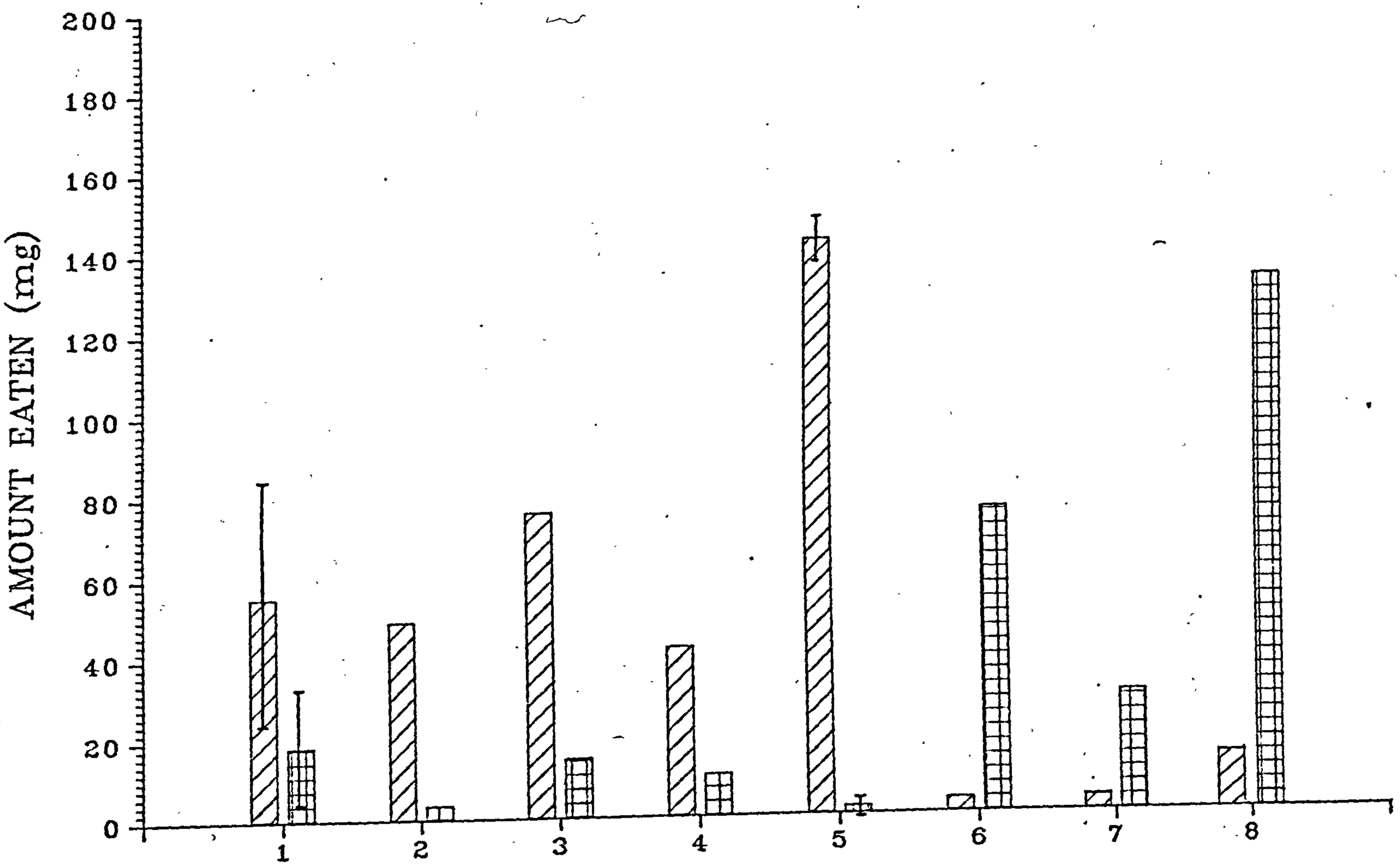
TABLE 24 FEEDING TESTS WITH HOPS AND HOPPED WORT

FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
Hopped wort		Bran			
104.9	80	37.4	50	2.80	142.3
32.1	80	21.7	50	1.48	53.8
40.0	90	18.3	40	2.19	58.3
65.9	100	10.3	30	6.40	76.2
33.1	70	3.4	10	9.74	36.5
$\bar{x}$ 55.2	84	18.22	36	3.03	73.42
Hopped wort		F2			
49.0	90	3.4	10	14.41	52.4
Hopped wort		F3			
75.8	90	14.8	30	5.12	90.6
Hopped wort		W7			
42.1	70	10.3	30	4.09	52.4
Hopped wort		Hop solution			
138.6	100	0.0	0	-	138.6
146.0	100	3.4	10	0.02	149.4
$\bar{x}$ 142.3	100	1.7	5	0.01	144.0
S.D. 5.23		2.40			
Hop solution		Bran			
3.4	10	75.8	90	0.04	79.2
Hop solution		F2			
3.4	10	29.7	60	0.11	33.1
Hop solution		F3			
13.8	40	132.0	90	0.10	145.8

FIG 32 CONSUMPTION OF FOOD BY Deroceras
TESTS WITH HOPS AND HOPPED WORT

KEY		hatching	squares
x axis	I	hopped wort	wheatbran
	2	hopped wort	F2
	3	hopped wort	F3
	4	hopped wort	W7
	5	hopped wort	hop solution
	6	hop solution	wheatbran
	7	hop solution	F2
	8	hop solution	F3

CONSUMPTION OF FOOD BY DEROCERAS
TESTS WITH HOPS AND HOPPED WORT



yeast samples as food B. The percent response was also high in each test with an average value of 84% for the number of slugs feeding on hopped wort discs and 36% feeding on bran. An average percent response of 83.33% was obtained by animals feeding on hopped wort discs when these were offered in combination with yeast discs - this latter food was eaten by an average of 23.33% of the slugs.

The hop solution, food A, proved to be unattractive when compared with either bran, F2, F3 or hopped wort discs as food B. The A/B values of 0.04, 0.11, 0.10 and 0.01 respectively indicate that the hop solution discs were highly unpalatable. Total meal size varied in these tests with the amount of F3 consumed being much greater than the amount of the older F2 discs consumed The hopped wort however appeared to be a highly attractive food since total meal size averaged almost 150mg. This suggests that although the hops do not produce a water soluble compound which is capable of increasing food consumption in Deroceras, some component formed after the bittering process contributes to the attractiveness of the pressed yeast mixture.

4.15 FEEDING TESTS WITH SUCROSE AND A FERMENTATION PRODUCT

Sucrose was assayed against bran and pressed yeast controls for its effect as a feeding stimulant. A sucrose solution (5g. in 100ml water) was combined with agar to form a sucrose/agar sheet from which discs could be cut. A fermentation concentrate - a by-product from molasses fermentation was obtained from B.P. Chemicals and similarly

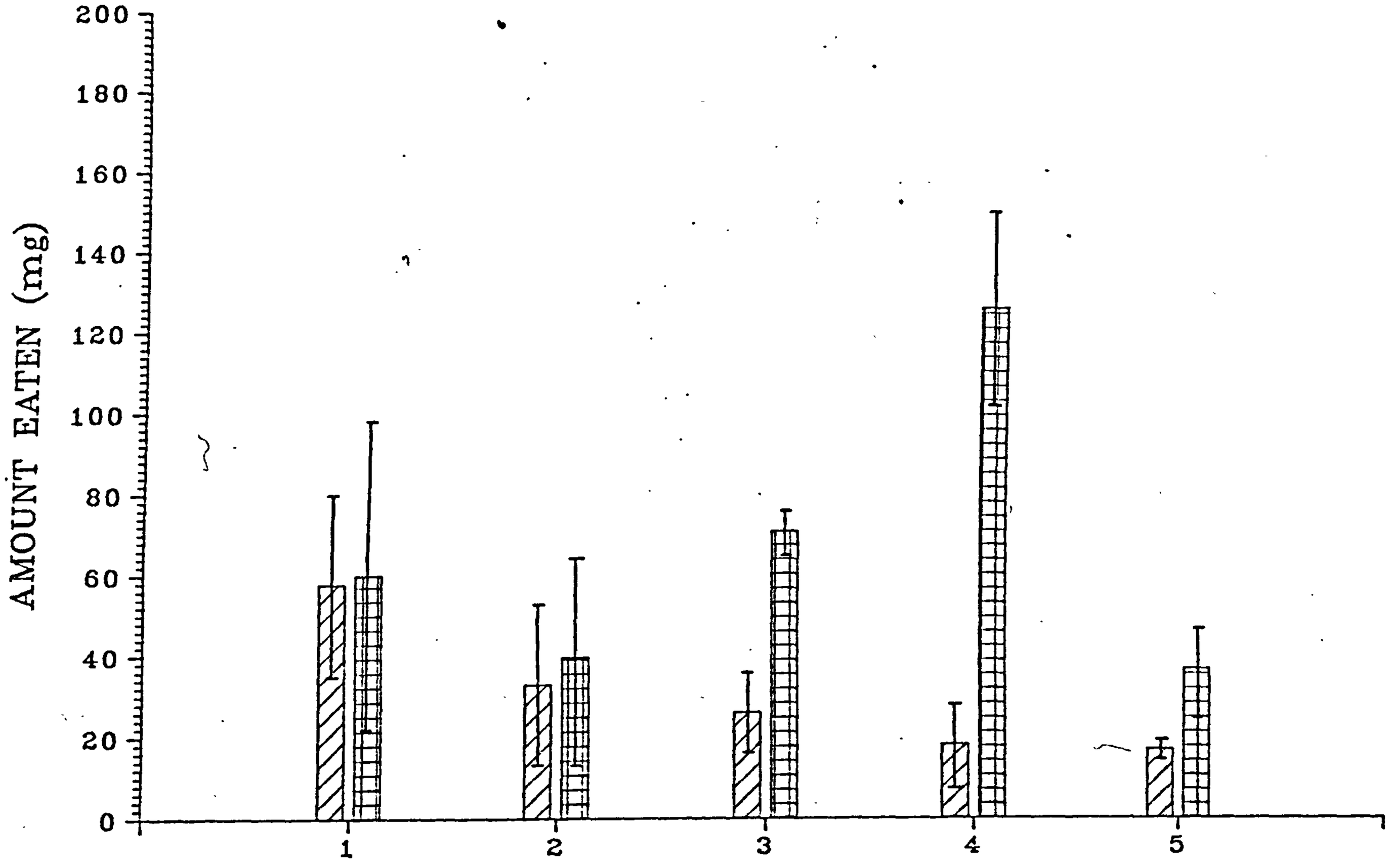
TABLE 25 FEEDING TESTS WITH SUCROSE AND A FERMENTATION BY-PRODUCT

FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
Sucrose		Bran			
73.6	90	104.0	90	0.71	177,6
31.8	40	38.6	60	0.82	70.4
68.1	100	37.6	70	1.81	105.7
$\bar{x}$ 57.83	76.67	60.07	73.33	0.96	117.9
S.D. 22.71		38.05			
Sucrose		W8			
62.5	90	25.2	60	2.48	87.7
22.8	40	54.5	80	0.42	77.3
21.7	50	80.3	90	0.27	102.0
10.3	30	21.7	50	0.47	32.0
47.7	80	17.2	50	2.77	64.9
$\bar{x}$ 33.0	58	39.78	66	0.83	72.78
S.D. 21.41		26.96			
Sucrose		W8Bran			
33.1	70	73.6	90	0.45	106.7
14.8	30	75.7	60	0.20	90.5
30.7	50	64.4	70	0.48	95.1
$\bar{x}$ 26.2	50	71.23	73.33	0.37	97.43
S.D. 9,95		6.01			
B.P. molasses		Bran			
10.3	30	109.4	80	0.09	119.7
26.2	50	143.2	90	0.18	169.4
$\bar{x}$ 18.25	40	126.3	85	0.14	144.55
S.D. 11.24		23.90			
B.P. molasses		F2			
19.3	30	27.3	40	0.71	46.6
14.8	30	46.6	70	0.32	61.4
$\bar{x}$ 17.05	30	36.95	55	0.46	54.0
S.D. 3.18		13.65			

FIG. 33 CONSUMPTION OF FOOD BY Deroceras
TESTS WITH SUCROSE AND FERMENTATION PRODUCTS

KEY		hatching	squares
x axis	1	sucrose	wheatbran
	2	sucrose	W8
	3	sucrose	W8Bran
	4	B.P. molasses	wheatbran
	5	B.P. molasses	F2

CONSUMPTION OF FOOD BY DEROCERAS
TESTS WITH SUCROSE AND FERMENTATION PRODUCTS



made up into agar sheets. 5g. wet weight of this compound, which has a similar texture to the pressed yeast obtained from breweries, was used to prepare the agar sheets. Sugars are normally considered to be attractive to slugs and to provide the major feeding stimulus in many foods (Stephenson 1980). A sucrose solution proved to be slightly less attractive than the yeast sample W8, with an A/B value of 0.83 for five tests, but of equivalent attraction to bran discs (A/B of 0.96). A combination of W8 and bran in the same disc proved more attractive so that when the W8 bran was offered as food B, with sucrose as food A, the A/B value was 0.37. This increase in the consumption of food B resulted in a decrease in the amount of sucrose discs eaten, so that in this case, the total meal size was consistently low for all the tests with sucrose.(Table 25,Fig 33)

A molasses fermentation concentrate, obtained from B.P. Ltd. proved less attractive than the sucrose although the sugar content of the concentrate is high. Meal sizes with bran were high giving an average A/B value of 0.14. The yeast sample was less attractive yet it was only two days old and the animals were recently collected. The yeast remained more attractive than the B.P. molasses, however, with an A/B value of 0.46 and the yeast being eaten by a larger number of slugs.

4.16 FEEDING TESTS WITH BEER

A comparison of a yeast sample, W5, with 100ml commercial beer fermentation into an agar sheet indicated that the pressed yeast was significantly more attractive than the

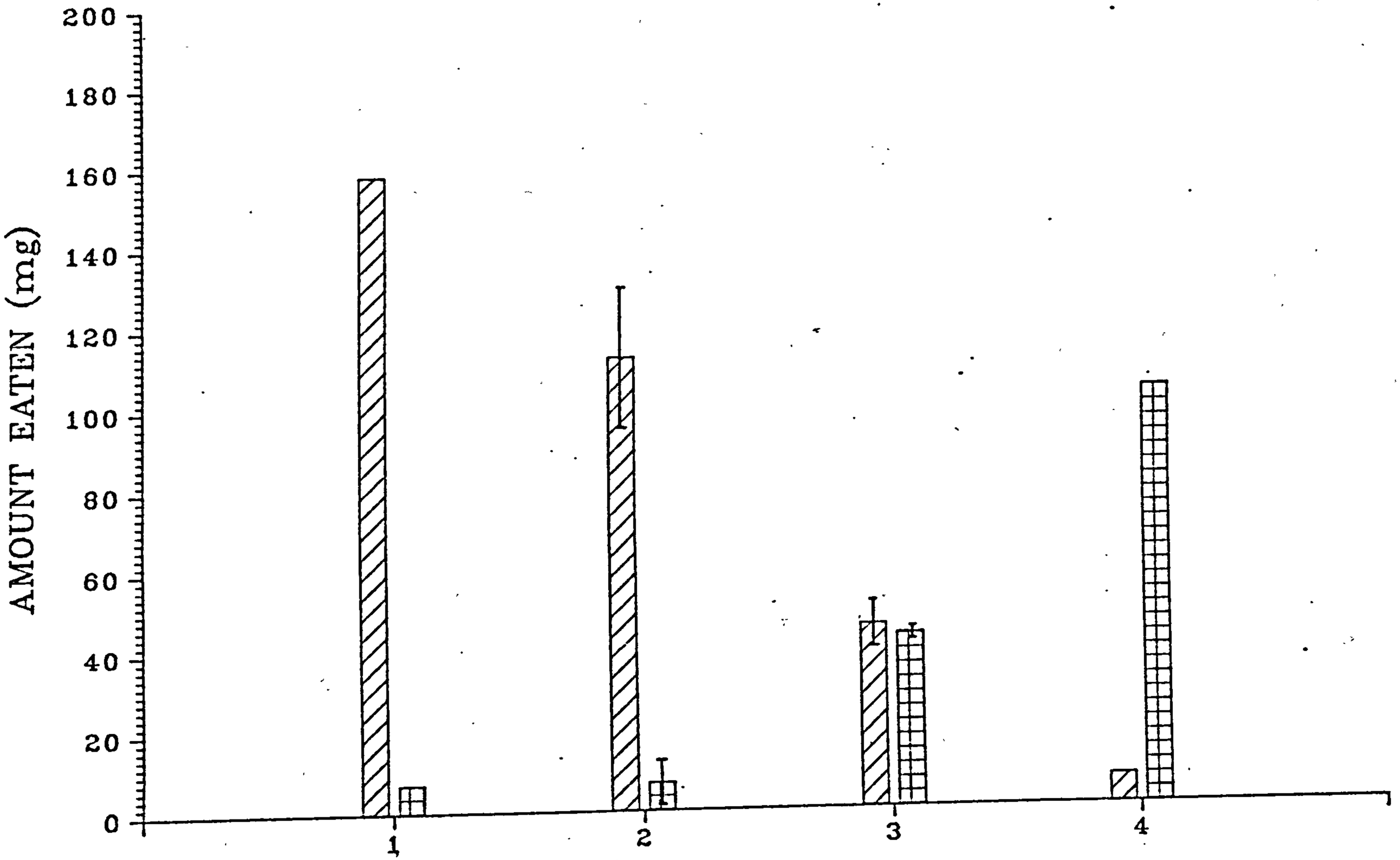
TABLE 26 FEEDING TESTS WITH BEER

	FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
	W5		Beer			
	157.8	100	6.9	20	22.87	164.7
	Beer sediment		Beer supernatant			
	125.3	100	3.4	10	36.85	128.7
	99.6	80	10.3	30	9.67	109.9
$\bar{x}$	112.45	90	6.85	20	16.42	119.3
S.D.	18.17		4.88			
	Beer sediment		W5			
	42.1	70	42.1	70	1.00	84.2
	49.0	90	44.3	70	1.11	93.3
$\bar{x}$	45.55	80	43.20	70	1.05	88.75
S.D.	4.88		1.56			
	Beer supernatant		W5			
	6.9	20	103.8	100	0.07	170.7

FIG. 34 CONSUMPTION OF FOOD BY Deroceras
TESTS WITH BEER

KEY		hatching	squares
x axis	I	W5	beer
	2	beer sediment	beer sediment
	3	beer sediment	W5
	4	beer supernatant	W5

CONSUMPTION OF FOOD BY DEROCERAS
TESTS WITH BEER



beer which has presumably lost some of its attractiveness in the refining process. This confirms the preliminary tests on samples from the brewing process which had suggested that the by-products formed at a later stage in the fermenting process were less palatable in feeding tests than pressed yeast. The samples originally tested - yeast slurry, yeast plus finings and breakings - are true waste products and contain diluted levels of yeast metabolites. This may have acccounted for their reduced palatability but the small consumption of the commercial beer sample suggests that the unrefined fermentation product, pressed yeast is more attractive. (See Table 26, Fig. 34)

Feeding tests to compare beer sediment and beer supernatant indicated that the attractiveness of the beer resides in the sediment. An average total meal size of 119.3mg with an A/B value of 16.42 indicated that the sediment contributed the most to the diet and was a highly palatable food.

The beer supernatant, as expected, proved unattractive when compared with pressed yeast, W5, giving an A/B value of 0.07. The beer sediment compared well with the yeast sample W5 to give an A/B of 1.05 and an average total meal size of 88.75mg in 24 hours. Three further tests with these two foodstuffs were left for 48 hours and showed some interesting results. In these tests, the sediment proved slightly more attractive than the yeast discs but the total meal size had almost doubled. An average meal size of just over 300mg for the 48 hour period gives a value of 150.93mg food consumed in 24 hours. When the slugs were

left undisturbed therefore, meal size was increased twofold.

4.17 FEEDING TESTS WITH A COMBINATION YEAST/BRAN FOOD

A combination of two attractive feeding compounds was made by dissolving the pressed yeast in a bran extract of water and formulating this into agar sheets. This could then be assayed against bran or pressed yeast alone to ascertain whether the combination of feeding attractants increased the amount eaten. The results of these feeding tests shown in Table 27 and Fig. 35 .

Feeding tests in which the combination of W8Bran, F5Bran or F6Bran was offered to Deroceras with wheatbran discs, produced consistently high meal sizes. Total meal size was over 100mg in most cases and the combination food proved more attractive in all but one test. The number of animals consuming some of each disc was high for both foods so the preferential feeding on the yeast/bran/agar discs is likely to be due to an increased intake of this food.

Combination of bran and yeast into the same disc, F5Bran, proved to be more attractive than hopped wort discs. An A/B value of 0.42 was obtained in a feeding test where hopped wort was offered as food A together with F5bran as food B. Hopped wort had previously been shown to be a highly palatable food for Deroceras.

The feeding tests in which the combination agar disc is offered with a disc of yeast alone also indicate that the combination diet is preferentially eaten. The total meal size and the percent response are very similar to the

TABLE 27 FEEDING TESTS WITH A COMBINATION YEAST/BRAN FOOD

	FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
	W8Bran		Bran			
	46.4	50	36.3	60	1.28	82.7
	151.9	100	62.1	70	2.45	214.0
	33.1	70	18.3	40	1.81	51.4
$\bar{x}$	77.13	73.33	38.90	56.67	1.98	116.03
S.D.	65.09		22.02			
	F5Bran		Bran			
	95.8	90	39.0	70	2.46	134.8
	89.1	90	50.0	80	1.78	139.1
	57.7	70	88.3	90	0.65	145.0
	92.7	100	35.2	50	2.63	127.9
$\bar{x}$	83.83	87.5	53.13	72.5	1.16	156.33
S.D.	17.63		24.28			
	F6Bran		Bran			
	104.9	80	62.3	90	1.68	167.2
	61.0	60	48.8	70	1.25	109.8
	91.5	90	32.9	50	2.78	124.4
$\bar{x}$	85.8	76.67	48.0	70	1.79	133.8
S.D.	22.50		14.72			
	F5Bran		F5			
	88.2	100	27.3	40	3.23	115.5
	119.5	90	25.2	60	4.74	144.7
	97.1	100	37.6	70	2.58	134.7
	131.2	90	40.8	60	3.22	172.0
	54.4	80	46.4	50	1.17	100.8
	90.2	80	56.7	80	1.59	146.9
$\bar{x}$	96.77	80	39.0	60	2.48	135.77
S.D.	26.88		11.84			
	F6Bran		F6			
	64.6	90	24.1	70	2.68	88.7
	89.1	90	29.7	60	3.00	118.8
	30.7	50	41.0	80	0.75	71.7
	90.2	80	68.9	70	1.31	159.1
$\bar{x}$	68.65	77.5	40.93	70	1.68	109.58
S.D.	27.92		19.93			

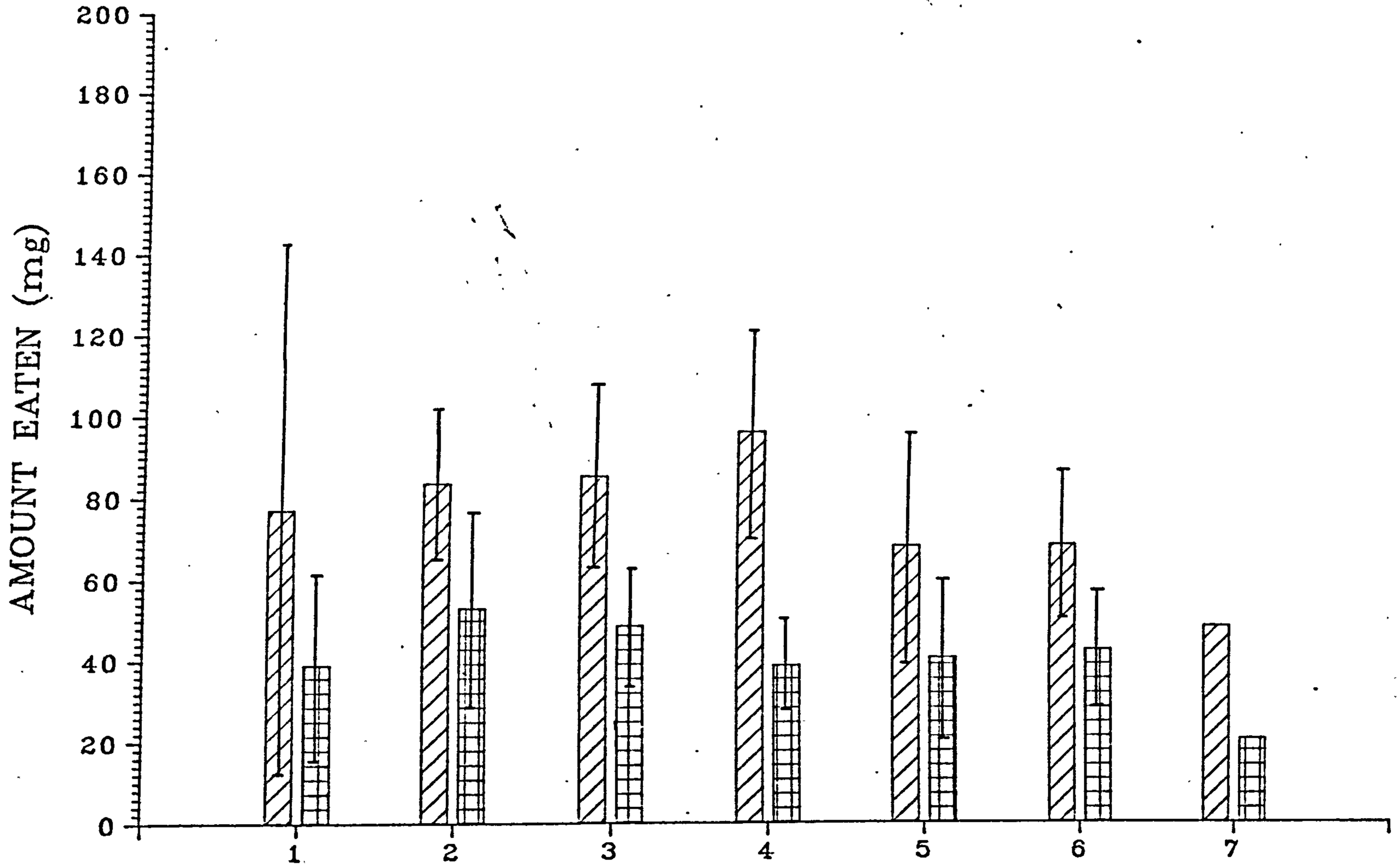
TABLE 27 (continued). FEEDING TESTS WITH A COMBINATION YEAST/BRAN FOOD

FOOD A amount eaten mg	percent response	FOOD B amount eaten mg	percent response	A/B	total eaten mg
W8Bran		W8			
48.5	50	27.3	40	1.78	75.8
63.4	80	42.1	70	1.51	105.5
69.1	90	57.6	40	1.20	126.7
68.9	70	61.0	60	1.13	129.9
94.9	100	26.2	50	3.62	121.1
$\bar{x}$ 68.96	78	42.84	52	1.61	111.8
S.D. 16.75		16.33			
F5Bran		Hopped wort			
48.8	70	20.7	60	1.97	69.5

FIG 35 CONSUMPTION OF FOOD BY Deroceras
TESTS WITH A COMBINATION YEAST/BRAN FOOD

KEY		hatching	squares
x axis	I	W8Bran	wheatbran
	2	F5Bran	wheatbran
	3	F6Bran	wheatbran
	4	F5Bran	F5
	5	F6Bran	F6
	6	W8Bran	W8
	7	F5Bran	Hopped wort

CONSUMPTION OF FOOD BY DEROCERAS
TESTS WITH A COMBINATION YEAST/BRAN FOOD



tests in which wheatbran was offered as the alternative food and the consistently large meal sizes of over 100mg in 24 hours suggest that a choice diet such as this may encourage the slugs to consume a larger meal. The feeding results with this combination food were less variable than many other tests and satisfied the requirements of a large meal being consumed by a large number of animals which is the most important factor in the step towards increasing bait consumption.

4.18 Conclusions

The nature of the bioassay indicated that the attractant(s) responsible for the high palatability of the yeast must be water soluble, heat stable and non-volatile. The yeast was analysed to compare the attraction of constituents and the whole sample. The results suggest that a number of compounds may be involved so that the attraction results from a synergistic effect since the individual components were insufficient to account for the attraction of the combined product.

The yeast cells themselves are also important since a sediment of yeast sample proved more attractive than the corresponding supernatant which would contain intercellular material. A commercial debittered yeast sample was attractive to the animals but this material has several flavour components of its own which may be responsible for the palatability of the material. A yeast sample which was debittered in the laboratory appeared to be unattractive and the hopped wort is certainly attractive to Deroceras so

it is probable that the hops do contribute to the appeal of the yeast sample.

Sugars are known to be attractive to slugs and probably contribute to the palatability of the sample but the carbohydrate present at the start of fermentation is metabolised by the yeast to amino acids and the quantity of sugars in the yeast sample is therefore less than in the malt. A simple sucrose solution was not sufficiently attractive to maintain feeding in Deroceras when tested in a feeding bioassay. Ingledew et al (1977) sampled a 130mg/ml brewers yeast slurry at intervals for a period of 336 hours and analysed protein and carbohydrate fractions. Their results indicated a decline in carbohydrate levels and a rise in amino acid levels as the yeast metabolism continues once the beer has been drawn off. Work on the attractive nature of feeding stimulants in marine molluscs such as Aplysia suggests that amino acids may be responsible for the feeding response and the high level of amino acids in the brewers yeast suggests this is an attractive class of chemicals for terrestrial slugs also.

It appears that the attraction of the yeast changes with time so that it becomes unpalatable to slugs, even when the material has been stored at low temperatures or formulated into agar sheets. Spoilage chemicals form which are obviously repellent to Deroceras and mask the attractive compounds which can stimulate feeding. The chemical diacetyl which was suggested by Selim as a possible attractive component of beer, was unattractive to Deroceras when combined with agar and this is one of many chemicals which are regarded as spoilage

components in some beers.

A concentration of 5g wet weight yeast per 100ml water produced a food of high palatability. Increasing this to 20g wet weight per 100ml caused a twofold increase in the amount of this food eaten though the total meal size was not correspondingly increased. This suggests that the concentration of the attractant can be altered to improve the amount of a particular food eaten but that total meal size is limited perhaps, as Gelperin suggests, by gut stretch receptors which monitor food intake.

The addition of an attractant can, it appears, improve the consumption of a food material quite considerably. Optimum results in increasing meal size are obtained by varying the diet regularly and by offering foods to the animals at the same time - a bran and a yeast disc therefore seen to be more palatable than two bran discs or two yeast discs. It is more difficult to rank the foods compared in the bioassays as the palatability of each is so dependant on the type of food the animals has consumed previously. It does appear, however, that brewers yeast is a palatable food for Deroceras and can be used to encourage feeding.

4.19 REPELLENTS

In the past decade, there have been several reports of domestic pets being poisoned by metaldehyde baits, e.g. Turner (1968). Maddy (1975) reported 20 - 30 cases of metaldehyde poisoning per week in California. Commercial pellets contain a nutritive substance such as bran which acts as a carrier and an attractant to the slugs and snails.

It appears however, that other animals are attracted to this food and toxic symptoms can be produced if a large quantity is consumed. Kitchell et al (1978) compared standard slug and snail bait bases (SSBB) with the least palatable dog food in a behavioural test which involved a choice between two foods. The SSBB was less palatable to the dogs than the least palatable dog food and addition of 1% SSBB to the dog food reduced palatability of the latter. In practice however, this does not deter dogs from feeding on pellets placed in the garden or left in an opened package, The symptoms are usually general sickness and several forms of treatment have been suggested but ingestion of metaldehyde by small dogs can be fatal and a repellent has been sought to deter domestic animals from eating bait pellets.

Legislation is now in force in many countries demanding that a mammal repellent be included in all slug and snail baits. The time at which these laws must be implemented varies but it is inevitable that as control of pesticides becomes more stringent many other chemicals will require repellents to protect non-target organisms. The precise nature of the chemicals used as repellents is protected by company confidentiality but one, at least, is a compound which irritates the mucus membrane of warm blooded animals (Lonza, British Patent No 1434900).

Tests were conducted to ensure that the repellents incorporated into the baits do not deter slugs from approaching and feeding upon the pellets. Slug movements were recorded in the vicinity of these baits and feeding

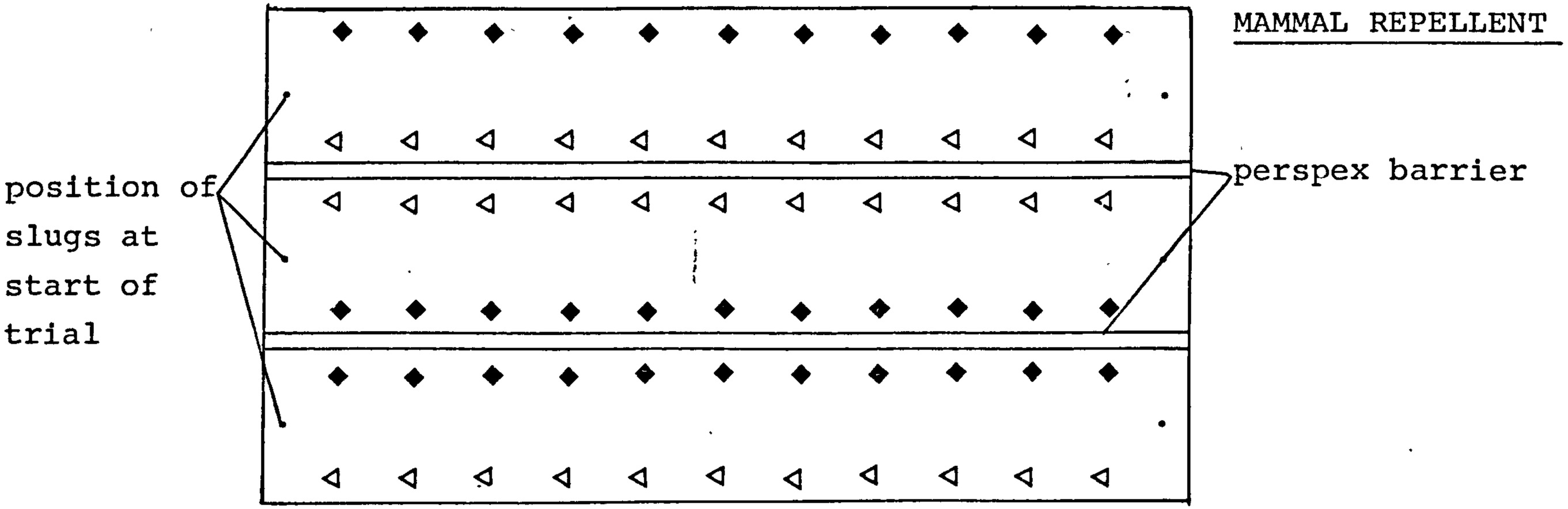
activity observed. Field tests to compare the effectiveness of the traditional formulation and the mammal repellent formulation are reported in Section 5. In France, a bait containing a mammal repellent (Bait F in Section 5) has been marketed since 1978 but there are no reports of how this compares with the original (Bait E in Section 5) - these two formulations were therefore compared in these trials.

EXPERIMENTAL PROCEDURE

The experimental apparatus was a perspex arena 50 x 75 x 5cm^3 which was divided by two longitudinal barriers to provide three tracks, each 17cm wide and 75cm long. The floor of the apparatus was lined with blotting paper (Whatman No 1) which was changed after each trial and moistened before use to provide conditions of high humidity. The two baits - bait E, the original formulation and bait F, the formulation incorporating a mammal repellent - were placed at 5cm intervals along the length of each track (Fig. 36). Each row contained pellets of one type of bait only and was placed approximately 1cm from the wall of the track, giving a 15cm distance between the two baits in each track. Adjacent rows were of the same type so that the response of the animals would not be affected by intermingling of odours from another track.

An animal was placed at the beginning of each track and allowed to move freely for 30 minutes. A positive score was recorded each time an animal reached a bait pellet and remained there for at least two minutes. This improved the likelihood that the animal actually fed on the bait. In

FIG. 36 PLAN OF BAIT DISTRIBUTION IN TESTS TO ASSESS THE ATTRACTIVE QUALITIES OF A TRADITIONAL BAIT FORMULATION AND ONE INCORPORATING A MAMMAL REPELLENT



◆ traditional formulation (bait E in Section 5)

◁ formulation containing repellent (bait F in Section 5)

none of the tests did an animal move away from a pellet once it had started to feed. Each animal was tested once only to eliminate learning responses and the filter paper and bait pellets were replaced after each trial to prevent interference from directional cues in the slime trails. The position of each animal was marked on the roof of the apparatus at two minute intervals to facilitate the tracing of the path and the time taken to reach a bait was recorded. The animals had been well fed on lettuce and kept under reverse daylight conditions. They were tested in the initial stages of scotophase, using a red light for observation to minimise interference with their activity.

RESULTS

	Number of tests	Positive responses			
		Bait E	Bait F	Total	Percent
Helix aspersa	75	27	I5	40	53.3
D. reticulatum	75	30	2I	5I	68.0
D. caruanae	24	8	5	I3	54.I7
Otala lactea	24	7	6	I3	54.I7

All four species tested showed a response of over 50% and the average time taken to reach the bait pellets was similar for both types of bait.

	BAIT E	BAIT F
Helix aspersa	I7.8I minutes	I9.07 minutes
D. reticulatum	I2.00 minutes	9.42 minutes
D. caruanae	9.75 minutes	II.00 minutes
Otala lactea	I4.85 minutes	I0.00 minutes

The results suggest that the two formulations are similar in their ability to attract slugs and stimulate them to feed. The addition of the mammal repellent in this formulation does not therefore have any deleterious effect on the efficiency of the bait. Further tests to confirm this are presented in Section 5.

SECTION 5 FIELD TRIALS WITH MOLLUSCICIDE FORMULATIONS

INTRODUCTION

Field trials were made to compare ten different bait formulations for their ability to attract and kill an indigenous population of slugs. The slug population in the field was assessed for seven days before baiting by counting the number of animals under refuge traps but without the use of attractant baits since residues may have affected subsequent trapping figures. A true estimate of bait effectiveness can only be obtained by careful sampling of the population to establish the initial level of infestation. The reduction in slug numbers can then be calculated by similar trapping after baiting. Post baiting estimates of the population provided an independent check on the reliability of the mortality estimates made from counts of dead animals from the soil surface.

A site with a uniform topography and a high slug population was sought so that conditions in each of the treatment and replicate plots were similar.

5.1 SITE DESCRIPTION

The trials took place in late August and early September 1979 at Upper Maxted Street Farm, Elmsted, near Canterbury, Kent. (Nat. Grid Ref. 135522). The site was recommended by the M.A.F.F. (A.D.A.S. division) as having a high slug population and preliminary baiting tests on a small area suggested a population of 90 *Deroceras reticulatum* per metre^2. equivalent to 370,000 per acre.

Two possible fields were available for use - the one finally chosen, Bigfield, appeared to have the highest slug population and a less aggregated distribution of slugs. Although the number of slugs was high, the area had not been baited in recent years and thus provided an ideal site as there were no residues from other baits.

Bigfield has a medium to heavy loam soil, with a ridge running across the centre of the field which is slightly drier than the surrounding soil. Sheep congregate along this line and as larger amounts of dung are therefore deposited here, it may be expected that soil fertility would differ. Bigfield is bounded on three sides by a hedge of trees and the fourth side is separated from a wheat field by a wire fence. Although the plant cover was fairly uniform over the field, there were district patches of bare ground, partly overlain by unharvested clover which had been missed by the combine harvester.

The field was sown with a mixture of wild white clover(Trifolium repens) and perennial rye grass (Lolium perenne) and had been heavily grazed by sheep for several months. A wet summer had encouraged the growth of a large slug population and although the yield of clover seed was high - over 150 tons - almost all the growing clover was found to be grazed by slugs. The field was harvested on 10th August, though the straw lay on the surface for two weeks after this as rainfall was high in this period - 12.5cm. - and it had no chance to dry. This waste material provided an ideal environment for the slugs but unfortunately encouraged an aggregated distribution and would have made searching rather difficult. On 25th and

FIG. 37 EXPERIMENTAL SITE FOR FIELD TRIALS

6470
1·587ha
3·92

159·9m

Upper Maxted Street Farm

BM 160·16m

Maxted Street Cottage

6466
·210ha
·52

Maxted Street

7269
·875ha
2·16

5866
·413ha
1·02

159·5m

7162
·624ha
1·54

9067
4·723ha
11·67

LOWER MAXTED STREET

6061
·202ha
·50

7654
2·998ha
7·41

LONGFIELD

a b c d e

study area

6745
3·443ha
8·51

BIG FIELD

8143
·061ha
·15

9844
2·941ha
7·27

THISTLES

BRIDLE ROAD

5630
·705ha
6·68

8934
3·006ha
7·43

SEEDS

JIMMERS

6924
3·605ha
8·9

SIXTEEN

26th August it was moved from the plots into the guard rows, leaving the experimental area clear. In fact, the straw from the entire field was baled mechanically on 27th August, thereby reducing the possibility of the guard rows harbouring an artificially high slug population.

5.2 PLOTS

The experimental site is shown in Fig. 37 . Fifty plots were marked out with a bamboo cane at each corner. Each plot was 10m x 10m and bounded on all sides by guard rows of 5m. The whole treatment area was therefore 12,000m^2 and laid out in a plan of five columns ("a" - "e") by ten rows (0-9) - this was later extended by the addition of another row (0 - 10) when the population at the top of the field was found to be lower than the rest of the field. Each of the ten treatments, A - K, was assigned to the area five times using tables of random numbers in a randomised block design so that each treatment occurred once in each column. This minimised any inherant differences in slug distribution in the field. The plots were then colour coded for easy identification and a hessian sack placed in the centre of each, held down by two four inch nails. This acted as a refuge trap so that the population in each plot could be assessed before the area was baited. Table 28 illustrates the allocation of each treatment to the plots and the resultant plan of treatment distribution.

Fig. 38 shows the view looking down the field from row 0, near the road to row 10 with the bamboo canes marking the boundary of each plot.

TABLE 28

ALLOCATION OF THE TREATMENTS TO PLOTS

Treatment	Plot Number				
A	a5	b8	c2	d2	e10
B	a2	b9	c2	d8	e1
C	a4	b2	c10	d7	e3
D	a9	b5	c7	d1	e8
E	a1	b1	c4	d9	e6
F	a3	b6	c8	d6	e2
G	a6	b4	c1	d10	e9
H	a10	b7	c5	d4	e4
J	a8	b10	c3	d3	e7
K	a7	b3	c6	d5	e5

PLAN OF EXPERIMENTAL PLOTS - RANDOMISED BLOCK DESIGN

	column	a	b	c	d	e
row	1	E	E	G	D	B
	2	B	C	A	A	F
	3	F	K	J	J	C
	4	C	G	E	H	H
	5	A	D	H	K	K
	6	G	F	K	F	E
	7	K	H	D	C	J
	8	J	A	F	B	D
	9	D	B	B	E	G
	10	H	J	C	G	A

Each vertical column represents the 10 treatments in randomised positions.

Each horizontal row represents one of the five replicates.

FIG 38 VIEW OF BIGFIELD FROM ROW O (FOREGROUND) TO ROW IO SHOWING BAMBOO CANES MARKING THE BOUNDARY OF PLOTS. GUARD ROW VISIBLE TO RIGHT

FIG 39 TYPICAL APPEARANCE OF Deroceras FOUND UNDER TRAPS DURING PRE-BAITING SAMPLING

5.3 POPULATION DATA

The estimation of slug populations is notoriously difficult and in a trial where one is attempting to search a large area, methods are further limited. Mechanical extraction is laborious and tends to distort the slugs so accurate work on size or weight is not possible. Hunter (1966) found that Deroceras reticulatum trapped in such a way lost approximately 34% of their weight during the soil washing process. Surface searching was the only feasible method to sample a large area and allow division of the animals into poisoned and unpoisoned slugs. Barnes (1944) found that searching by torchlight for a specific period at night he was able to obtain reasonably reproduceable data on slug numbers. Surface searching estimates of the population are dependent on the activity of the slugs which is in turn dependent on the weather. Traps were therefore laid to provide shelter and to encourage the slugs to collect and every attempt was made to standardise the time spent searching each plot. Fig. 39 is typical of the number and appearance of Deroceras found under traps in this trial. The level of infestation was high and the animals were active in the early morning. The weather was quite stable throughout the trial with heavy rain on only two nights - the other nights were mainly clear with a heavy dew in the mornings. Cooler days did produce higher slug counts with the juveniles more susceptible to fluctuations than adults.

Predetermined sampling sites were taken as representative areas for searching each plot since the experimental area was too large to be examined thoroughly

FIG. 40 HESSIAN GRAIN SACK SERVING AS A TRAP IN THE CENTRE OF EACH PLOT - UNBAITED THROUGHOUT TRIAL

FIG 4I ONE OF FOUR STRAW TRAPS IN EACH PLOT. USED FOR POST-BAITING SAMPLING ON DAYS 9 - I6 AND RESIDUAL POPULATION SAMPLING ON DAYS 2I AND 22

each morning. Three types of trap were used on these sampling sites - hessian grain sacks, Marley tiles and straw. The hessian sack (65cm x 100 cm), Fig. 40 , in the centre of each plot was a control trap from which no animals were removed either before or after baiting. The Marley tiles, each 22.5cm x 22.5cm, were used as traps on day 8, the first day after baiting, and on day 22 to estimate the residual population on each plot two weeks after baiting. Straw traps, 50cm x 50cm, Fig. 41, were used as sampling sites on days 9-16 since these areas were known to contain baits and problems arose from wind drift of pellets on uncovered areas in the plots.

Young slugs were commonly found grazing the tips of stalks while the adults usually remained at the base even in dry weather when the juveniles disappeared underground. This difference in behaviour may have contributed to a bias in the records of adult and juvenile numbers in favour of more adults.

5.4 POPULATION COUNT

Although population data provided an indication of the level of slug infestation, it was more important in providing a relative estimate of slug numbers before and after baiting. The population count was begun on the morning of 31st August (day 1). The area under each sack was searched by separating the plant cover and methodically moving up and down the exposed ground. Animals on the underside of the sack were included also - these were almost exclusively juveniles. The slugs were assigned to one of the following five length classes:

TABLE 29 - TEMPERATURE AND WEATHER CONDITIONS

	Temperature °C - average for 22 day trial period		
	a.m.	midday	p.m.
air	I3.36 ± 2.43	20.I4 ± 2.32	I4.89 ± 2.5I
surface	I2.45 ± 2.29	2I.93 ± 4.00	I4.04 ± 2.34
3cm depth	I3.95 ± I.96	22.04 ± 6.29	I5.7I ± 2.46

DAY	TEMPERATURE °C (AIR)			WEATHER
	a.m.	midday	P.M.	
I	I7	-	I6	heavy dew, dry and sunny
2	I5	23	-	dry, warm day
3	I0	-	-	¾ inch. rain in a.m., cold and sunny later
4	I2.5	-	I6	rain in a.m., windy & showery
5	-	-	20.5	dry, sunny
6	I7	22	-	dry, sunny
7	I6	20.5	I6	light dew, ground very dry
8	I2.5	20	I3.5	misty, heavy dew, dry & hot day
9	I2	-	-	light dew, dry day
I0	I5	20.5	-	misty, overcast a.m. sunny p.m.
II	I5	-	-	misty a.m., damp & overcast
I2	II.5	23	I5	dry, sunny but windy
I3	I3.5	I6	-	windy, overcast
I4	I3	20	I5	dry but overcast
I5	I5	-	I5	dry, cool
I6	I3	I8.5	9.5	windy, overcast, cool
I7	9	23	I2	dry, bright afternoon
I8	I2	22	I4.5	sunny, windy
I9	I5	I7	I6	light dew, cloudy & windy
20	I6	I7.5	I6.5	heavy rain & wind all day
2I	I2	I9	I3	overcast but dry
22	8.5			

0 - 10mm 11 - 20mm 21 - 30mm 31 - 40mm 41mm+

The last two categories were obviously adults and most of these would be laying eggs during September/October. The two smallest groups were recently hatched individuals while the middle size class may have contained the largest of these and possibly slugs which had hatched in the spring. These three groups were collectively termed juveniles in the population analysis.

5.5 WEATHER CONDITIONS

Temperature was recorded on the surface of the soil. at 3cm depth and in the air. This was repeated at midday and early evening for the rest of the trial whenever possible - temperature and weather conditions are presented in Table 29 . The counting was started at 07.00 while the dew was still heavy and the animals abundant, for as the day progressed the slugs disappeared underground. Judge (1972) also found the time of trap counting to be especially important in August and September, when traps with a large number of slugs at 09.00 would be empty in the afternoon. The weather throughout the trial was dry and warm with an average air temperature of 14°C in the morning. By lunchtime this had increased to over 20°C, the dew had disappeared and the number of slugs under the traps greatly decreased. No animals were removed from the plots during the seven day pre-baiting period. This criterion of minimal interference with the population limited the method of estimating population numbers to searching in the early part of the day.

Table 30

TREATMENTS APPLIED TO EXPERIMENTAL PLOTS

BAIT	TOXICANT	FORMULATION	DOSAGE
A	non-toxic control	pellet wheat	6.25kg/ha 62.5g/plot
B	4% methiocarb	pellet wheat carrier commercial	3kg/ha 30g/plot
C	10% metaldehyde	pellet + repellent inorganic carrier commercial	3kg/ha 30g/plot
D	6% metaldehyde	pellet wheat carrier commercial	6.25kg/ha 62.5g/plot
E	5% metaldehyde	pellet wheat carrier commercial	5kg/ha 50g/plot
F	5% metaldehyde	pellet + repellent wheat carrier commercial	5kg/ha 50g/plot
G	7.5% metaldehyde	fine grains inorganic carrier experimental	10kg/ha 100g/plot
H	non-toxic control	semi-solid	Ikg/ha 10g/plot
J	6% metaldehyde	pellets wheat carrier experimental	6.25kg/ha 62.5g/plot
K	6% metaldehyde	pellets + attractant wheat carrier experimental	6.25kg/ha 62.5g/plot

5.6 BAITS AND BAIT APPLICATION

Table 30 lists the formulation and dosage rate of the ten baits used. The baits were compared for their ability to attract slugs and kill them and for their persistence in field conditions. Baits B, C, D, E and F were commercial formulations, baits G, J and K were experimental formulations and baits A and B were non-toxic controls. Two of the experimental baits were manufactured industrially to ensure that size,hardness and shape were consistent. Bait J was a simple metaldehyde/wheat mixture with no other additives while bait K contained 1% of a yeast compound which had shown promise as a feeding stimulant in laboratory feeding tests. These pellets also contained a fungicide which was the same as that used in the commercial formulation, bait D. The other experimental formulation, bait G, was a laboratory mixture of metaldehyde and sand (sieved through a O.1mm mesh) which had been combined in an electric blender. The metaldehyde was absorbed onto the surface of the sand grains, thus presenting a large surface area and so increasing the availability of the toxicant.

The two controls provided a source of food only and therefore allowed a comparison between the two attractants combined into the other baits - wheat and yeast. The pressed yeast was applied in the form collected from the brewery - a sticky, semi-solid mixture which was formed into small lumps of approximately 5mm diamter - whilst the wheat grains were prepared commercially and thus combined into pellets of similar structure to the poison baits.

On the evening of day seven, each plot was baited with the recommended dosage rate of the bait assigned to that plot. The baits were hand broadcast, but as the evening was windy, it was difficult to achieve an even distribution. With this in mind, four piles of straw, each approximately 50cm x 50cm, were placed equidistant from the central sack and a few pellets scattered under each. These four smaller traps provided at least four areas in each plot which could be examined for aggregations of poisoned slugs. The central sacked area was left unbaited so that it could be searched at a later date and provide some data on the natural population change over the baiting period.

5.7 POST-BAITING SAMPLING

The plots were sampled for the following nine days to assess the number of animals killed by each treatment. Both poisoned and unpoisoned slugs were removed from under the traps and kept in plastic seed boxes so that any recovery could be monitored. Laboratory tests to compare the effectiveness of bait formulations usually involve the use of recovery boxes in which all the animals trapped are placed for observation. These boxes offer conditions designed to encourage maximum recovery - an equable temperature and a high relative humidity. Examination of the animals at regular intervals can reveal whether they consumed a sublethal dose and were thus able to recover when placed in optimum conditions. Unfortunately, there was no suitable constant environment in which to place the recovery boxes in this trial. The temperature

rose markedly most afternoons and many of the slugs which had been recorded as unpoisoned died during the 12 hours in the recovery boxes. It was unclear whether this was from belated effects of poisoning or from the high temperature but the slug deaths may well have been due to a combination of these two factors.

The recount of poisoned animals was discontinued after three days since it proved impossible to distinguish temperature deaths from deaths due to chemical treatment. The figures presented in all the tables therefore refer to animals recorded as poisoned in the field and there is no estimate of the recovery of slugs when removed to optimum conditions or the belated effects of chemical poisoning. Since the two toxicants metaldehyde and methiocarb produce different symptoms of poisoning (see p 243), this may have led to an underestimation of metaldehyde poisoned slugs.

Each plot was subdivided into 100 units of $1m^2$ and numbered consecutively. With the aid of random number tables, two units in each plot were selected for sampling each day and a trap placed in the appropriate place on the evening prior to sampling. It had been anticipated that in this way a representative analysis of slug deaths in each plot could be obtained. On the morning of day 8, the area under the two traps in each plot was examined for slugs but a count of the number of bait pellets in the same area indicated a range of 0 - 15. This suggested that the wind had caused pellets to drift and sampling random areas each day would give a biased view of bait effectiveness. For the following eight days therefore, (days 9 - 16), the area under the straw traps was searched for poisoned

and unpoisoned slugs. One trap per plot was examined each day and the searching rotated so that each of the four traps was searched twice during the following eight days. By day 16, nine days after baiting, the numbers of poisoned animals had decreased greatly and some of the baits had weathered so the daily counts were discontinued as they were increasingly unproductive.

The residual population on each plot was examined at the end of the trial to compare the number of unpoisoned slugs remaining after treatment. On day 19, the sacked unbaited area in the centre of each plot was searched to investigate natural changes in the slug population for comparison with the seven day pre-baiting estimates. On days 21 and 22, the area under each of the four straw traps was similarly searched to compare slug numbers on the baited areas two weeks after bait application. On day 22, the population throughout the plots was estimated by trapping under two Marley tiles randomly placed in each plot. These sampling areas were small compared to the total area of each plot but the constraint of searching the traps in the morning, when the animals were above ground, limited the area which could be searched by one person.

5.8 PREBAITING POPULATION ESTIMATE

Preliminary population work suggested that Deroceras reticulatum was certainly the most prominent slug pest. A total of only 124 Arion ater, all juveniles, was recorded during the entire trial period compared with 10,651 Deroceras. No Limax or Milax spp. were found during the plot searching but were seen in the long vegetation at

the perimeter of the field. Several fungi here may have attracted these species but Deroceras is generally regarded as the most active species and as the weather was so dry, other species may have moved underground.

Jennings & Barkham (1979) studied niche separation in 8 species of slug and found that a vertical separation of the feeding population was sufficient to avoid competition between most species. Deroceras reticulatum and Arion ater were the closest, however, and the authors suggest that a temporal separation may reduce competition between these two species caused by similar food preferences. Barnes & Weil (1944) also indicated temporal separation between different slug species.

Since all the Arion ater trapped in this trial were juveniles, it does appear that the two species are separated by the seasonal difference in the attainment of their maximum populations.

Trapping records for the seven day prebaiting period are presented in the Appendix 2 & Table 3I summarises these results. The largest number of slugs recorded under the hessian sacks on any one day was taken as the value for transforming into an estimate of the slug population for the field. This value of 1443 slugs (day 4) represents a minimum level of slugs present in the $25m^2$ sampled area i.e. 57.72 Deroceras per $metre^2$. The number of adults trapped was 793 and the number of juveniles 650 which is equivalent to a population level of 31.72 adults and 26.0 juveniles per $metre^2$. Rain had fallen the previous night, encouraging activity of the slugs, and the day was damp so the number of juveniles was particularly high.

TABLE 3I SUMMARY OF PRE-BAITING TRAPPING DATA

DAY	I	2	3	4	5	6	7
No. of plots	50	50	50	50	50	55	55
Deroceras							
Juveniles	I96	290	505	650	347	248	204
Adults	280	400	68I	793	533	54I	538
Total	476	690	II86	I443	880	789	742
No. per m^2	I9.04	27.60	47.52	57.72	35.20	28.69	28.92
Arion ater							
Total	I	2	O	O	O	4	2

TABLE 32 PRE-BAITING TRAPPING FIGURES FOR DAY 6 REARRANGED INTO TREATMENT GROUPS.

COLUMN / BAIT	a	b	c	d	e	TOTAL	MEAN	S.D.
A	22	29	7	8	8	74	I4.8	IO.08
B	I2	25	23	II	7	78	I5.6	7.94
C	25	2I	6	I4	II	77	I5.4	7.64
D	22	37	I3	I	I2	85	I7.O	I3.44
E	I	2	IO	I2	I9	44	8.8	7.46
F	I3	3I	I6	I7	5	82	I6.4	9.42
G	34	2I	8	II	II	85	I7.O	IO.70
H	I8	I7	IO	8	9	62	I2.4	4.72
J	36	I7	23	5	I2	93	I8.6	II.76
K	27	I7	22	2O	23	IO9	2I.8	3.7O
TOTAL	2IO	2I7	I38	IO7	II7	789		

Analysis of variance (Appendix 2) shows a significant difference between the number of slugs trapped on the columns "a" to "e" (P = O.OI) but no significant difference between the treatment groups (P = O.O5).

Distribution of the slugs varied both across and down the field, with the number of animals highest in column "a" and rows 4 - 9. The greatest number of slugs was in plot a7 (subsequently baited with bait H) with 64 slugs trapped on day 4 in the $0.5m^2$ sacked area - this is equivalent to a population of 138 Deroceras per $metre^2$. As the upper rows appeared to be sparsely populated, another row (row 10) was added to the bottom of the set of plots but only the pre-baiting data from days 6 and 7 include this row.

The trapping figures for rows 1 - 10 on day 6 have been rearranged into the treatment groups so that the prebaiting population figures are in the same form as the post-baiting figures (Table 32). Analysis of variance on these data indicate that there is no significant difference between the row totals ($P = 0.05$) although the column totals are significantly different ($P = 0.01$). Therefore, although some areas of the field may have suggested an aggregated distribution, this is not significant within the treatment rows and the inherent slug population can be assumed to be similar for each treatment.

5.9 PLANT COVER

The ground cover was assessed using a $30cm^2$ quadrat which was thrown five times in each plot. The cover under each of the straw traps was also recorded since the main post-baiting population counts were from these areas. Ground cover was divided into clover, perennial rye grass, straw (lying on the surface) and bare ground - occasionally small fungal fruiting bodies and mosses were

FIG 42

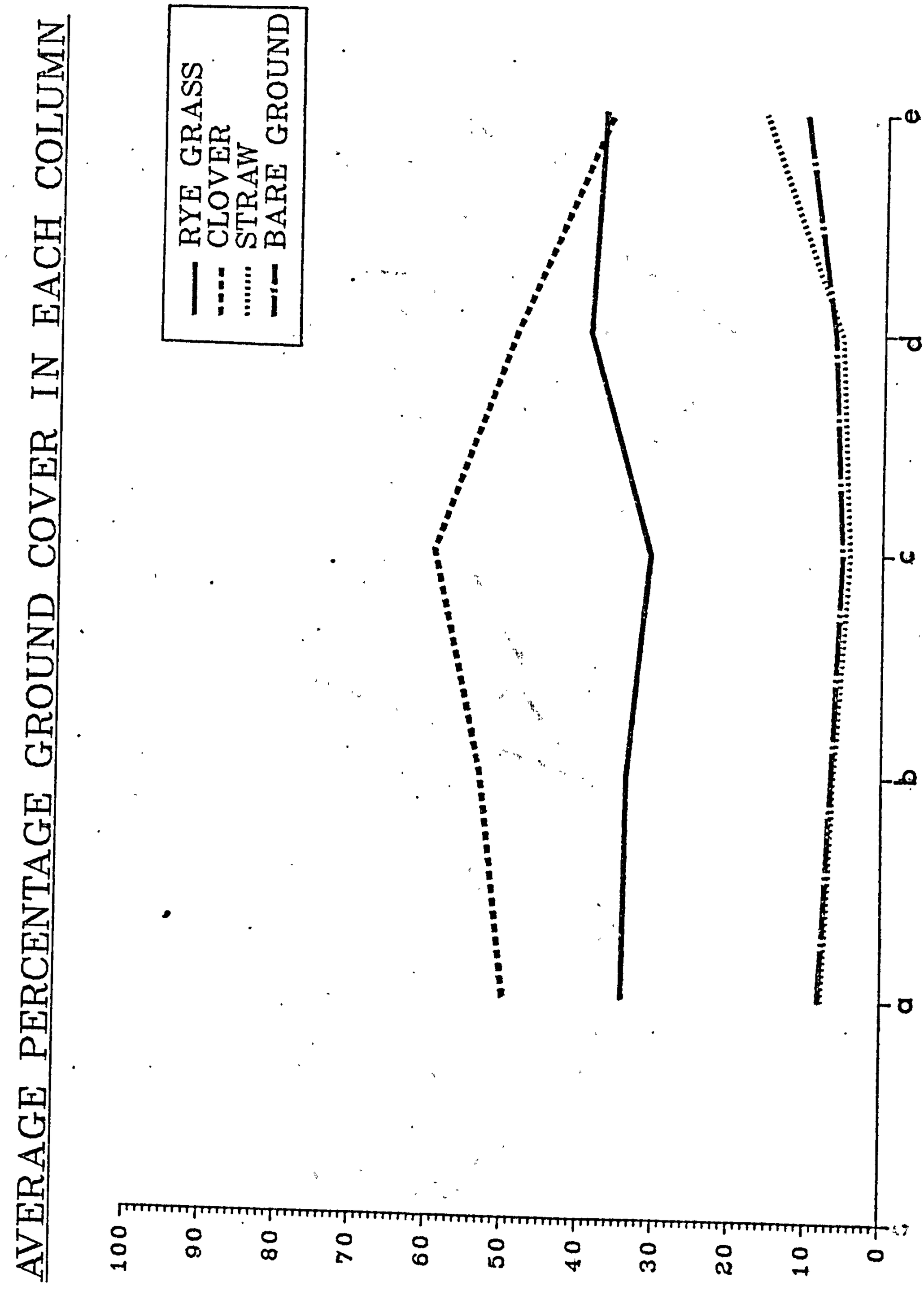

AVERAGE PERCENTAGE GROUND COVER IN EACH COLUMN
RYE GRASS
CLOVER
STRAW
BARE GROUND
a
b
c
d
e
100
90
80
70
60
50
40
30
20
10
0
PERCENTAGE COVER %

FIG 43

AVERAGE PERCENTAGE DISTRIBUTION OF CLOVER IN EACH ROW

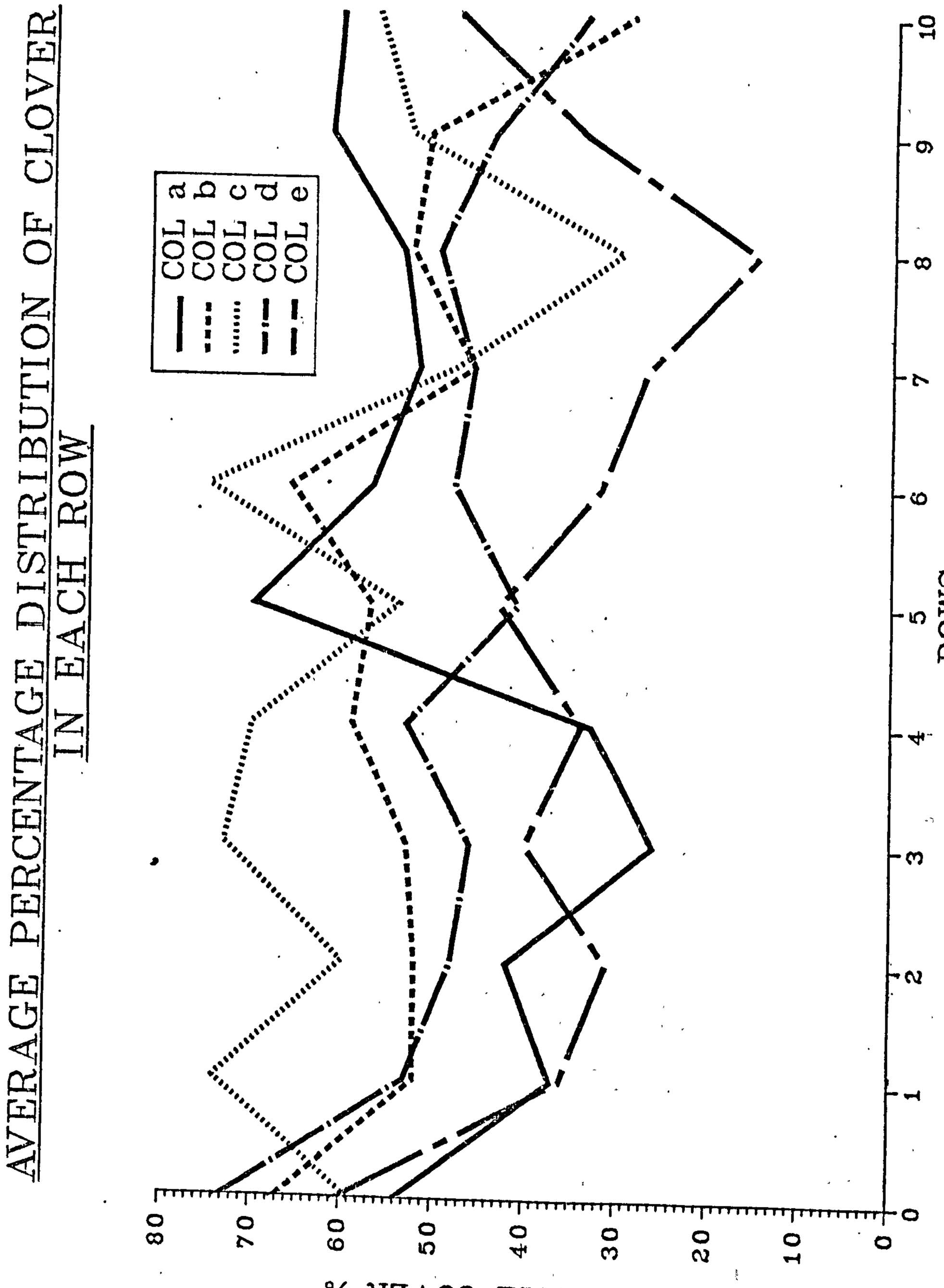
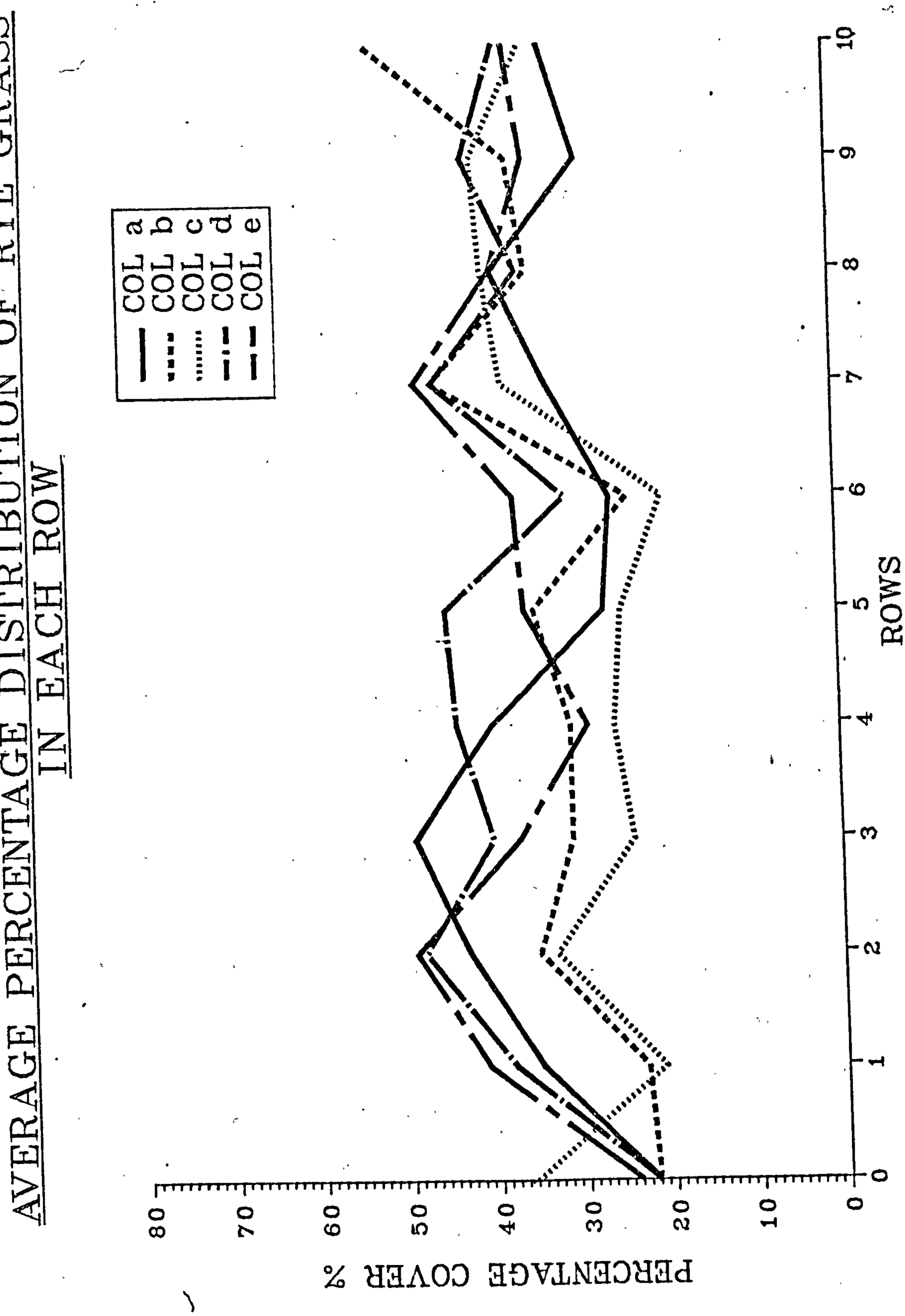


FIG 44


AVERAGE PERCENTAGE DISTRIBUTION OF RYE GRASS
IN EACH ROW
COL a
COL b
COL c
COL d
COL e
PERCENTAGE COVER %
80
70
60
50
40
30
20
10
0
ROWS
0
1
2
3
4
5
6
7
8
9
10

present in the quadrats but these were not included as the main purpose of this exercise was to highlight any major trends in the distribution of the dominant plant species. Both the clover and the rye grass provide food and shelter for the slugs, so only variations in the total percentage cover of these two species would be likely to affect slug distribution.

The average percentage cover for the four principal componenents of the ground layer was plotted for each column, Fig. 42. Although there was a fall in the clover cover across the field with column "a" higher than column "e", the rye grass cover was more even. The distribution of clover in each row is shown in Fig. 43. and that of rye grass in Fig. 44. No major trend is apparent in the percentage cover of either plant species down the field. As slug distribution was different between both columns and rows, it is unlikely that plant cover was sufficiently important to account for trends in slug distribution.

5.10 POST BAITING SAMPLING

A summary of the trapping data collected after baiting is given in Table 33. Deroceras reticulatum was the only slug species whose dead bodies were collected under the traps though other dead invertebrates were found on the plots treated with bait B. All the animals captured under the traps were removed and placed in plastic 'recovery boxes', but since there was a four day interval between searching the straw traps, this allowed time for attraction to the bait and poisoning effects to take place. Slugs were classified as either adults or juveniles and

Table 33

SUMMARY OF SLUGS TRAPPED IN NINE DAY POST-BAITING PERIOD

DAY	8	9	10	11	12	13	14	15	16
Deroceras									
UNPOISONED JUVENILES	85	184	170	203	152	81	118	73	49
UNPOISONED ADULTS	52	166	141	210	127	66	78	85	70
TOTAL	137	350	311	413	279	147	196	158	119
POISONED JUVENILES	8	4	23	14	16	2	1	4	7
POISONED ADULTS	96	102	173	218	198	54	44	34	61
TOTAL	104	106	196	232	214	56	45	38	68
Arion ater									
UNPOISONED JUVENILES	2	9	12	8	13	6	17	11	7
POISONED JUVENILES	0	1	0	1	3	1	2	0	0
TOTAL	2	10	12	9	16	7	19	11	7

NUMBER OF Deroceras TRAPPED UNDER EACH OF THE FOUR STRAW TRAPS

DAYS	9 + 13	10 + 14	11 + 15	12 + 16
TRAP	1	2	3	4
UNPOISONED	497	507	571	398
POISONED	162	241	270	282
TOTAL	659	748	841	680

TABLE 34 TOTAL NUMBER OF ALL SLUGS (POISONED AND UNPOISONED TRAPPED ON EACH TREATMENT

DAY / BAIT	8	9	IO	II	I2	I3	I4	I5	I6	TOTAL	MEAN
A	24	63	48	98	56	I8	24	28	23	328	42.44
B	23	37	82	98	IIO	22	28	37	32	469	52.II
C	24	38	44	37	27	I5	I2	I9	I8	234	26.OO
D	2O	55	46	54	34	I5	26	I7	I3	28O	3I.II
E	I9	4I	45	38	48	I6	32	I9	I7	275	3O.56
F	27	I7	57	48	44	2O	I7	I6	I3	259	28.78
G	28	57	46	93	45	4I	4O	25	25	4OO	44.44
H	8	59	6O	77	53	33	36	2I	33	37O	4I.II
J	3I	49	38	42	43	I2	22	8	IO	255	28.33
K	39	5O	53	69	49	I8	23	I7	2O	338	37.56
TOTAL	243	466	5I9	654	5O9	2IO	26O	2O7	I94	3262	

Analysis of variance shows a significant difference between both treatments and days (P = O.OI). See Appendix 2

TABLE 35 TOTAL NUMBER OF POISONED Deroceras TRAPPED ON EACH PLOT IN THE NINE DAY POST-BAITING PERIOD

COLUMN / BAIT	a	b	c	d	e	TOTAL	MEAN
A	4	O	3	2	2	II	2.2
B	5I	87	66	7I	5I	326	65.2
C	IO	8	8	IO	I3	49	9.8
D	I5	I7	I4	I2	IO	68	I3.6
E	IO	I3	46	29	2I	II9	23.8
F	4I	26	2O	I7	I4	II8	23.6
G	42	29	6	2I	28	I26	25.2
H	4	I	O	2	O	7	I.4
J	34	27	36	I3	2O	I3O	26.O
K	4I	I6	8	23	I7	IO5	2I.O
TOTAL	252	224	2O8	2OO	I76	IO59	

Analysis of variance shows no significant difference between the columns "a" to "e" (P = O.O5) but a significant difference between the treatments (P = O.OI).

poisoned or unpoisoned so that the results for either sector of the population could be analysed separately.

Table 34 provides a breakdown of the number of slugs trapped on each treatment during the nine day post-baiting period. The figures represent the sum of the five replicates and analysis of variance indicates a significant difference between the treatments (P = 0.01) and between the numbers trapped on different days (P = 0.01).

5.11 POISONING SYMPTOMS

The criterion used to assess whether or not a slug was dead was the ability to respond to mechanical stimuli. Many slugs were seen on all the metaldehyde plots (baits C, D, E, F, G, J, K) to secrete a thick mucus - a sign of irritation - yet were fully capable of moving when prodded. These animals were not recorded as poisoned but, as all the animals trapped were collected, it was hoped that examination 12 hours later would reveal whether or not the slugs exhibiting symptoms of irritation were poisoned. As the recount was unsuccessful in differentiating poisoned slugs from temperature deaths, this plan was unfulfilled. This probably led to an under-estimation of the number of metaldehyde poisoned slugs since those recorded as poisoned were completely dead and usually in a dehydrated condition. A count of the residual population on each treatment was therefore planned to determine whether an anomaly existed between the actual number of deaths from metaldehyde baits and the number indicated by sampling.

Methiocarb poisoned slugs, on plots baited with bait B, had an entirely different appearance. Their bodies

FIG. 45 METHIOCARB POISONED SLUG

Typical features include bloated body, extended tentacles, everted buccal mass, blue tinge to soft,jelly-like body.

FIG 46 METALDEHYDE POISONED SLUG

Typical features include hard, shrunken body, dark appearance, tentacles withdrawn.

were bloated, rather transparent and frequently tinged with blue - presumably bait in the gut. Poisoned earthworms found on methiocarb baited plots were also extended and, like the slugs showed no ability to crawl away when prodded with a mounted needle. Many of these poisoned animals were not completely dead since they could make uncoordinated movements although they were unable to initiate coordinated locomotion when stimulated. Figs $_{45}$ and $_{46}$ illustrate the typical appearance of a methiocarb poisoned slug and a metaldehyde poisoned slug.

The action of the two toxicants metaldehyde and methiocarb therefore produce different symptoms of poisoning which may have introduced a bias in the trapping records. Physiological and behavioural differences may have caused discrepancies in the number of slugs present on each plot and their conspicuousness. Methiocarb poisoned animals were bright, expanded and therefore clearly visible while metaldehyde poisoned animals were generally dark, dehydrated and shrivelled so less easy to see in the field. Metaldehyde poisoned animals may also have been able to move away from the bait since they were not immobilised and the daily mortality counts give no indication of the numbers of these slugs. In a trial where poisoning is accepted as the means of assessing bait efficiency, methiocarb poisoned slugs are easily classified as such but the interpretation of symptoms in metaldehyde poisoned slugs is rather ambiguous and estimates of bait efficiency less accurate.

If the methiocarb bait was significantly more effective than the metaldehyde formulations, as the number of

dead slugs found suggests, one would expect the population of slugs on these plots to be greatly reduced at the end of the trial. Field trials to assess bait efficiency are normally concerned with the number of dead slugs found on treated areas and the residual population is ignored. Evidence from this trial suggests that this may be an important omission and the population count was continued several days after the termination of the daily mortality counts to assess whether the treatments differed in the residual population level.

5.12 POISONED SLUGS

Table 35 shows the deaths for each treatment rearranged into the columns "a" - "e". Each value therefore represents the total number of dead slugs found on that plot on days 8 - 16. The column totals do indicate a decrease across the field with more slugs killed in column "a" than column "e", ($P = 0.05$). This corresponds to the pre-baiting population figures which suggested a higher level of slugs in column "a" (though this difference in the number of dead slugs is not significant.) The treatment totals for the five replicas do show distinct differences ($P = 0.01$) and since pre-baiting analysis had indicated that there was no difference in the inherent slug population between the treatment rows, this variability in the number of dead slugs trapped on each treatment can be attributed to the effects of the treatments themselves.

The figures for the numbers of dead slugs trapped on days 8 - 16 are presented in the Appendix 2 together with the analysis of variance for each day. There was no significant

TABLE 36 NUMBER OF POISONED Deroceras TRAPPED ON EACH TREATMENT

DAY / BAIT	8	9	IO	II	I2	I3	I4	I5	I6	TOTAL	MEAN
A	2	O	4	3	I	O	O	O	I	II	I.22
B	I5	I8	7O	8O	9O	IO	II	IO	22	326	36.22
C	6	6	8	II	4	5	I	2	6	49	5.44
D	IO	I5	I3	I2	II	4	O	I	2	68	7.56
E	II	I5	22	I6	29	8	3	5	IO	II9	I3.22
F	I2	7	25	I7	22	5	6	8	6	II8	I3.II
G	3	7	I3	38	I7	I8	I5	7	8	I26	I4.OO
H	4	I	O	O	O	O	O	2	O	7	O.78
J	I5	26	24	I9	25	4	8	2	7	I3O	I4.44
K	26	II	I7	26	I5	2	I	I	6	IO5	II.67
TOTAL	IO4	IO6	I96	232	2I4	56	45	38	68	IO59	

The table includes natural mortality since 'poisoned' slugs were recorded on the control treatments, A and H

Analysis of variance shows a significant difference in the number of poisoned slugs trapped on each treatment ($P = O.OI$) and on each day ($P = O.OI$).

difference between the number of dead slugs found on each replicate ("a" - "e") on days 8, 9, 11, 12, 13, 14 and 15 - days IO and 16 showed a significant difference (P = 0.05). The treatment differences were significant on all days except day 8 and day 15. The difference between baits was therefore not apparent immediately after baiting (day 8) and when the baits had decreased in effectiveness (day 15).

The results are summarised in Table 36 which gives the combined value for all five replicates for the nine day period. Dead animals on the two control treatments could not be victims of poisoning since the guard rows were too wide to allow migration from baited areas - these dead slugs therefore represent the natural mortality. Analysis of variance on the combined data indicates that the differences between treatments and between days are highly significant (P = 0.01).

A "t" test has been used to compare the number of dead slugs trapped at individual treatments in the nine day post-baiting period (See Appendix 2). The values obtained have been incorporated into a table for each day which gives the probability of similarity between any two treatments. Any bait which has a high probability of being similar to either of the control baits A or H is not effective at killing slugs - both of these control treatments show a high degree of similarity to one another on all nine days.

On days 10 and 12, the effectiveness of the methiocarb formulation is apparent, as none of the other formulations show greater than a 0.5% probability of being similar to it - on day 11, only the sand based formulation bait G breaks this pattern. After this three day period,the

other baits appear to improve, in particular the two commercial formulations E and F, so that on day 13 and day 15 respectively these baits show more than a 50% probability of similarity to the methiocarb formulation. The performance of these two baits is comparable - at least 25 to 50% similarity on all days but one - indicating that the mammal repellent added to the basic formulation does not affect the number of slugs killed.

Most baits declined in effectiveness after an initial rise so that on day 15, for example, baits C, D, J and K had a greater than 50% chance of being similar to either of the non-toxic controls. The sand based formulation, G, however, showed a reverse trend and gradually improved in performance. The two other experimental formulations J and K were less constant and fluctuated in performance. Bait J generally performed well but comparison with bait K which contained the yeast attractant was not really possible. Bait K was generally less attractive than bait J though it had a high degree of similarity with bait D, the commercial formulation of similar constituents.

A matrix to compare the treatments on different days has been compiled from the combined data of Table 36 The most striking feature of this matrix (Table 37) is that none of the baits has a greater than 0.5% probability of similarity to bait B, the methiocarb treatment. The trapping figures indicate that this bait produced the greatest number of dead animals which gives the impression that this formulation is superior to the others. However, the results presented in 5.I3 and in Fig. 48 conflict with

TABLE 37 MATRIX TO COMPARE THE POISONED DEROCERAS TRAPPED ON EACH TREATMENT

BAIT	A	B	C	D	E	F	G	H	J	K
A										
B	-0.5%									
C	25 - 50%	-0.5%								
D	25%	-0.5%	+50%							
E	5%	-0.5%	IO - 25%	25 - 50%						
F	5%	-0.5%	IO - 25%	25 - 50%	+50%					
G	2.5 - 5%	-0.5%	IO - 25%	25%	+50%	+50%				
H	+50%	-0.5%	25 - 50%	IO - 25%	2.5 - 5%	2.5 - 5%	2.5 - 5%			
J	2.5 - 5%	-0.5%	IO - 25%	IO - 25%	+50%	+50%	25 - 50%	2.5%		
K	5 - IO%	-0.5%	25%	25 - 50%	+50%	+50%	+50%	5 - IO%	+50%	

The percentages indicate the probability of similarity between any two treatments in the number of poisoned slugs trapped on days 8 - I6. Values calculated from the $S.E._d$ (TABLE 36).

this interpretation. Baits E, F, G, J and K had a greater than 50% chance of being similar to one another and only a small probability of being similar to either of the non-toxic controls. Baits C and D, two of the commerical treatments, were similar to one another and to the controls, but both appeared to perform less well than other metaldehyde formulations.

JUVENILE AND ADULT DEATHS

Timing of bait application is considered to be of great importance in the success of a treatment. Baits are normally applied in early spring and in mid summer to kill the adult slugs before they lay eggs and thereby prevent or reduce juvenile recruitment. To minimise interference with the farming practices on Bigfield, baits in this trial were applied in early September and, as the prebaiting trapping data indicated, a juvenile population was already present.

A change in the population structure (see P 26I) was anticipated as more eggs hatched and recruitment continued so the adult and juvenile figures were treated separately. It was also apparent that juvenile and adult mortality was very different with the number of juvenile deaths much lower than those of the adult slugs. As adult slugs are the normal target of baiting programmes, emphasis on the adult death rate can highlight the trends in bait effectiveness which are likely to be obtained when treatments are applied earlier in the year.

Tables 38and 39 show the number of adults and juveniles poisoned in the nine day period after baiting.

TABLE 38 NUMBER OF POISONED Deroceras TRAPPED PER DAY - JUVENILES

DAY / BAIT	8	9	IO	II	I2	I3	I4	I5	I6	TOTAL	MEAN
A	O	O	O	O	O	O	O	O	O	O	O.OO
B	5	I	I6	IO	I4	2	I	3	6	58	6.44
C	O	O	I	O	O	O	O	O	O	I	O.II
D	O	O	O	I	O	O	O	O	O	I	O.II
E	I	I	I	I	O	O	O	O	O	4	O.44
F	I	I	O	O	O	O	O	O	O	2	O.22
G	O	O	I	O	O	O	O	O	I	2	O.22
H	O	O	O	O	O	O	O	O	O	O	O.OO
J	I	I	I	O	2	O	O	I	O	6	O.67
K	O	O	2	3	O	O	O	O	O	5	O.56
TOTAL	8	4	23	I4	I6	2	I	4	7	79	

Analysis of variance shows that the difference between days is significant (P = O.OI) and the difference between treatments also significant (P = O.OI). See Appendix 2

TABLE 39 NUMBER OF POISONED Deroceras TRAPPED PER DAY - ADULTS

DAY / BAIT	8	9	IO	II	I2	I3	I4	I5	I6	TOTAL	MEAN
A	2	O	4	3	I	O	O	O	I	II	I.22
B	IO	I7	54	7O	76	8	IO	7	I6	268	29.78
C	6	6	7	II	4	5	I	2	6	48	5.33
D	IO	I5	I2	I2	II	4	O	I	2	67	7.44
E	IO	I4	2I	I5	29	8	3	5	IO	II5	I2.78
F	II	6	25	27	22	5	6	8	6	II6	I2.89
G	3	7	I2	38	I7	I8	I5	7	7	I24	I3.78
H	4	I	O	O	O	O	O	2	O	7	O.78
J	I4	25	23	I9	23	4	8	I	7	I24	I3.78
K	26	II	I5	23	I5	2	I	I	6	IOO	II.II
TOTAL	96	IO2	I73	2I8	I98	54	44	34	6I	980	

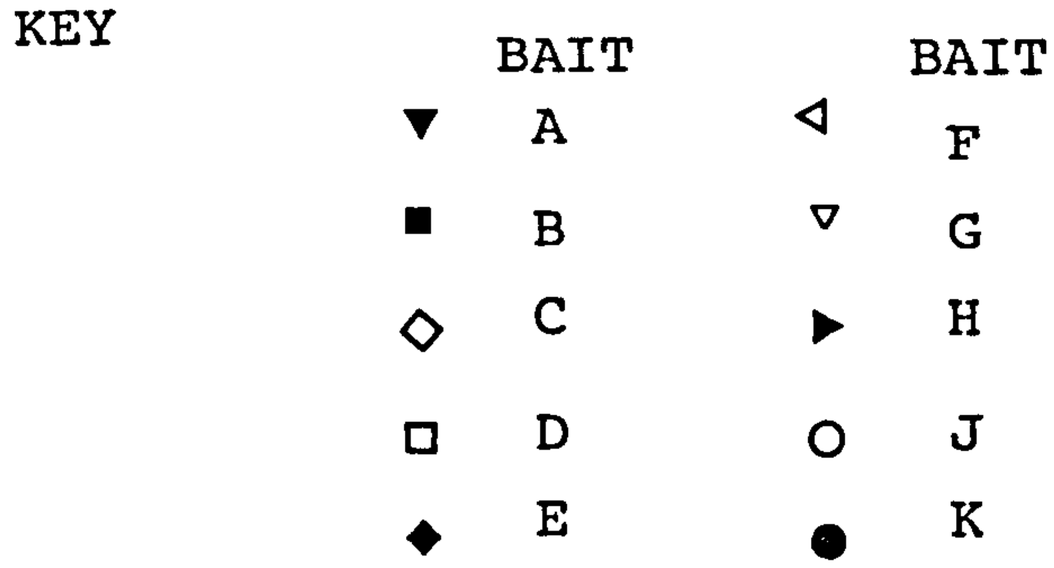
Analysis of variance shows that the difference between days is significant (P = O.OI) and the difference between treatments is also significant (P = O.OI). See Appendix 2.

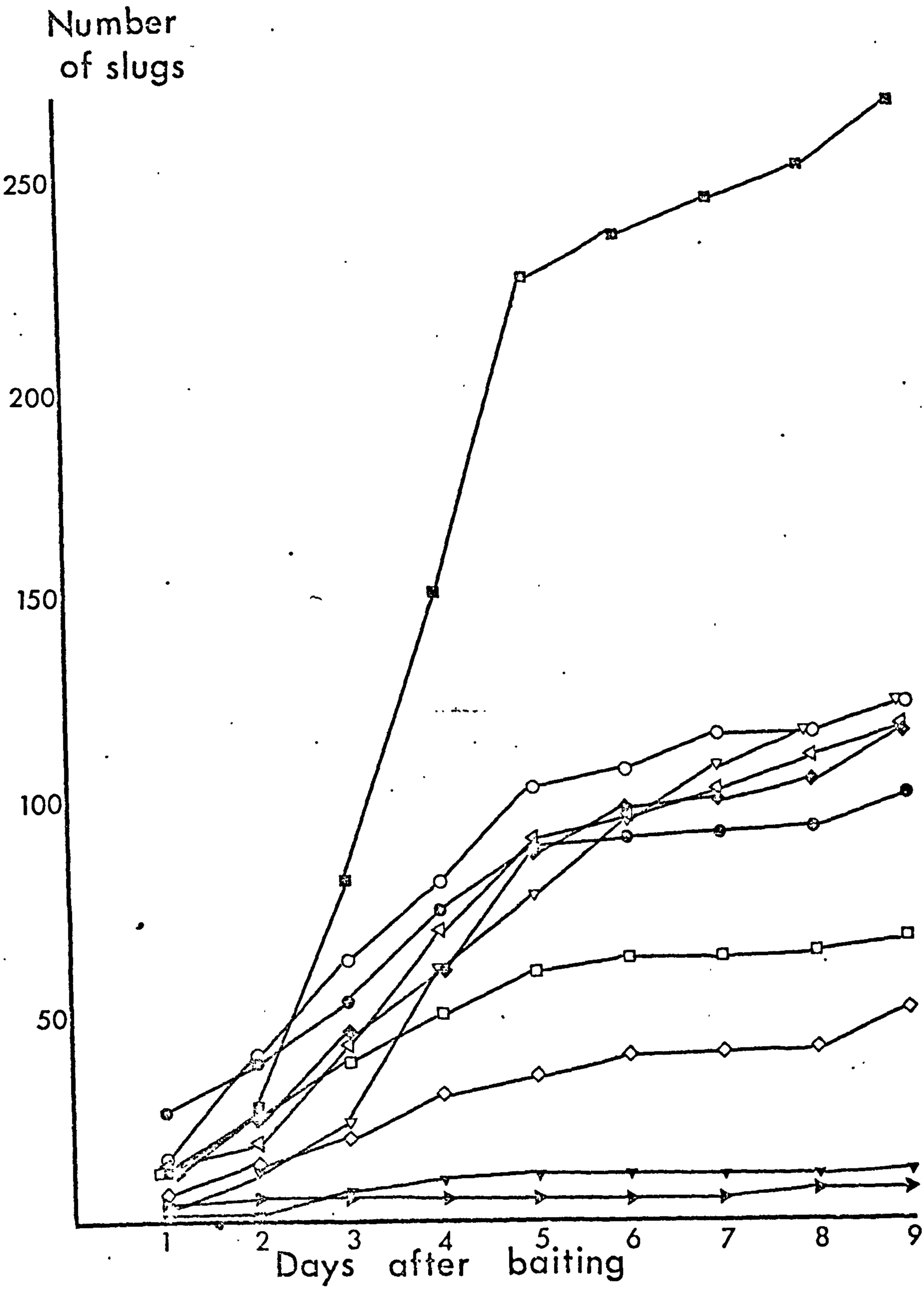
Analysis of variance on the figures for juveniles indicates a significant difference between treatments (P = 0.05) with the methiocarb formulation, bait B, proving the most effective. The difference between the number of juveniles on successive days is not significant (P = 0.05) but the number of dead juveniles found was extremely small - only 7% of the total number of juveniles trapped were dead.

Adult deaths from baits show a significant difference between treatments (P = 0.01) and between days (P = 0.01). A graph of cumulative kill for the dead adult Deroceras found on each treatment in the nine days after baiting (days 8 - 16) is presented in Fig. 47 . As expected the two control treatments have few dead animals. The metaldehyde treatments fall into two groups - baits C and D which trapped 50 -70 slug bodies in this period and baits E, F, G, J and K which all produced approximately 100 dead adult Deroceras in the same period. Bait G, the sand based formulation is seen to improve in performance rather later than the other treatments. Bait B, the methiocarb treatment, trapped over 250 dead adult slugs suggesting that this treatment is the most efficient. The effectiveness of methiocarb is questioned, however, when the residual population is estimated, (see P 264).

The results do indicate that an age related susceptibility exists, with juveniles apparently quite resistant to the toxic effects of bait. This has been indicated by Crawford-Sidebotham (1971) and others though Daxl (1970) suggested that the small size of juveniles makes them more susceptible to poisoning. These trials indicate that young slugs are relatively unaffected by

FIG. 47 CUMULATIVE TOTAL OF POISONED ADULT *Deroceras* TRAPPED IN NINE DAY POST-BAITING PERIOD (days 8 - I6)




Number of slugs
250
200
150
100
50
1
2
3
4
5
6
7
8
9
Days after baiting

bait application and highlight the importance of baiting at the correct time of year - before adult slugs lay eggs. The reason for such diminished juvenile mortality is very probably due to the fact that they are less mobile than the adults and encounter the bait less frequently. They are also active only in cool, damp weather, remaining underground for much of the time and are known to have different eating habits to the adult slugs which undoubtedly affects the palatability of the toxic baits.

5.13 UNPOISONED SLUGS

The number of unpoisoned animals trapped at each plot is some measure of the relative attractiveness of each bait but also its inefficiency at killing the slugs. The control plots, although acting as standards for toxicity measurements, do provide food materials and as such are likely to attract slugs. For the other eight baits, the number of unpoisoned slugs trapped in the nine day post-baiting period is an important factor in comparing bait effectiveness. These data have been used to compile the efficiency ratio for each bait which is presented in the following section. Table 40 summarises the trapping figures for unpoisoned slugs during this period and Table 41 and 42 divides this into adult and juvenile slugs. Analysis of variance on these data indicates a significant difference between treatments ($P = 0.01$) and between days ($P = 0.01$) for both the combined data and separate treatment of adult and juvenile figures. A large number of juveniles were trapped which confirms the hypothesis that juvenile mortality was low because the baits were ineffective

TABLE 40 NUMBER OF UNPOISONED Deroceras TRAPPED ON EACH TREATMENT

DAY / BAIT	8	9	IO	II	I2	I3	I4	I5	I6	TOTAL	MEAN
A	22	63	43	95	55	I8	23	28	22	369	4I.OO
B	8	I9	I2	I7	I6	I2	I3	27	IO	I34	I4.89
C	I8	3I	33	24	22	IO	9	I6	II	I74	I9.34
D	IO	39	32	4O	2I	II	23	I3	II	2OO	22.22
E	8	26	22	22	I7	7	26	IO	5	I43	I5.89
F	I4	IO	29	I9	I9	I2	IO	8	7	I28	I4.22
G	25	48	32	55	26	22	23	I8	I6	265	29.44
H	4	57	6O	77	53	33	36	I9	22	36I	4O.II
J	I5	2O	I3	22	I8	8	I3	6	3	II8	I3.II
K	I3	37	35	42	32	I4	2O	I3	I2	2I8	24.22
TOTAL	I37	35O	3II	4I3	279	I47	I96	I58	II9	2IIO	

Analysis of variance shows a significant difference between the number of unpoisoned slugs trapped on each treatment (P = O.OI) and on each day (P = O.OI).

TABLE 4I NUMBER OF UNPOISONED Deroceras TRAPPED PER DAY - JUVENILES

DAY / BAIT	8	9	IO	II	I2	I3	I4	I5	I6	TOTAL	MEAN
A	I3	34	IO	28	I8	6	I4	7	2	I32	I4.67
B	4	7	8	I4	I2	IO	IO	23	8	96	IO.67
C	I6	I6	25	I7	I8	6	6	I3	6	I23	I3.67
D	6	24	I7	26	IO	6	9	5	6	IO9	I2.II
E	5	II	I7	I8	IO	5	2I	2	3	92	IO.22
F	I3	5	22	9	II	6	8	4	5	83	9.22
G	I8	22	I4	27	I5	I4	IO	3	7	I3O	I4.44
H	3	3I	26	32	33	I6	2O	6	6	I73	I9.22
J	2	II	9	I2	IO	4	7	5	O	6O	6.67
K	5	23	22	2O	I5	8	I3	5	6	II7	I3.OO
TOTAL	85	I84	I7O	2O3	I52	8I	II8	73	49	III5	

Analysis of variance shows that the difference between days is significant (P =O.OI) and the difference between treatments is also significant (P = O.OI). See Appendix 2.

TABLE-42 NUMBER OF UNPOISONED Deroceras TRAPPED PER DAY - ADULTS

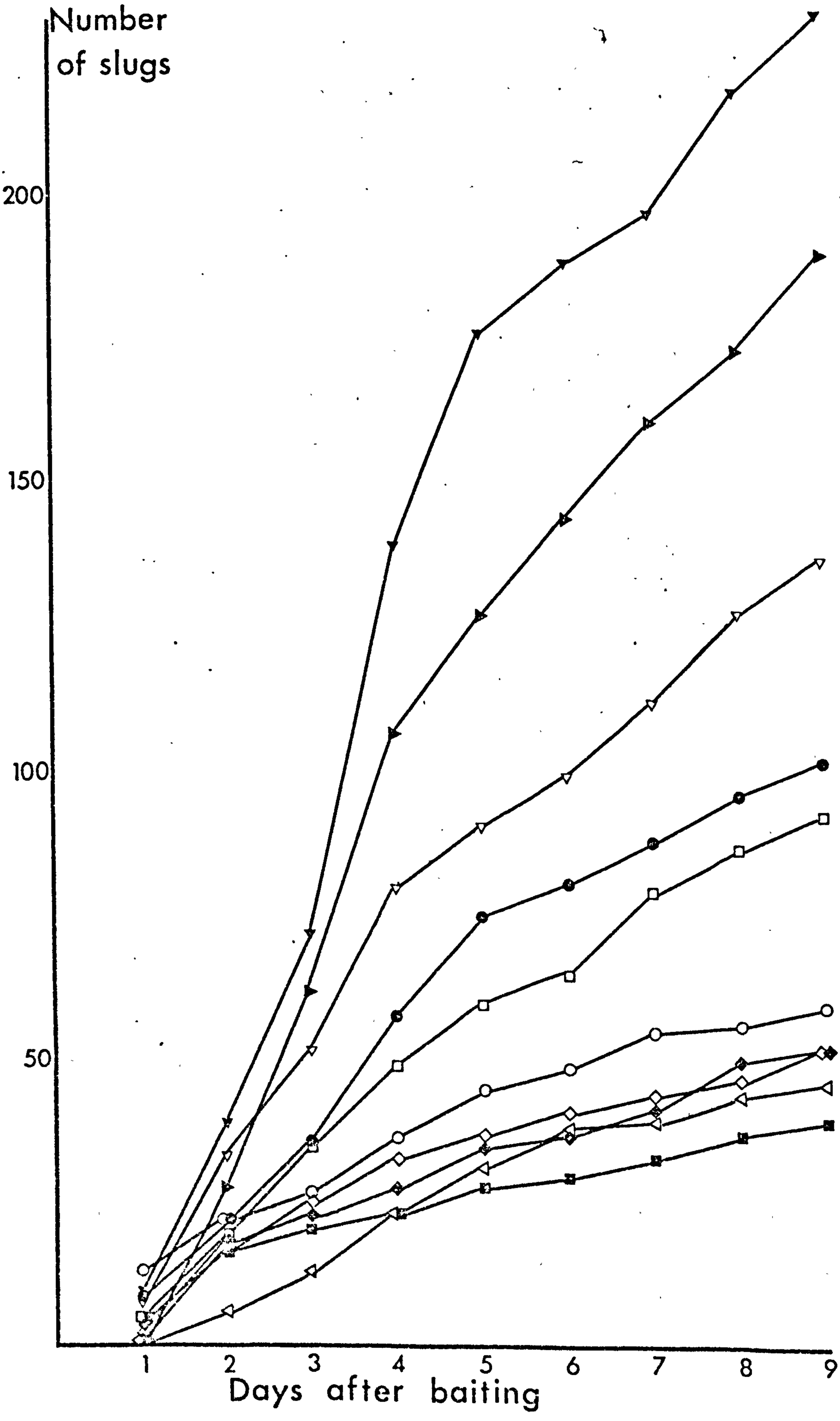
DAY / BAIT	8	9	IO	II	I2	I3	I4	I5	I6	TOTAL	MEAN
A	9	29	33	67	37	I2	9	2I	2O	237	26.33
B	4	I2	4	3	4	2	3	4	2	38	4.22
C	2	I5	8	7	4	4	3	3	5	5I	5.67
D	4	I5	I5	I4	II	5	I4	8	5	9I	IO.II
E	3	I5	5	4	7	2	5	8	2	5I	5.67
F	I	5	7	IO	8	6	2	4	2	45	5.OO
G	7	26	I8	28	II	8	I3	I5	9	I35	I5.OO
H	I	26	34	45	2O	I7	I6	I3	I6	I88	2O.89
J	I3	9	4	IO	8	4	6	I	3	58	6.44
K	8	I4	I3	22	I7	6	7	8	6	IOI	II.22
TOTAL	52	I66	I4I	2IO	I27	66	78	85	7O	995	

Analysis of variance shows that the difference between days is significant (P = O.OI) and the difference between treatments is is also significant (P = O.OI). See Appendix 2.

FIG. 48 CUMULATIVE TOTAL OF UNPOISONED ADULT *Deroceras* TRAPPED IN NINE DAY POST-BAITING PERIOD (days 8 - 16)

KEY

	BAIT		BAIT
▼	A	◁	F
■	B	▽	G
◇	C	▶	H
□	D	○	J
◆	E	●	K


Number of slugs
200
150
100
50
1
2
3
4
5
6
7
8
9
Days after baiting

at poisoning young slugs rather than any bias in the sampling. The two control treatments had the greatest number of unpoisoned slugs but the sand based formulation, G, also had a large number - this pattern persists when the adults and juveniles are considered separately.

The difference between individual treatments in the number of unpoisoned animals trapped after baiting has been compared in a matrix (Table 43)using the combined data for days 8 - 16. There is a greater than 50% probability of similarity between baits B, the methiocarb formulation, and the metaldehyde formulations E, F and J and a less than 0.5% chance of either of these baits being similar to the control. There is therefore no evidence to discriminate between these four treatments on the basis of the number of unpoisoned slugs trapped after baiting. A graph of the cumulative totals of unpoisoned slugs (Fig 48) also indicates that these four baits, and bait C have very similar levels of unpoisoned slugs. This evidence must be reconciled with the trapping figures for the number of dead slugs found in this period which had suggested that the methiocarb bait, B, killed far more slugs than any metaldehyde formulation.

Since the initial prebaiting trapping figures indicated that the distribution of slugs was similar for all treatments and bait B apparently trapped more poisoned slugs, one would expect that the number of live slugs found on plots baited with this treatment would be fewer than the number trapped on metaldehyde baited plots. Four of the metaldehyde baits were however, very similar to bait B in the numbers of unpoisoned slugs they trapped. This is an

TABLE 43 MATRIX TO COMPARE THE UNPOISONED DEROCERAS TRAPPED ON EACH TREATMENT

BAIT	A	B	C	D	E	F	G	H	J	K
A										
B	-0.5%									
C	0.5 - I%	25 - 50%								
D	I - 2.5%	25 - 50%	+50%							
E	-0.5%	+50%	+50%	25 - 50%						
F	-0.5%	+50%	25 - 50%	I0 - 25%	+50%					
G	5 - I0%	2.5 - 5%	I0 - 25%	25 - 50%	5 - I0%	I0 - 25%				
H	+50%	0.5 - I%	0.5 - I%	I - 2.5%	-0.5%	2.5 - I%	I0 - 25%			
J	-0.5%	+50%	25 - 50%	I0 - 25%	+50%	+50%	2.5 - 5%	-0.5%		
K	I - 2.5%	I0 - 25%	25 - 50%	-50%	I0 - 25%	I0 - 25%	25 - 50%	2.5 - 5%	I0 - 25%	

The percentages indicate the probability of similarity between any two treatments in the number of unpoisoned slugs trapped on days 8 - I6. Values calculated from the $S.E._d$ (TABLE 40).

important sector of the postbaiting population which is generally ignored yet these results indicate that valuable evidence on the effectiveness of each treatment is available by analysing these data.

5.14 BAIT EFFICIENCY ESTIMATES

An efficiency ratio was compiled for each treatment, summarising the results from the nine day post-baiting period and the five replicates. Since this includes both poisoned and unpoisoned components of the population, it is a more accurate estimate of bait effectiveness than any estimate solely relying on the number of dead bodies found. The efficiency ratio (E.R.) was derived from adult trapping figures for days 8 - 16 since adult deaths occurred in all plots and represent the situation which would be achieved by baiting a crop before juvenile recruitment takes place. Table 44 shows the E.R. for each bait.

5.15 NATURAL POPULATION CHANGE

On day 19, the population under each hessian sack was assessed to account for any natural changes in the population over the baiting period - this area had been left untouched from day 7 to day 19. The trapping data revealed an entirely different age structure from the prebaiting counts with a marked increase in the number of juveniles. A population level of 55.88 Deroceras per metre^2, equivalent to 41.16 juveniles and 14.72 adults per metre^2, was recorded indicating that the juvenile : adult ratio had changed from 1 ; 2.64 on day 7, to 1 : 0.36 on day 19.

The fall in the number of adults can be attributed

TABLE 44 EFFICIENCY RATIOS OF TEST TREATMENTS

$$E.R. = \frac{\text{Total number of poisoned adult } \underline{\text{Deroceras}} \text{ trapped}}{\text{Total number of } \underline{\text{Deroceras}} \text{ trapped}}$$

BAIT	TOTAL DEAD	TOTAL ALIVE	TOTAL TRAPPED	E.R.
A	II	237	248	0.044
B	268	38	306	0.876
C	48	5I	99	0.485
D	67	9I	I58	0.424
E	II5	5I	I66	0.693
F	II6	45	I6I	0.72O
G	I24	I35	259	0.479
H	7	I88	I95	0.036
J	I24	58	I82	0.68I
K	IOO	IOI	2OI	0.498

TABLE 45 TOTAL NUMBER OF DEROCERAS TRAPPED UNDER SACKS ON DAY I9

COLUMN / BAIT	a	b	c	d	e	TOTAL	MEAN
A	29	44	28	2O	56	I77	35.4
B	8	3O	4I	36	28	I43	28.6
C	I9	29	39	29	22	I38	27.6
D	2I	38	2O	I3	26	II8	23.6
E	5	I2	36	44	39	I36	27.2
F	22	26	34	2O	I2	II4	22.8
G	29	22	I9	3I	45	I46	29.2
H	3	26	2I	29	3I	IIO	22.O
J	I7	3O	22	I4	I8	IOI	2O.2
K	4I	23	23	34	34	I55	3I.O
TOTAL	I94	28O	283	27O	3II	I338	

Analysis of variance shows no signifwcant difference between the columns (P = 0.05) or between bait treatments (P = 0.05). See Appendix 2.

FIG. 49 POPULATION STRUCTURE OF Deroceras

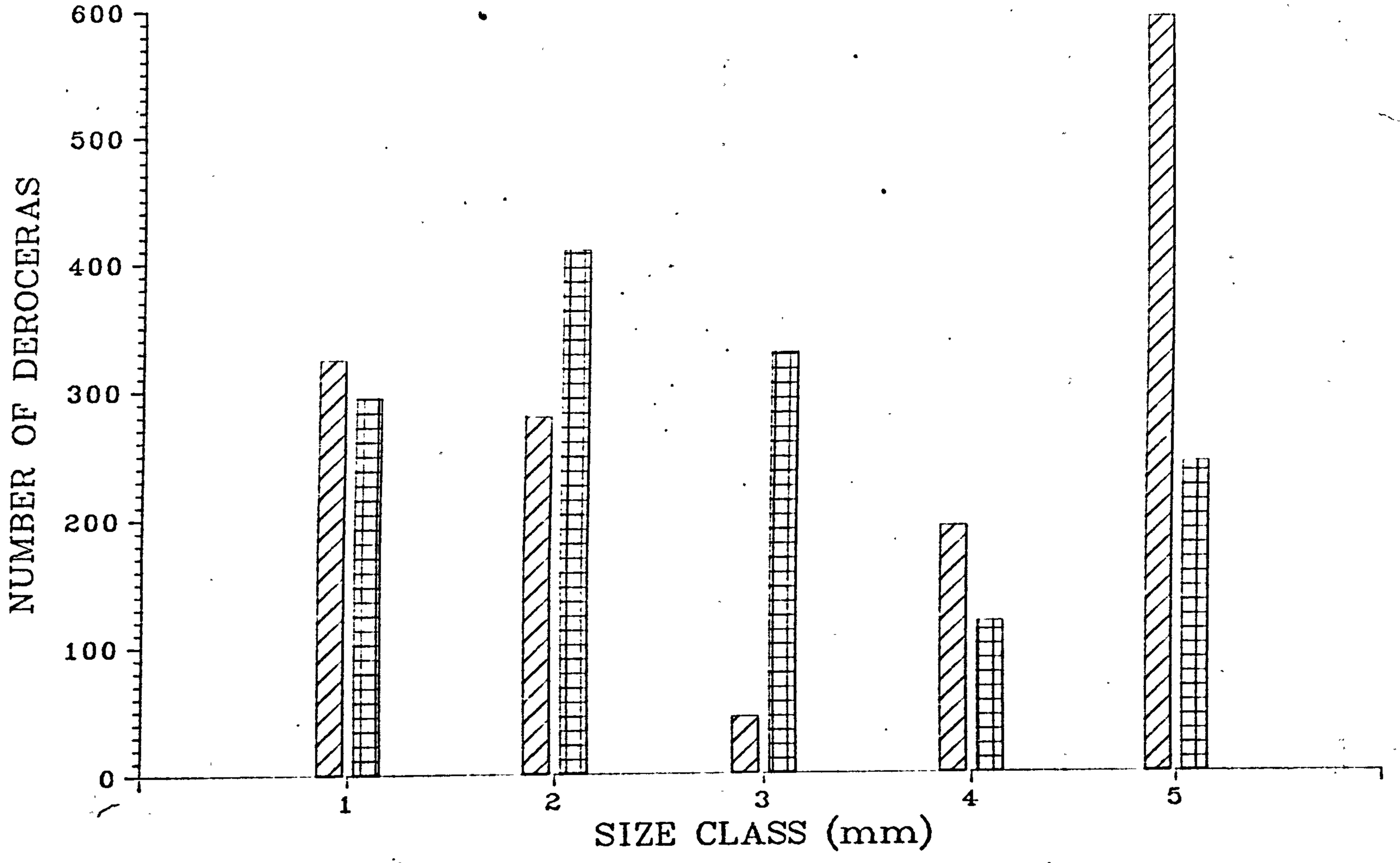
KEY

x axis	I	size class	0 - 10 mm
	2	size class	11 - 20 mm
	3	size class	21 - 30 mm
	4	size class	31 - 40 mm
	5	size class	41 mm +

Day 4 - hatched

Day 19 - squares

POPULATION STRUCTURE OF DEROCERAS ON DAY 4 (HATCH) & DAY 14 (SQUARES)



both to natural mortality and migration into baited ground around the sack. Since natural mortality on the two control treatments was low throughout the post-baiting sampling (less than 3%), this was probably not a major factor in reducing the adult population though more animals would continue to die after laying eggs. The rise in the juvenile population is almost certainly due to the large number of eggs which hatch at this time of year as the number of juveniles present was 30% higher than the pre-baiting estimate of the population. There may also have been some effect from the low level of juvenile mortality on the treated plots.

Table 45 presents the combined trapping figures for day 19 and indicates no significant difference between either columns or treatment rows ($P = 0.05$). Fig 49 shows the distribution of the five size classes of Deroceras on days 4 and 19 and illustrates how the juveniles have increased in both number and size during the fifteen days inbetween.

5.16 RESIDUAL POPULATION ANALYSIS

The post-baiting analysis of population on days 20 and 21 was designed to examine the residual population on each plot and to compare any long term treatment effects. The population count was carried out under the straw covered areas from which both poisoned and unpoisoned slugs had been removed during the nine day post-baiting period. On day 20, heavy rain fell throughout the day and many slugs were active, so searching was continued into the afternoon and only column "a" was completed. Day 21 was

TABLE 46 POST-BAITING ANALYSIS OF POPULATION

Unpoisoned Deroceras trapped on day 20 (column a) and day 2I (columns b - e)

JUVENILES

COLUMN / BAIT	a	b	c	d	e	TOTAL	MEAN
A	68	I8	20	30	43	I7I	34.2
B	58	29	29	55	I7	I88	37.6
C	63	30	I9	33	33	I78	35.6
D	9I	I6	I9	I8	39	I83	36.6
E	I6	I3	35	38	39	I4I	28.2
F	67	9	I2	67	22	I77	35.4
G	78	50	2I	38	52	239	47.8
H	IOO	20	38	26	6I	245	49.0
J	94	I5	32	34	37	2I2	42.4
K	IO3	37	25	35	23	223	44.6
TOTAL	730	237	250	374	366	I957	

TABLE 47 POST-BAITING ANALYSIS OF POPULATION

ADULTS

COLUMN / BAIT	a	b	c	d	e	TOTAL	MEAN
A	IO	I2	5	5	I2	44	8.8
B	5	8	9	8	6	36	7.2
C	8	3	5	7	5	28	5.6
D	20	3	6	5	2	36	7.2
E	4	I	5	6	5	2I	4.2
F	IO	2	4	4	6	26	5.2
G	26	3	2	2	I5	48	9.6
H	6	I9	9	7	5	46	9.2
J	8	O	3	3	6	20	4.0
K	II	4	I	4	2	22	4.4
TOTAL	IO8	55	49	5I	64	327	

Analysis of variance on the results for adult and juvenile trapping figures shows a significant difference between the columns (P = O.OI). If column 'a' is excluded, blocks 'b' - 'e' show no difference (P = O.O5). There is no significant difference between treatments (P = O.O5).

TABLE 48 POST-BAITING ANALYSIS OF POPULATION.

Unpoisoned Deroceras trapped on day 22

JUVENILES

COLUMN / BAIT	a	b	c	d	e	TOTAL	MEAN
A	IO	I2	3	2	I2	39	7.8
B	7	7	7	8	3	32	6.4
C	I3	IO	7	9	8	47	9.4
D	4	7	5	I	3	20	4.0
E	6	5	6	IO	9	36	7.2
F	I2	3	4	IO	4	33	6.6
G	II	6	7	7	9	40	8.0
H	4	3	2	I	IO	20	4.0
J	9	4	5	7	9	34	6.8
K	I	I2	3	2	9	27	5.2
TOTAL	77	69	49	57	76	328	

TABLE 49 POST-BAITING ANALYSIS OF POPULATION.

Unpoisoned Deroceras trapped on day 22

ADULTS

COLUMN / BAIT	a	b	c	d	e	TOTAL	MEAN
A	3	2	I	I	3	IO	2.O
B	3	4	O	O	O	7	I.2
C	4	3	I	I	3	I2	2.4
D	O	O	2	O	I	3	O.6
E	O	4	2	I	I	8	I.6
F	I	3	O	I	I	6	I.2
G	4	2	O	2	O	8	I.6
H	4	7	I	I	6	I9	3.8
J	2	I	3	O	2	8	I.6
K	2	3	2	O	2	9	I.8
TOTAL	23	3I	I2	7	I9	90	

Analysis of variance on the data for the number of juveniles trapped shows no significant difference between the block or treatment totals. (P = O.O5) See Appendix 2

Analysis of variance on the data for the number of adults trapped shows a significant difference between the columns (P = O.OI) and between the treatments (P = O.O5). See Appendix

drier and warmer, so fewer animals were active when columns "b" - "e" were searched. Tables 46 & 47 present the number of adults and juveniles trapped on these two days and as expected, the results indicate a higher number of animals in column "a". Analysis of variance on these data suggests that there is no difference between the number of animals trapped on each treatment (P = 0.05) although the difference between columns is signifcant (P = 0.01). If these data for day 20 (column a) are treated separately, then the difference between columns is no longer apparent and the difference between treatments remains significant.

The total number of Deroceras trapped on the 50 baited plots in these two days was 2328 - of these only 44 were poisoned (6 juveniles and 38 adults). These numbers were too low to compare different treatments as many of the deaths may be due to natural mortality but long term poisoning effects seem to be minimal - 49 dead earthworms were found on methiocarb baited plots though. The slugs trapped represent recruitment into the areas in a five day period and indicate a similar level of slugs on all treatments including the control plots.

Trapping on day 22 (14 days after baiting) provided an opportunity to sample a different area in each plot as two Marley tiles were placed at specified random positions. The traps were laid in areas which had been baited but left uncovered and not searched previously. The time available for searching limited the traps to two per plot and since the actual area sampled was small, the trapping figures are low (Table 48 and 49). Only two dead slugs

were found suggesting that the bodies had disintegrated or the bait pellets lost their effectiveness - pellets were found under the traps but they may have lost their attractiveness to the slugs.

The overall population for the field was estimated at 83.6 Deroceras per metre^2 (18.0 adults and 65.6 juveniles per m^2) but the importance of this estimate lies in the information it gives about the residual population on each treatment. Analysis of variance shows that there is no signifcant difference between treatments (P = 0.05) for the number of juveniles trapped on this day. This again confirms that the treatments were ineffective in reducing the juvenile population since the two control treatments should have had a higher residual population than the toxic treatments. The adult population does however demonstrate a difference between treatments (P = 0.05) and a highly significant difference between columns (P = 0.01). If the two control treatments are withdrawn from the analysis, the eight toxicant containing treatments no longer show this difference although the column variabilities still exist.

The residual adult population demonstrates that the eight toxic treatments are comparable in performance and the overall differences in numbers trapped on different treatments can be attributed to the higher population remaining on the control plots. However one would expect the methiocarb plots to have a lower residual population since more poisoned animals were found on them and the initial population was similar for all treatments. It would appear that this discrepancy between the similarity in the number of live animals remaining at the end of the trial on each of

the plots baited with a toxic treatment and the large number of poisoned animals trapped on methiocarb baited plots is due to the different effects of the two types of toxicant.

5.17 BAIT PERSISTENCE AND POISONING CHARACTERISTICS

Three test formulations were prepared industrially so that the pellets were of similar structure to the commercial baits. Only one of these, bait K, had binders and fungicides incorporated though all commercial formulations have these additives. The untreated pellets were the first to show signs of fungal attack - the two control treatments, baits A and H, and the test bait J had disintegrated somewhat after 21 days in the field. The pellets swell when they become damp and crumble easily, the other formulations lasted well throughout the trials when undisturbed.

The methiocarb bait, B, appeared to be the most effective at killing slugs but post-baiting analysis questions this conclusion. Other non-pest invertebrate species were also killed by this bait - a total of 49 earthworms, 20 beetles, 1 earwig and 1 caterpillar. This is from an area of $5m^2$ and such indiscriminate poisoning is obviously not acceptable. No dead animals other than slugs were found on any of the other treatments.

A more specific bait is required to control the slug population without interference with other soil fauna but the variation in the results obtained with the metaldehyde baits indicates the importance of correct formulation and dosage. Bait C had a high toxicant concentration but was

less effective than most of the other metaldehyde baits. The reasons for this are not clear but the dosage rate may have been too low for these conditions. This inorganic bait was one of the most resistant to decay despite the fact that the colour had worn in some places. An earlier trial using snail mortality to estimate the effectiveness of this bait and a methiocarb formulation (Cardew & Newell 1976) suggested that the former was more efficient. Methiocarb content is recorded at 2%, whereas present day bait contains 4% methiocarb - if this twofold increase in concentration has occurred, it would certainly account for the improvement in methiocarb poisoning in these trials made 3 years later. There may also be a difference in the attractancy of the bait to snails and slugs. Thomas (1948) found that some snails were apparently more susceptible to direct toxic action of metaldehyde than slugs and many trials have indicated a species related susceptibility in slugs e.g. Crawford-Sidebotham (1971).

The baits D, J and K were manufactured by the same company, presumably using the same attractant so it is possible that the binders, fungicides or other additives in the commercial bait caused a reduction in the attractancy of the basic metaldehyde/wheat complex. Since the pellets of bait J were the first to show signs of decay, there is obviously a need to improve the keeping qualities, but another fungicide may prove to have less influence on bait attractancy. Bait K, containing a binder, fungicide and 5% freeze-dried brewers yeast complex, seemed to be intermediate between the basic metaldehyde/wheat mixture (bait J) and the commercial formulation (bait D). The

yeast, a suggested attractant, may have been responsible for the improvement of this formulation over the commercial bait, but some of the additives, possibly binder or fungicide, have reduced the attractancy compared to the basic metaldehyde/wheat bait. The unpredicted high performance of bait J has prevented any true estimate of the yeast attractant in the field and further tests, at a range of attractant concentration, must be carried out to elucidate the effects of the yeast complex in the field.

Baits E and F were effective toxicants, trapping similar numbers of slugs and thereby indicating that the addition of a mammal repellent to bait F has no adverse effect on bait attractancy. This is supported by film evidence. The number of animals trapped per day was very consistent suggesting that the baits retained their effectiveness while other baits declined.

Bait G, the sand metaldehyde mixture, was also effective, particularly towards the end of the trial. The total number of slugs trapped on plots baited with this treatment was high suggesting that the formulation is attractive to slugs - this would support the idea that metaldehyde itself is attractive since the odour was unmasked by carrier or attractants. The number of slugs actually killed was not correspondingly high however but the time lag in maximum poisoning may be due to the fact that contact poisoning is a more probable mode of action - the slugs pick up the metaldehyde and sand mixture as they move and it is difficult to see why they should ingest more of the non-nutritive inorganic sand than a wheat carrier. Experiments by Henderson (1968, 1969) showed

that metaldehyde is more toxic as a stomach poison than as a contact poison and peak mortality with the contact action bait in these trials occurred after 6 - 8 days compared with 3 - 5 days for a traditional stomach action bait. The dosage rate and toxicant concentration applied in this trial may well be inadequate for effective control but enhancement of the bait with suitable attractants would make it worthwhile for further study as this could perhaps combine a contact and stomach action. The structure of this bait makes it particularly suitable for mechanical application and as the carrier is inexpensive the production could be cost-effective.

Formulation of each bait is of great importance in improving efficiency. The variation in the results obtained with the metaldehyde formulations indicates that additives to some commercial baits reduce the effectiveness of the basic metaldehyde/wheat complex. The addition of a mammal repellent does not, however, appear to have any deleterious effects. The effect of the yeast attractant in bait K was not easy to ascertain since the commercial formulation which the attractant had been incorporated into proved to be less effective than the basic metaldehyde/wheat pellets of bait J.

Further field trials are necessary to determine whether the attractive nature of the brewers yeast can be maintained in the field but the formulations now available for slug control require standardisation. The disparate baits examined in this trial ranged from those which give little improvement over non-toxic controls to those which were, at the very least, equivalent to a methiocarb

formulation. None of the treatments gave 100% kill, however, suggesting that the recommended dosage rates may be too low.

5.18 CONCLUSIONS

The results of this field trial indicate that work on a field population of slugs is affected by many factors which must be fully investigated before adequate interpretation of the trapping results is possible. The evidence obtained from any trial is therefore applicable only to the conditions present at that time, though inferences can be drawn which may be applied to other crops and conditions.

Despite time constraints and availability problems associated with the farmer's timetable, the site was ideal since it provided a large area - the plots and guard rows were therefore large enough to discount the possibility of migration between plots - and a high slug population. Pre-baiting data suggested a minimum level of 57.77 Deroceras reticulatum per metre^2 which rose to 83.60/m^2 by day 22 with juvenile influx. As slugs are very susceptible to weather fluctuations, the number of active animals varied daily and there is no estimate of the inactive slugs which remain underground. It was found though, that sampling early in the morning provided reasonably consistent results as the dew was generally heavy and the traps provided protection from the early morning sun. Most of the daily fluctuations in slug numbers were in fact due to movements of juveniles and the adult population remained more stable.

The trial reinforced several ideas proposed by other workers. All the baits were relatively ineffective

against juvenile slugs and against Arion ater individuals though this may be due to the fact that all of this species trapped were juveniles also. It is not clear whether this difference in age susceptibility is due to the variations in behaviour - juveniles were seen to eat at the tips of plant stems and may thus avoid the pellets which fall to the base - or to a difference in attraction of the bait - many workers have noted that food preferences of slugs change as they age. This does,however,emphasise the importance of baiting at the correct time of year since application of the bait when the population is largely adult not only reduces the risk of a second generation but also achieves its aim as the adults do appear to be attracted to the bait and will ingest it.

An efficiency ratio comparing the number of dead adult slugs trapped per treatment with the total number of adults trapped on that treatment suggested that bait B, the methiocarb formulation, was the most efficient bait. This treatment was shown to be an indiscriminate poison, killing non-pest invertebrate species, particularly earthworms - dead earthworms were found on methiocarb baited plots two weeks after bait application when the number of dead slugs had fallen to a minimum level.

The appearance and behaviour of methiocarb poisoned slugs may have exaggerated their importance against metaldehyde poisoned slugs. The total number of slugs trapped after baiting should be similar for each treatment as both poisoned and unpoisoned animals were recorded and prebaiting analysis suggested that there was no significant difference in the population level of the different

treatment plots.

Analysis of the residual population at the end of the trial strengthens the hypothesis that the metaldehyde baits poisoned more slugs than the number of bodies found suggested. This is the sector of the population which remains to damage the crop and produce a new generation of slugs so the bait treatment which produces the lowest residual population is the most effective. The better metaldehyde formulations, baits E, F and J, produced low residual populations and one must therefore assume that the records of poisoned slugs on these plots are incomplete. The efficiency ratios for these metaldehyde baits would then be a minimum value only.

Methiocarb acts directly on the nervous system, paralysing the animal and thereby preventing movement away from the toxic baits. These animals are therefore always recorded as poisoned and their bloated appearance makes them easy to spot in the field. Metaldehyde is primarily a desiccant and poisoned animals may well move underground to seek shelter. As paralysis gradually occurs, the slugs become incapable of movement and may never reemerge. This theory would account for the fact that few dead bodies were recovered from metaldehyde baited plots yet the number of unpoisoned slugs trapped indicated that the animals were being killed at a rate similar to those on methiocarb baited plots. Relative humidity was not high during the experimental period and it is unlikely that the slugs could have recovered from metaldehyde poisoning. It is thus extremely difficult to obtain a precise estimate of the number of slugs killed in the field by application of

metaldehyde baits. The efficacy must be confirmed by measuring the residual population and relating this to each treatment. This may have important implications on the results of other field trials where only the number of poisoned animals has been recorded and the effectiveness of the metaldehyde formulations underestimated.

There are few indications that any assessment of the residual population has ever been made in other field trials designed to compare bait effectiveness, though some authors have hinted that a large residual population may be present by suggesting that baits were ineffective at reducing damage. Rings et al (1975) applied methiocarb bait to several field crops and recorded high kills on these plots when compared to metaldehyde baits. They were unable,however,to detect a corresponding reduction in injury to the vegetables as a result of the treatment and despite the large number of dead slugs found, there was no difference in the level of damage on treated and untreated areas.

Crowell (1977) compared three different baits to field trials on fallow ground. The number of slugs trapped under boards baited with bran was assessed before bait application, 72 hours after treatment and 7 days after treatment. A comparison of the number of dead slug bodies found on the plots had indicated that a methiocarb spray was the most effective treatment. However, after 7 days the number of live slugs trapped on this treatment was high, with an overall reduction in population of 31% compared with 61% for the untreated control plot. The two other treatments - a candidate bait and a commercial combination of metaldehyde

and Carbaryl - were intermediate in the percent reduction of the slug population. The author attributes the apparent paradox in the results to the fact that large numbers of slugs were migrating onto plots from an adjacent wheat stubble field. This is difficult to verify,however,and he does not include the figures for the number of poisoned slugs trapped at each treatment so there is no estimate of the efficiency of the other baits. Since the initial population level differed on each treatment the post-baiting population is also subject to inherent variation though this is accounted for in the term percent kill.

Such discrepancies indicate the importance of sampling the animals remaining after baiting if the trial is to provide an accurate analysis of the effectiveness of each treatment. Dead slugs are not the only criterion for estimating bait effectiveness and the different symptoms of poisoning produced by the two toxicants metaldehyde and methiocarb may have led to false conclusions in many trials where the residual population has not been examined at the end of the trial.

BIBLIOGRAPHY

ACRAMAN A.R. 1966 Processing brewer's yeast
Process Biochemistry. September 1966

ACRAMAN A.R. & SMITH A.J. 1976 Problems encountered in the industrialisation of brewers yeast for human food purposes
The Brewer. October 1976 pp 318 - 323

ADAMS L. 1898 Sense of small in Limax maximus.
J. Conchology 9 p24

AMOORE J.E., JOHNSTON J.W. Jr & RUBIN M. 1964 The stereochemical theory of odour.
Scientific American 210 pp 42 - 49

ARIAS R.O. & CROWELL H.H. 1963 A contribution to the biology of the gray garden slug.
Bull. S. Californian Acad. Sci. 62 (2) pp 83 - 97

AUDESIRK T.E. 1975 Behavioural and histological localisation of distance chemoreceptors in Aplysia californica
American Zoologist 15 (3) p796

AUDESIRK T.E. 1977 Chemoreception in Aplysia californica III. Evidence for pheromones influencing reproductive behaviour.
Behavioural Biology 20 pp235 - 243

AUDESIRK T.E. & AUDESIRK G.J. 1977 Chemoreception in Aplysia californica II. Electrophysiological evidence for the detection of the odor of conspecifics.
Comp. Biochem. Physiol. 56A (3) pp267 - 270

BAILEY D.F. & LAVERACK M.S. 1966 Aspects of the neurophysiology of Buccinum undatum L. (Gastropoda). I. Central responses to stimulation of the osphradium.
J. Exp. Biol. 44 pp131 - 148

BAKER A.N. 1973 Factors contributing towards the initiation of slug activity in the field.
Proc. Malac. Soc. Lond. 40 (4) pp329 - 333

BARDACH J. 1975 Chemoreception of aquatic animals. In: Olfaction and Taste V. DENTON D.A. & COGHLAN P. (eds.) Proc. 5th International Symposium at Univ. Melbourne. October 1974 Academic Press, London.

BARNES H.F. 1944 Discussion on slugs. I. Introduction: Seasonal activity of slugs .
Ann. Appl. Biol. 31 (2) pp160 - 163

BARNES H.F. & WEIL J.W. 1940 Meta slug control: Experiments on possible substitutes for bran.
Gardeners Chronicle 108 p20

BARNES H.F. & WEIL J.W. 1942 Baiting slugs using metaldehyde mixed with various substances.
Ann. Appl. Biol. 29 (1) pp56 - 68

BARNES H.F. & WEIL J.W. 1944 Slugs in gardens: their numbers, activities and distribution. Part I.
J. Anim. Ecol. 13 pp140 - 175

BARONIO P. 1974 The insect enemies of gasteropodous molluscs
Boll. dell'Instituto di Entomol.della Univ. Bologna 32 pp169 - 187

BEETS M.G.J. 1977 Structural aspects of chemoreception pp71 - 89. In: Sensory properties of foods BIRCH G.G., BRENNAN J.G., PARKER K.J. (eds.)
Applied Science Publishers Ltd. London

BEISWANGER C.M. & PRIOR D.J. 1976 Light entrainment of the circadian locomotor rhythum of the slug Limax maximus
Anat. Record. 184 (3) p356

BEYER W. & SAARI D.M. 1977 Effect of tree species on the distribution of slugs.
J. Anim. Ecol. 46 pp 697 - 702

BEYER W. & SAARI D.M. 1978 Activity and ecological distribution of the slug Arion subfuscus (Draparnaud) (Stylommatophora - Arionidae).
American Midland Naturalist 100 (2) pp359 - 367

BISHOP L.R. 1971 The evaluation of beer flavours
J. Inst. Brew. 77 pp389 - 397

BOECKH J., KAISSLING K.E. & SCHNEIDER D. 1965 Insect olfactory receptors
Cold Spring Harbour Symp. on Quantitative Biol. 30 pp263 - 280

BOER H.H. & JOOSSE J. 1975 Endocrinology pp245 - 307 In: Pulmonates Vol. I - Functional anatomy and physiology FRETTER V. & PEAKE J. (eds.) Academic Press. London

BOVBJERG R.V. 1965 Feeding and dispersal in the snail Stagnicola reflexa (Basommatophora : Lymnaeidae)
Malacologia 2 (2) pp199 - 207

BROWN A.C. 1961 Chemoreception in the sandy beach snail Bullia
S. Afr. J. Lab & Clin. Med. 7 (4) pp160 - 161

van den BRUEL W.E. & MOENS R. 1957 Les propriétés des hélicides et la protection des cultures.
Lutte contre les ennemis des plantes 9eme Congrès Hamburg pp1255 - 1275

BRUGGER H. 1974 Lonza Information - Metaldehyde, slug and snail control agent for use in agriculture and horticulture
Unpublished 30pp

BURKE W.R. 1964 Chemoreception by Tegula funebralis (Moll:Gast)
Veliger 6 (Suppl.) pp17 - 20

CAIRASCHI E. & LECOMTE V. 1973 Observations sur Euparypha (Theba) pisana Muller en luzernières à graines.
Haliotis 3 pp33 - 37

CAMERON A.E. 1939 The control of potato slugs.
J. Min. Agric. 46 pp454 - 462

CAMERON R.A.D. & CARTER M.A. 1979 Intra- and interspecific effects of population density on growth and activity in some helicid land snails (Gastropoda : Pulmonata).
J. Anim. Ecol. 48 (1) pp237 - 246

CARDEW J.St E. & NEWELL P.F. 1976 Field trials with metaldehyde
Unpublished report 33pp.

CARR W.E.S. 1967a Chemoreception in the mud snail Nassarius obsoletus L. I. Properties of stimulatory substances extracted from the shrimp
Biol. Bull. 133 (1) pp90 - 105

CARR W.E.S. 1967b Chemoreception in the mud snail Nassarius obsoletus L. II. Identification of stimulatory substances
Biol. Bull. 133 (1) pp106 - 127

CARR W.E.S., HALL E.R. & GURIN S. 1974 Chemoreception and the role of proteins : a comparative study
Comp. Biochem. Physiol. 47A pp559 - 566

CARRICK R 1939 The life history and development of Agriolimax agrestis L., the grey field slug.
Trans. R. Soc. Edinb. 59 pp563 - 597

CARRICK R 1942 The grey field slug Agriolimax agrestis and its environment.
Ann. Appl. Biol. 29 pp43 - 55

CARTHY J.D. 1958 An introduction into the behaviour of invertebrates
George Allen & Unwin Ltd. London

CATES R.G. 1975 The interface between slugs and wild ginger ; some evolutionary aspects.
Ecology 56 pp391 - 400

CATES R.G. & ORIANS G.H. 1975 Successional status and the palatability of plants to generalised herbivores.
Ecology 56 pp410 - 418

CHARLES G.H. 1966 Sense organs (less cephalopods). In: Physiology of Mollusca Vol II. Academic Press, London pp455 - 521

CHARLTON J.F.L. 1978 Slugs as a possible cause of establishment failure in pasture legumes oversown in boxes.
N.Z. J. Exp. Agric. 6 (4) pp313 - 318

CHASE R. & BOULANGER C. 1978 Attraction of the snail Achatina fulica to extracts of conspecific pedal glands.
Behavioural Biol. 23 pp107 - 111

CHASE R., PRYOR K., BAKER R. & MADISON D. 1978 Responses to conspecific chemical stimuli in the terrestrial snail Achatina fulica.
Behavioural Biol. 22 pp302 - 315

CHATFIELD J.E. 1973 Aspects of feeding and growth in land snails.
Malacologia 14 pp391 - 392

CHATFIELD J.E. 1976 Studies on food and feeding in some European land molluscs.
J. Conchology 29 pp5 - 20

CLAPPERTON J.F. 1978 Fatty acids contributing to caprylic flavour in beer. The use of profile and threshold data in flavour research.
J. Inst. Brew. 84 (2) pp107 - 112

COOK A. 1977 Homing in Gastropoda. p87
Abstacts 6th European Malacological Congress 15th - 20th August 1977, Amsterdam

COOK A. 1979a Homing in the Gastropoda.
Malacologia 18 (1 - 2) pp315 - 318

COOK A. 1979b Homing by the slug Limax pseudoflavus
Anim. Behav. 27 (2) pp545 - 552

COOK A. 1980 Field studies of homing in the pulmonate slug Limax pseudoflavus (Evans)
J. Moll. Stud. 46 (1) pp100 - 105

COOKE A.H., SHIPLEY A.E. & REED F.R.C. 1895 Molluscs and Brachiopods
Cambridge Natural History III. MacMillan, London. 595pp

CRAGG J.B. & VINCENT M.H. 1952 The action of metaldehyde on the slug Agriolimax reticulatus
Ann. Appl. Biol. 39 pp396 - 406

CRAWFORD-SIDEBOTHAM T.J. 1970 Differential susceptibility of species of slugs to metaldehyde/bran and to methiocarb baits.
Oecologia (Berl.) 5 pp303 - 324

CRAWFORD-SIDEBOTHAM T.J. 1971 Studies on aspects of slug behaviour and the relation between molluscs and cyanogenic plants.
Ph.D. Thesis Univ. Birmingham

CRAWFORD-SIDEBOTHAM T.J. 1972 The influence of weather upon the activity of slugs
Oecologia (Berl.) 9 pp141 - 154

CROLL R.P. & CHASE R. 1977a Food odor preferences in juvenile snails, Achatina fulica.
American Zoologist 17 (4) p949 (Abs. 498)

CROLL R.P. & CHASE R. 1977b A long term memory for food odors in the land snail Achatina fulica.
Behavioural Biol. 19 pp261 - 268

CROWELL H.H. 1967 Slug and snail control with experimental poison baits.
J. Econ. Entomol. 60 (4) pp1048 - 1049

CROWELL H.H. 1977 Chemical control of terrestrial slugs and snails
Agric. Exp. Stat. Oregon State Univ., Corvallis.
Bulletin 628. 70pp

DAINTON B.H. 1954a The activity of slugs. I. The induction of activity by changing temperatures.
J. Exp. Biol. 31 pp165 - 187

DAINTON B.H. 1954b The activity of slugs. II. The effect of light and air currents.
J. Exp. Biol. 31 pp188 - 197

DAVIDSON D.H. 1976 Assimilation efficiencies of slugs on different food materials.
Oecologia (Berl.) 26 pp267 - 273.

DAVIES J.T. 1965 A theory of the quality of odours.
J. Theoretical Biol. 8 (1) pp1 - 7

DAVIS B.N.K. 1968 The soil macrofauna of organochlorine insecticide residues at 12 agricultural sites near Huntingdon.
Ann. Appl. Biol. 61 pp29 - 45

DAVIS B.N.K. & FRENCH M.C. 1969 The accumulation of organochlorine insecticide residues by beetles, worms and slugs in sprayed fields.
Soil Biol. Biochem. 1 pp45 - 55

DAVIS W.J. 1976 Behavioural and neuronal plasticity in mollusks.
In: Simpler networks: An approach to patterned behaviour and its foundations. pp224 - 238 FENTRESS J.C. (ed)
Sinauer Associates, Sunderland, Massachusetts

DAXL R. 1970 Der Einfluss von Temperatur und relativer Luftfeuchte auf die molluskizide Wirkung das Metaldehyd, Isolan und Ioxynil auf Limax flavus L. und dessen Eier.
Z. Angew. Entomol. 67 (1) pp57 - 87

DAXL R. 1971 Das Verhalten der molluskiziden Verbindungen Metaldehyd, Isolan und Ioxynil gegen Nachtschnecken unter Freilandbedingungen.
Z. Angew. Zool. 58 pp203 - 241

DETHIER V.G. 1947 Chemical insect attractants and repellents.
The Blakiston Co., Toronto. 289pp

DINDAL D.L. & WURZINGER K.H. 1971 Acclimation and excretion of DDT by the terrestrial snail Cepaea hortensis.
Bull. Environ. Contam. Toxicol. 6 (4) pp362 - 371

DMITRIEVA E.F. & SHAPIRO Y.S. 1975 Some functional changes in the field slug (Deroceras reticulatum) following poisoning with metaldehyde.
Zap. Leningr. S-kh Inst. 270 pp115 - 118

DRAVNIEKS A. 1968 Comparison of theories on relations between odor parameters and other properties of odorants. In: Theories of odor and odor measurement. TANYOLAC N. (ed) Technivision. pp11 - 31

DUNDEE D.S., TIZZARD M. & TRAUB M. 1975 Aggregative behaviour in veronicellid slugs.
The Nautilus 89 (3) pp69 - 72

DUVAL D.M. 1971 A note on the acceptability of various weeds as food for Agriolimax reticulatus (Muller).
J. Conchology 27 pp249 - 251

DUVAL D.M. 1972 A record of slug movements in late summer.
J. Conchology 27 pp505 - 508

EDELSTAM C. & PALMER C. 1950 Homing behaviour in gastropods.
Oikos 2 pp259 - 270

ELLISON J. 1974 The commercial utilisation of waste brewery yeast.
Internat. Bottler & Packer January 1974 pp54 - 62

ENGAN S. 1974 Higher alcohols in beers.
The Brewers Digest August 1974 pp52 - 59

ETGES F. 1962 Inhibition of chemokinetic responses of the Schistosome vector, Australorbis glabratus, by some chemical agents.
American Zoologist 2 p521

FARKAS S.R. & SHOREY H.H. 1976 Anemotaxis and odour trail following by the terrestrial snail Helix aspersa. Anim. Behav. 24 (3) pp686 - 689

FISHER T., MOORE I., LEGNER E.F. & ORTH R.E. 1976 Ocypus olens: A predator of brown garden snail. Calif. Agric. 30 (3) pp20 - 21

FISHER T. & ORTH R.E. 1975 Differential susceptibility of brown garden snail to metaldehyde. Calif. Agric. 29 (6) pp7 - 8

FOG K. 1979 Studies on decomposing wooden stumps.III. Different relations among some gastropod species and species groups to the stump microflora , weather changes and pH. Pedobiologia 19 (3) pp200 - 212

FOUNTAIN R.L. 1974 Higher order control of centrally programmed feeding behaviour in the snail Helisoma. American Zool. 14 (4) p1273

FRÖMMING E. 1939 Sind unsere milchsaftführenden Pflanzen vor Tier, insbesondere Schneckenfrass geschützt? Angew. Botanik 21 pp177 - 189

FRÖMMING E. 1940 Über das Verhalten unserer Nachtschnecken gegenüber den Blätter- und Löcherpilzen. Angew. Botanik 22 pp157 - 167

FRÖMMING E. 1954 Biologie der mitteleuropäischen Landgastropoden. Duncker & Humbolt, Berlin. 404pp

FRÖMMING E. 1957 Nachtschnecken als Schädlinge in Mehl-Vorratskellern und über den Einfluss d ser Ernäbrung auf die Körperfarbe. Z. Angew. Zool. 44 pp349 - 357

FURNESS G.O. 1977 A survey of snails on citrus in the riverland of southern Australia. Aust. J. Exp. Agric. & Anim. Husb. 17 No. 89 pp1036 - 1039

GAIN W.A. 1891 Notes on the food of some of the British molluscs. J. Conchology 6 pp349 - 361

GELPERIN A. 1974 Olfactory basis of homing behaviour in the giant garden slug Limax maximus. Proc. Nat. Acad. Sci. U.S.A. 71 (3) pp966 - 970

GELPERIN A. 1975 Rapid food aversion learning by a terrestrial mollusc.
Science 189 pp567 - 570

GELPERIN A. 1976 Identified serotonergic neurons modulate feeding in the terrestrial mollusc: Limax maximus.
The Physiologist 19 p204

GELPERIN A. 1977 Behavioural studies of gastropod feeding.
The Veliger 20 (1) p58

GELPERIN A., CHANG J.J. & REINGOLD S. 1978 Feeding motor program in Limax. I. Neuromuscular correlates and control by chemosensory input.
J. Neurobiol. 9 (4) pp285 - 300

GELPERIN A. & FORSYTHE D. 1976 Neuroethological studies of learning in mollusks. pp 239 - 246. Simpler networks: An approach to patterned behaviour and its foundations. FENTRESS J.C. (ed). Sinauer Associates. Sunderland. Massachusetts.

GESTELAND R.C. 1964 Initial events of the electro-olfactogram.
Ann. N.Y. Acad. Sci. 116 pp440 - 447

GETZ L.L. 1963 Light preferences of molluscs.
Ecology 44 pp612 - 613

GODAN D. 1966 Untersuchungen über die molluskizide Wirkung der Carbamate.
Z. Angew. Zool. 53 pp418 - 436

GOLDSCHMEDING J.T. & JAGER J.C. 1973 Feeding responses to sucrose in the pond snail Lymnaea stagnalis after nerve section and tentacle amputation.
Neth. J. Zool. 23 (1) pp118 - 124

GOULD H.J. 1961 Observations on slug damage to winter wheat in E. Anglia, 1957 - 1959.
Plant Pathol. 10 (4) pp142 - 146

GRIFFITHS A.B. 1892 On the olfactory organs of Helix.
Proc. R. Soc. Edinb. 19 pp198 - 199

GURIN S. & CARR W.E. 1971 Chemoreception in Nassarius obsoletus: The role of specific stimulatory proteins.
Science 174 pp293 - 295

HAMILTON P.V. 1977 The use of mucus trails in gastropod orientation studies.
Malacological Review 10 (1 - 2) pp73 - 76

HANNA G.D. 1966 Introduced mollusks of Western North America.
Occasional Papers of Calif. Acad. Sci. 48 108pp

HENDERSON I.F. 1966 Studies on the laboratory assessment of the toxicity of chemicals to slugs, especially Agriolimax reticulatus.
Ph.D. Thesis London Univ.

HENDERSON I.F. 1968 Laboratory methods for assessing the toxicity of contact poisons to slugs.
Ann. Appl. Biol. 62 pp363 - 369

HENDERSON I.F. 1969 A laboratory method for assessing the toxicity of stomach poisons to slugs.
Ann. Appl. Biol. 63 pp161 - 171

HENDERSON I.F. 1970 The fumigant effects of metaldehyde.
Ann. Appl. Biol. 65 pp507 - 510

HENDERSON N.E. & PELLUET D. 1960 The effect of visible light in the ovotestis of the slug Deroceras reticulatum (Müller).
Can. J. Zool. 38 pp173 - 178

HERMANN G. 1971 Bekämpfung von Landschnecken mit Methiocarb.
Meded. Fac. Landbouwet Rijksuniv. Gent. 36 (1) pp197 - 206

HERMANN H.T. 1968 Optic guidance of locomotor behaviour in the land snail Otala lactea.
Vision Research 8 pp601 - 612

HODGSON E.S. 1965 Problems in invertebrate chemoreception.
Q. Rev. Biol. 30 (4) pp331 - 347

HOUGH J.S., BRIGGS D.E. & STEVENS K. 1971 Malting and brewing science. Chapman & Hall Ltd., London. 678pp.

HOWITT A.J. 1961 Chemical control of slugs in orchard grass - ladino white clover pastures in the Pacific northwest.
J. Econ. Entomol. 54 (2) pp778 - 781

HOWITT A.J. & COLE S.G. 1962 Chemical control of slugs affecting vegetable and strawberries in the Pacific northwest.
J. Econ. Entomol. 55 (3) pp320 - 325

HUNTER P.J. 1966 The distribution and abundance of slugs on an arable plot in Northumberland.
J. Anim. Ecol. 35 pp543 - 557

HUNTER P.J. 1968 Studies of slugs on arable ground. I. Sampling methods.
Malacologia 6 (3) pp369 - 377

HUNTER P.J. 1969a Slugs and their control.
Proc. 5th Brit, Insect. Fungic. Conf. pp715 - 719

HUNTER P.J. 1969b An estimate of the extent of slug damage to wheat and potatoes in England and Wales.
N.A.A.S. Quart. Rev. No. 85 pp31 - 36

HUNTER P.J. & JOHNSTON D.L. 1970 Screening carbamates for toxicity against slugs.
J. Econ. Entomol. 63 (1) pp305 - 306

HUNTER P.J. & SYMONDS B.V. 1970 The distribution of bait pellets for slug control.
Ann. Appl. Biol. 65 pp1 - 7

HUNTER P.J. & SYMONDS B.V. 1971 The leap-frogging slug.
Nature 229 p349

ICHINOSE L.Y. 1968 The role of the posterior tentacles of the giant African land snail Achatina fulica Bowdich (Gastropoda - Pulmonata) in olfaction and orientation.
Ph.D. Thesis Univ. Hawaii

INGLEDEW W.M., LANGILLE L.A., MENEGAZZI G.S. & MOK C.H. 1977 Spent Brewers Yeast - Analysis, improvement and heat processing considerations.
MRAA Technical Quarterly 14 (4) pp231 - 237

JAHAN-PARWAR B. 1972 Behavioural and electrophysiological studies on chemoreception in Aplysia.
American Zool. 12 (3) pp525 - 537

JAHAN-PARWAR B. 1975 Chemoreception in gastropods. In: Olfaction and Taste V. DENTON D.A. & COGHLAN J.P. (eds.) Proc. Int. Symp. at Univ, Melbourne. October 1974. pp133 - 139
Academic Press, London.

JENNINGS T.J. & BARKHAM J.P. 1979 Niche separation in woodland slugs.
Oikos 33 pp127 - 131

JENSON T.F. 1975 A tentative energy budget for a summer population of Arion ater.
Natura Jutlandica 18 pp10 -20

JONES D.A. 1962 Selective eating of the acyanogenic form of the plant Lotus corniculatus L. by various animals.
Nature 193 (4820) pp1109 - 1110

JUDGE F.D. 1969 Preliminary screening of candidate molluscicides.
J. Econ. Entomol. 62 (6) pp1393 - 1397

JUDGE F.D. 1972 Aspects of the biology of the grey garden slug (Deroceras reticulatum Müller).
Search. Agriculture (Entomology). 2 (19) pp1 - 18

KALMUS H. 1942 Anemotaxis in soft skinned animals.
Nature 150 (1) p524.

KARLIN E.J. 1961 Temperature and light as factors affecting the locomotor activity of slugs.
The Nautilus 74 (4) pp125 - 130

KEMPENDORFF W. 1942 Über das Fluchtphänomen und die Chemorezeption von Helisoma (Taphius) nigricans Spix.
Arch. Molluskenkunde 74 pp1 - 27

KIECKEBUSCH W. 1953 Beitrag zur Physiologie des chemischen Sinnes von Helix pomatia.
Zool. Jahrb. Abt. F. Allg. Zool. und Physiol. der Tiere 64 (2) pp153 - 182

KITCHELL R.V., SCHUBERT T.A., MULL R.L. & KNAAK J.B. 1978 Palatability studies of snail and slug poison baits using dogs.
J. Am. Vet. Med. Ass. 173 (1) pp85 - 90

KITTEL R. 1956 Untersuchungen über den Geruchs- und Geschmackssin bei den Gattungen Arion und Limax (Mollusca: Pulmonata).
Zool. Anz. 157 pp185 - 195

KNIGHTS R.N. 1979 Effects of experimental stress on the glycogen content of Cepaea hortensis (Mollusca). J. Zool. Lond. 189 pp485 - 491

KNUTSON L.V., STEPHENSON J.W. & BERG C,O. 1965 Biology of a slug killing fly, Tetanocera elata (Diptera: Sciomyzidae). Proc. Malac. Soc. Lond. 36 pp213 - 220

LANGE W.H. & MACLEOD G.F. 1941 Metaldehyde and calcium arsenate in slug and snail baits. J. Econ. Entomol. 34 (2) pp321 - 322

LANGE W.H. & SCIARONI R.H. 1952 Metaldehyde dusts for control of slugs affecting brussel sprouts in central California. J. Econ. Entomol. 45(5) pp896 - 897

LAVIOLETTE P. 1954 Rôle de la gonade dans le déterminisme humoral de la maturité glandulaire du tractus génital chez quelques gastéropodes - Arionidae et Limacidae. Bull. Biol. de France et Belg. 88 pp310 - 332

LAVIOLETTE P. 1956 Rôle de la gonade dans la maturation glandulaire du tractus génital chez quelques gastéropodes. Ann. des Sci. Nat. Zool. II^{e} Series 18 pp171 - 173

LEVY M.G., TUNIS M. & ISSEROFF H. 1973 Population control in snails by natural inhibitors. Nature 241 pp65 - 66

LEWIS H.C. & LA FOLLETTE J.R. 1942 Control of the brown snail in citrus orchards. J. Econ. Entomol. 35 (1) pp359 - 362

LEWIS R.D. 1969 Studies on the locomotor activity of the slug Arion ater L. I. Humidity, temperature and light reactions. Malacologia 7 (2 - 3) pp295 - 306

LONZA Ltd. Brtish Patent 1,434,899. Filed 5th July 1974 in Switzerland. Convention application filed 3rd March 1975 Complete specification published 5th May 1976

LUTMAN J. 1978 The role of slugs in an Agrostis - Festuca grassland In: Production ecology of British moors and montane grasslands. HEAL O.W. & PERKINS D.F. (eds.) pp332 - 347 Springer-Verlag, Berlin.

MACHIN J. 1964 The evaporation of water from Helix aspersa. I. The nature of the evaporating surface. J. Exp. Biol. 41 pp759 - 769

MADDY K.T. 1975 Poisoning of dogs with metaldehyde in snail and slug poison bait. Calif. Vet. 29 (3) pp27 and 28

MAGNENAT M. 1972 Les pulmones terrestres ravageurs des plantes cultiveés. Une analyse bibliographique. 72pp

MALEK E.A. & CHENG T.C. 1974 Medical and economic malacology. Academic Press, London. 398pp.

MALLET C. & BOUGARAN H. 1968 Détermination de la valeur attractive des appâts sur les limaces. Meded. Fac. Landb. Rij. Gent. 33 pp769 - 776

MALLET C. & BOUGARAN H. 1971 Action molluscicide de divers carbamates. Meded. Fac. Landb. Rij. Gent. 36 (1) pp207 - 215

MARTIN T.J., DAVIS M.E. & MORRIS D.B. 1969 Development work with methiocarb in Great Britain. Proc. 5th Brit. Insect. Fungic. Conf. 2 pp434 - 441

MASON C.F. 1970 Food, feeding rates and assimilation in woodland snails. Oecologia (Berl.) 4 pp358 - 373

MATTHEWS R.S. 1977 Chemotaxis and anemotaxis in the slug Limax maximus. Tennessee Acad. Sci, J. 52 (4) p150

MAYER K 1957 Die Schneckenbekämpfung mit Metaldehydpräparaten. Nachrichtenblatt Deutschen Pfanzenschutz. 9 pp36 - 41

M^{C}CORMACK G. 1970 Homing of the tiger slug Limax maximus L. Tane 16 pp 143-148

MEAD A.R. 1961 The giant African snail - a problem in economic malacology. Univ. Chigaco Press 257pp.

MEAD A.R. 1979 Economic Malacology - with particular reference to Achatina fulica. Vol 2B of Pulmonates FRETTER V. & PEAKE J. (eds.). Academic Press, London

MEENAKSHI V.R. & SCHEER B.T. 1969 Regulation of galactogen synthesis in the slug Ariolimax columbianus.
Comp. Biochem. Physiol. 29 pp841 - 845

MELLANBY K. 1961 Slugs at low temperatures.
Nature 189 (4768) p944

MILES P.M. 1969 Technique of recording slug movement and some results of tests with metaldehyde and bran and bran baits only.
Lab. Practice 18 pp437 - 440

MOENS R., FRANCOIS E., RIGA A & van den BRUEL W.E. 1967
A mechanical barrier against terrestrial gasteropods.
Parasitica 23 (1) pp22 - 27

MOQUIN-TANDON A. 1891 L'organe de l'odorat chez les gastéropodes.
Ann. des Sci. Nat. 3e Series (Zool) 15 - 16 pp151 - 158

MORETON B.D. 1953 Preliminary experiments with metaldehyde suspensions against slugs.
Plant Pathol. 2 pp49 - 51

MORETON B.D. 1979 The diurnal rhythm and the cycle of feeding and digestion in the slug Deroceras caruanae.
J. Zool. Lond. 187 (2) pp135 - 152

MORTON J.E. 1967 Molluscs 244p.
Hutchinson Univ. Press

MUSICK G.J. 1972 Efficacy of Phorate for control of slugs in field corn.
J. Econ. Entomol. 65 (1) pp220 - 222

NEWELL P.F. 1965a The behaviour and distribution of slugs
Ph.D. Thesis London Univ.

NEWELL P.F. 1965b Time lapse ciné recording the soil surface activity of slugs.
Anim. Behav. 13 p583

NEWELL P.F. 1966 The nocturnal behaviour of slugs.
Med. Biol. Illustr. 16 pp146 - 159

NEWELL P.F. 1968 The measurement of light and temperature as factors controlling the surface activity of the slug Agriolimax reticulatus (Muller). In: The measurement of environmental factors in terrestrial ecology. WADSWORTH R.M. (ed.)
Blackwell Sci. Pub. Oxford, pp 141 - 146

NEWELL P.F. 1973 Etude de l'ultrastructure de l'epithelium dorsal et pedieux des limaces Arion hortensis (Fer.) et Agriolimax reticulatus (Müll.)
Haliotis 3 pp131 - 142

NEWELL P.F. 1977 The structure and enzyme histochemistry of slug skin.
Malacologia 16 (1) pp183 - 195

NEWELL P.F. & NEWELL G.E. 1968 The eye of the slug Agriolimax reticulatus (Müll.)
Symp. Zool. Soc. Lond. 23 pp97 - 111

NORTHERN R.T. 1970 Slugs and beer - some can take it and leave it.
Am. Orchid Soc. Bull. 39 (3) pp221 - 223

OLESEN J.M. 1979 Differential anther eating by snails in the heterostylous plant species Primula elatior L. Hill.
Acta Bot, Neerlandica 28 (6) pp519 - 521

ORTH R.E., MOORE I., FISHER T.W. & LEGNER E.F. 1975 A rove beetle, Ocypus olens, with potential for biological control of the brown garden snail, Helix aspersa, in California, including a key to the nearctic species of Ocypus.
Can. Entomol. 107 (10) pp1111 - 1116

PALLANT D. 1969 The food of the grey field slug Agriolimax reticulatus (Müller) in woodland.
J. Anim. Ecol. 38 pp391 - 397

PALLANT D. 1970a A quantitative study of feeding in woodland by the grey field slug Agriolimax reticulatus (Müller).
Proc. Malac. Soc. Lond. 39 pp83 - 87

PALLANT D. 1970b A note on the faeces of Agriolimax reticulatus (Müller).
J. Conchology 27 pp111 - 113

PALLANT D. 1971 The food of the grey field slug Agriolimax reticulatus (Müller) on grassland.
J. Anim. Ecol. 41 pp761 - 769

PALLANT D. 1974 Assimilation in the grey field slug Agriolimax reticulatus (Müller).
Proc. Malac. Soc. Lond. 41 pp99 - 107

PALLANT D. 1975 Some aspects of the ecology of the grey field slug Agriolimax reticulatus (Müller).
Ph.D. Thesis York Univ.

PAPPAS J.L. 1971 Control of European brown garden snail in citrus.
The Calif. Citrograph 56 pp225 - 228

PAPPAS J.L. & CARMAN J.L. 1961 Control of European brown snail in citrus groves in southern Cailifornia with Guthion and metaldehyde sprays.
J. Econ. Entomol. 54 (1) pp152 - 155

PELLUET D. 1964 On the hormonal control of cell differentiation in the ovotestis of slugs (Gastropoda: Pulmonata).
Can. J. Zool. 42 pp195 - 199

PELLUET D. & LANE N.J. 1961 The relation between neurosecretion and cell differentiation in the ovotestis of slugs (Gastropoda: Pulmonata).
Can. J. Zool. 39 pp789 - 805

PHILLIPS D.W. 1975 Localisation and electrical activity of the distance chemoreceptors that mediate predator avoidance behaviour in Acmaea limulata and Acmaea scutum (Gastropoda: Prosobranchia).
J. Exp. Biol. 63 pp403 - 412

PICKETT J.A. & STEPHENSON J.W. 1980 Plant volatiles and components influencing behaviour of the field slug Deroceras reticulatum (Müller).
J. Chem. Ecol. 6 (2) pp435 - 444

PIERON H. 1908 Le sens chimique des Limnées.
Ass. Franc. Adv. Sci. 37e Session pp603 - 608

PINDER L.C.V. 1974 The ecology of slugs in potato crops with special reference to the differential susceptibility of potato cultivars to slug damage.
J. Appl.Ecol. 11 pp439 - 451

QUICK H.E. 1960 British slugs (Pulmonata: Testacellidae, Arionidae, Limacidae).
Bull. Br. Mus. Nat. Hist. Zool. 6 (3) pp 103 - 226

RAYNER J.M. 1975 Experiments on the control of slugs in potatoes by means of molluscicidal baits. Plant Pathol. 24 (3) pp167 - 171

RICHARDSON A.M.M. 1975 Food, feeding rates and assimilation in the land snail Cepaea nemoralis L. Oecologia (Berl.) 19 pp59 - 70

RICHTER K.O. 1979 Aspects of nutrient cycling by Ariolimax columbianus (Mollusca: Arionidae) in Pacific northwest coniferous forests. Pedobiologia 19 (1) pp60 - 74

RICOU G. 1961 Contribution à l'étude des mollusques nuisibles en Normandie. Rev. Soc. Sav. Hte. Normandie 21 (6) pp30 - 44

RICOU G. 1964 Relation entre l'activité des limaces grises et la température. Meded. Landbouwhogesch. Gent. 29 (3) pp1071 - 1080

RIDGWAY J.W. 1971 Studies on the nutrition of the slug Arion ater - the assessment of food consumption and utilisation in the slug, with particular reference to vitamin B and nitrogen requirements, using such techniques as the addition of chromic oxide to artificial diets. Ph.D. Thesis Univ. Bradford

RINGS R.W., LINDQUIST R.K., ARNOLD F.J. & KRUEGER H.R. 1975 Research on bionomics of cutworms and garden snails. Entomol. Soc. Am. - North Central Branch, Proc. 30 pp75 - 76

RITCHIE L.S., BERRIOS-DURAN L.A. & SIERRA R. 1969 A field screening test on a slow release formulation of sodium pentachlorophenate for molluscicidal use. Bull. W.H.O. 40 pp474 - 476

RITTER F.J. (ed,) 1979 Chemical ecology: odour communication in animals. Proc. Adv. Res. Inst. on chemical ecology Noordwijkerhaut, The Netherlands. Elsevier, N. Holland.

RODERICK W.R. 1966 Current ideas on the chemical basis of olfaction J. Chem. Education 43 (10) pp510-520

ROGERS D.C. 1968 Fine structure of smooth muscle and neuro-muscular junctions in the optic tentacles of Helix aspersa and Limax flavus.
Z. Zell. Mikrosk. Anat. 89 pp80 - 94

ROLLO C.D. 1978 The behavioural ecology of terrestrial slugs.
Ph.D. Thesis Univ. British Columbia

ROLLO C.D. & ELLIS C.R. 1974 Studies on slugs, Deroceras reticulatum, D. laeve and Arion fasciatus, occuring in field corn grown under various tillage regimes.
Entomol. Soc. Am. - North Central Branch, Proc. 29 p193

ROLLO C.D. & WELLINGTON W.G. 1975 Terrestrial slugs in the vicinity of Vancouver, British Columbia,
The Nautilus 89 (4) pp107 - 115

ROLLO C.D. & WELLINGTON W.G. 1979 Intra- and interspecific agnostic behaviour among terrestrial slugs (Pulmonata: Stylommatophora)
Can. J. Zool. 57 (4) pp846 - 855

ROLLO C.D. & WELLINGTON W.G. 1980 Environmental orientation by terrestrial molluscs with particular reference to homing behaviour.
In Press

RORKE M.A., GARDNER D.R. & GREENHALGH H.R. 1974 Lethality and behavioural symptoms produced by some organophosphorous compounds in the snail (Helix aspersa)
Bull. Environ, Contam. Toxicol. 11(5) pp417 - 424

RUNHAM N,W., BAILEY T.G. & LARYEA A.A. 1973 Studies on the endocrine control of the reproductive tract of the grey field slug Agriolimax reticulatus.
Malacologia 14 pp135 - 142

RUNHAM N.W. & LARYEA A.A. 1968 Studies on the maturation of the reproductive system of Agriolimax reticulatus (Pulmonata: Limacidae).
Malacologia 7 (1) pp93 - 108

RUPPEL R.F. 1959 Effectiveness of Sevin against the grey garden slug.
J. Econ. Entomol. 52 (2) p360

SCHNEIDER D. 1968 Basic questions of olfactory research. In: Theories of odour and odour measurement. TANYOLAC N. (ed.) Technivision, Maidenhead pp201 - 211

SELIM S.I. 1973 The control of snail and slug pests in agriculture. I. Analysis for and dissipation of metaldehyde in vegetable crops. II. Beer as a slug attractant. Ph.D. Thesis Univ, California, Davis.

SENSEMAN D.M. 1976 Neurobehavioural studies on feeding in the terrestrial slug Ariolimax californicus Cooper. Ph.D. Thesis Princeton Univ. N.J.

SENSEMAN D.M. 1977a Neurobehavioural studies on feeding in the terrestrial slug Ariolimax californicus. The Veliger 20 (1) pp56 - 57

SENSEMAN D.M. 1977b Starch - a potent feeding stimulant for the terrestrial slug Ariolimax californicus. J. Chem. Ecol. 3 (6) pp707 - 715

SENSEMAN D.M. 1977c Gastropod mollusks as model systems for the study of integrative mechanisms controlling feeding behaviour In: The chemical senses and nutrition. Vol 2. KARE M. (ed.) Academic Press, London.

SENSEMAN D.M. 1978a Short term control of food intake by the terrestrial slug Ariolimax. J. Comp. Physiol. (A) 124 pp37 - 48

SENSEMAN D.M. 1978b Chemosensory control of feeding in the snail Helisoma. 3rd E.C.R.O. Congress. 10th - 14th Sept. 1978. Univ. Pavia

SHARAF N.S. & GHALEB A.H. 1978 A laboratory immersion test for assessing the contact toxicity of bait molluscicides. Z. Pflanzenkrankheiten. Pflanzenschutz 85 (5) pp275 - 279

SHOREY H. 1976 Animal communication by pheromones. Academic Press, London 167pp

SIEGEL R.K. & JARVIK M.E. 1974 Learning in the land snail (Helix aspersa Muller). Bull. Psychonomic Soc. 4 (5A) pp476 - 478

SIMPSON A.W., THOMAS J.D. & TOWNSEND C.R. 1973 Social behaviour in the freshwater pulmonate snail Biomphalaria glabrata. Behav. Biol. 9 pp731 - 740

SIMPSON L., BERN H. & NISHIOKA R.S. 1966 Survey of evidence for neurosecretion in gastropod molluscs. Am. Zool. 6 pp123 - 138

SMITH B.J. 1967 Correlation between neurosecretory changes and maturation of the reproductive tract of Arion ater (Stylommatophora: Arionidae). Malacologia 5 (2) pp285 - 298

SMITH F.F. & BOSWELL A.L. 1970 New baits and attractants for slugs. J. Econ. Entomol. 63 pp1919 - 1922

SNYDER N,F.R. & SNYDER H.A. 1971 Pheromone mediated behaviour of Fasciolaria tulipa. Anim. Behav. 19 pp257 - 268

SOKOLOVE P.G., BEISWANGER C.M., PRIOR D.J. & GELPERIN A. 1977 A circadian rhythum in the locomotor behaviour of the giant garden slug Limax maximus. J. Exp. Biol. 66 pp47 - 64

SOUTH A. 1965 Biology and ecology of Agriolimax reticulatus (Müll) and other slugs - spatial distribution. J. Anim. Ecol. 34 pp403 - 417

SOUTHWICK C.H. & SOUTHWICK H.M. 1969 Population density and preferential return in the giant African snail Achatina fulica. Am. Zool. 9 p566

SPEYER W. 1954 Versuche mit Metaldehyd zur Nachtschneckenbekämpfung. Nachrichtenbl. Dtsch. Pfanzenschutzdienstes 6 pp134 - 137

STEPHENS G.J. & MCGAUGH J.L. 1972 Biological factors related to learning in the land snail Helix aspersa Müller. Anim. Behav, 20 pp309 - 315

STEPHENS G.J. & STEPHENS G.C. 1966 Photoperiodic stimulation of egg laying in the land snail Helix aspersa. Nature 212 p1582

STEPHENSON J.W. 1961 A culture method for slugs. Proc. Malac. Soc. Lond. pp43 - 45

STEPHENSON J.W. 1965 The effect of irrigation on damage to potato tubers by slugs.
Europe. Potato J. 8 (3) pp145 - 149

STEPHENSON J.W. 1970 Slugs
Rep. Rothampstead Exp. Sta. pp198 - 199

STEPHENSON J.W. 1972 Gelatin as a carrier for S^2- cyanoethyl N-methylcarbamoyl oxy thioacetimidate, an experimental molluscicide.
Pest. Sci. 3 pp81 - 87.

STEPHENSON J.W. 1979 The functioning of the sense organs associated with feeding behaviour in Deroceras reticulatum (Müll).
J. Moll. Stud. 45 p167 - 171

STOUT R. 1968 Answers that startled them most - Slugging beer slays slugs.
Org. Gard. Farm. 15 (1) p43

STRINGER A & MORGAN N.G. 1969 Population and control of snails in blackcurrant populations.
Proc. 5th Br, Insect. Fungic. Conf. 2 pp453 - 457

SUBRAMANYAM O.V. 1973 Neuroendocrine control of metabolism of a pulmonate snail, Ariophanta sp.
Experientia 29 (9) pp1150 - 1151

SUZUKI N. 1967 Behavioural and electrical responses of the land snail Ezohelix flexibilis (Fulton) to odours.
J. Fac. Sci. Hokkaido Univ. Ser.6 Zool. 16 pp174 - 185

SUZUKI N. 1968 Odour discrimination in the land snail.
Zool. Mag. (Tokyo) 77 pp178 - 184

SYMONDS B.V. 1975 Evaluation of potential molluscicides for the control of the field slug Agriolimax reticulatus (Müll).
Plant Pathol. 24 (1) pp1 - 9

TAKEDA N. 1977 Stimulation of egg laying by nerve extracts in slugs.
Nature 267 (5611) pp513 - 514

TAKEDA N. & TSURUOKA H. 1979 A sex pheromone secreting gland in the terrestrial snail Euhadra peliomphala.
J. Exp. Zool. 207 (1) pp17 - 25

TAYLOR J.W. 1894 Monograph of the land and freshwater Mollusca of the British Isles. 312pp.
Taylor Bros., Leeds

TERRIERE L.C. 1974 A summary of information on metaldehyde pertaining to its use on strawberries for slug control.
Unpublished report. Dept. Agric. Chem. Oregon State Univ.

THOMAS D.C. 1947 Some observations on damage to potatoes by slugs.
Ann. Appl. Biol. 34 pp246 - 251

THOMAS D.C. 1948 The use of metaldehyde against slugs.
Ann. Appl. Biol. 35 pp207 - 227

TILEMANS E. & DORMAL S. 1952 Toxicité des products phytopharmaceutiques envers l'homme et les animaux à sang chaux.
Parasitica (Gembleux) 8 pp64 - 91

TOWNSEND C.R. 1973 The role of the osphradium in chemoreception by the snail Biomphalaria glabrata (Say).
Anim. Behav. 21 pp549 - 556

TOWNSEND C.R. 1974a Mucus trail following by the snail Biomphalaria glabrata (Say).
Anim. Behav. 22 pp170 - 177

TOWNSEND C.R. 1974b The chemoreceptor sites involved in food finding by the freshwater pulmonate snail Biomphalaria glabrata (Say), with particular reference to the function of the tentacles.
Behavioural Biol. 11 pp511 - 523

TURNER T. 1968 Treatment for poisoning by slug bait.
The Vet. Record 83 p180

VERSCHUUREN H.G., KROES R., ENGLINA M. den TONKELAAK, BERKVENS J.M. HELLEMAN P.W. & VAN ESCH G.J. 1975 Long term toxicity and reproduction studies with metaldehyde in rats.
Toxicology 4 (1) pp97 - 115

WALLIS D.I. & WRIGHT B.R. 1971 The tactile sense of the tentacles of the common slug Arion ater L.
J. Physiol. 213 8P

WARLEY A.P. 1970 Some aspects of the biology, ecology and control of slugs in S.E. Scotland, with particular reference to the potato crop.
Ph.D. Thesis Univ. Edinburgh

WATTEZ C. 1977 The control of sexual differentiation by the cephalic complex in the slug Arion subfuscus Drap. p99 (Gastropoda: Pulmonata) : Cytological and in vitro studies.
Abs. 6th European Malac. Cong. 15th - 20th Aug. 1977 Amsterdam

WATTEZ C. & DURCHON M. 1972 Influence des tentacles oculaires dans la differenciation génitale chez Arion subfuscus Draparnaud. (Mollusque, Gasteropode, Pulmoné).
C.R. Acad. Sci. Paris Series D 274 (16) pp2328 - 2331

WEBLEY D. 1963 Experiments with slug baits in 1959.
Plant Pathol. 12 pp19 - 20

WEBLEY D. 1964 Aspects of trapping slugs with metaldehyde and bran.
Ann. Appl. Biol. 56 pp37 - 45

WEBLEY D. 1966 Waterproofing of metaldehyde on bran baits for slug control.
Nature 212 pp320 - 321

WEBLEY D. 1970 Observations on the effects of distribution and numbers of slug pellets on the catch of slugs.
Ann. Appl. Biol. 66 pp347 - 352

WEISS J.C. 1973 La lutte anti limaces: historiques et evolution.
Haliotis 3 pp107 - 111

WELLS M.J. & BUCKLEY S.K.G. 1972 Snails and trails.
Anim. Behav, 20 pp345 - 355

WHARTON A.L. & ENSOR H. 1969 The slug problem in peas for processing
Proc. 5th Br. Insect. Fungic. Conf. 2 pp445 - 452

WIJDENES J. 1977 Some aspects of the control of the reproductive system of Agriolimax reticulatus. p94
Abs. 6th European Malac. Cong. 15th - 20th August 1977 Amsterdam

WIJDENES J. & RUNHAM N.W. 1976 Studies on the function of the dorsal bodies of Agriolimax reticulatus (Mollusca:Pulmonata).
Gen. Comp. Endocrin. 29 pp545 - 551

WIJDNES J. & RUNHAM N. W. 1977 Studies on the control of growth in Agriolimax reticulatus. (Mollusca: Pulmonata). Gen. Comp. Endicrin. 31 (1) pp154 - 156

WILLIAMS R. 1972 The effects of molluscicide concentration on slug bait consumption. M.Sc. Thesis Univ. Bradford

WILLOWS A.O.D. 1973 Learning in gastropod molluscs. In: Invertebrate learning Vol. 2: Arthropods and gastropod molluscs. DYAL J.A. & WILLOWS A.O.D. (eds) pp187 - 274 Plenum Press, N.Y.

WILSON E.O. 1970 Chemical communication in animal species. In: Chemical ecology. SONDHEIMER E. & SIMEONE J.B. (eds) Academic Press, London

WOLFF H.G. 1969 Einige Ergebnisse zur Ultrastruktur der Statocysten von Limax maximus, L.flavus und Arion empiricorum. Z. Zell. Mikrosk. Anat. 100 pp251 - 270

WRIGHT A.A. 1973 Evaluation of a synthetic diet for the rearing of the slug Arion ater L. Comp. Biochem. Physiol. 46A pp593 - 603

WRIGHT B.R. 1972 Sensory structure and function of Arion ater L. tentacles. Ph.D. Thesis Univ. Wales

WRIGHT R.H. & BURGESS R.E. 1975 Molecular coding of olfactory specificity. Can. J. Zool. 53 (9) pp1247 - 1253

YAMASHITA Y., JONES R.M. & NICHOLSON C.H.L. 1979 Feeding of slugs (Deroceras sp. and Lehmannia nyctelia) on subtropical pasture species, particulatly Kenya white clover (Trifolium semipilosum) J. Appl. Ecol. 16 (1) pp307 - 318

APPENDICES

FOOD CONSUMPTION WITH DIFFERENT BATCHES OF WATNEY'S YEAST - YEAST CONSUMPTION. ANALYSIS OF VARIANCE. SEE TABLE 9

	W1	W2	W5	W6	W7	TOTAL
	89.2	253.1	76.9	73.7	80.3	
	256.8	102.9	131.9	93.6	113.6	
	210.6		79.2	59.0	35.2	
			69.8	27.6	49.0	
			59.1		57.7	
Sum T	556.6	356.0	416.9	253.9	335.8	1919.2
n	3	2	5	4	5	19
$\bar{y}$	185.53	178.0	83.38	63.48	67.16	101.01
T^2	309803.56	126736.0	173805.61	64465.21	112761.64	
T^2/N	103267.85	63368.0	34761.12	16116.30	22552.33	240065.6

Correction Factor, $\Sigma x^2/N$ = 193859.4

Batch S.S. $\Sigma(T^2/n) - C$ = 46206.2

Total S.S., $\Sigma y^2 - C$ = 81750.36

Total d.f. = 18, Batch d.f. = 4

S.S.		d.f.	M.S.	V ratio	F value	P
Batch	46206.2	4	11551.55	4.55	3.11	5%
Residual	35544.16	14	2538.87			
Total	81750.36	18				

The V ratio exceeds the tabled value for the appropriate degrees of freedom.

The Null Hypothesis that the mean consumption of each batch of yeast is the same can thus be rejected.

$S.E._d$ = RMS $(1/n + 1/n)$

W1 v W2 = 46.00

W2 v W5 = 42.16

W5 v W6 = 33.80

W6 v W7 = 33.80

FOOD CONSUMPTION WITH DIFFERENT BATCHES OF WATNEY'S YEAST - BRAN CONSUMPTION. ANALYSIS OF VARIANCE. SEE TABLE 9

	W1	W2	W5	W6	W7	TOTAL
	3.4	10.3	0.0	26.2	31.8	
	17.2	3.4	3.4	22.8	24.1	
	3.4		28.6	40.0	18.3	
			24.1	36.5	36.5	
			31.0		57.7	
Sum T	24.0	13.7	87.1	125.5	168.4	418.7
n	3	2	5	4	5	19
$\bar{y}$	8.0	6.85	17.42	31.38	33.68	22.04
T^2	576.0	187.69	7586.41	15750.25	28358.56	
T^2/N	192.0	93.85	1517.28	3937.56	5671.71	11412.4

Correction Factor, $\Sigma x^2/N = 9226.83$

Batch S.S. $\Sigma(T^2/n) - C = 2185.57$

Total S.S., $\Sigma y^2 - C = 4308.12$

Total d.f. = 18, Batch d.f. = 4

S.S.		d.f.	M.S.	V ratio	F value	P
Batch	2185.57	4	546.39	3.60	3.11	5%
Residual	2122.55	14	151.61			
Total	4308.12	18				

The V ratio exceeds the tabled value for the appropriate degrees of freedom.

The Null Hypothesis that the mean consumption of bran with each batch of yeast is the same can thus be rejected.

FOOD CONSUMPTION WITH DIFFERENT BATCHES OF FULLER'S YEAST - YEAST CONSUMPTION ANALYSIS OF VARIANCE. SEE TABLE I2

F2	F3	F4	F5	F6	TOTAL
29.7	94.9	43.1	104.8·	35.2	
	91.5	68.9	44.3	83.7	
	43.1	26.2	68.1	48.8	
	38.6	74.5	25.2	34.2	
	34.2	53.0		40.0	
	40.0	72.4			
		40.8			
		138.6			
		74.5			
		33.1			
		67.9			
Sum T	29.7	342.3	693.0	242.4	241.9	1549.3
n	1	6	11	4	5	27
$\bar{y}$	29.7	57.05	63.0	60.6	48.38	57.38
T^2	882.09	117169.29	480249.0	58757.76	58515.61	
T^2/N	882.09	19528.22	43659.0	14689.44	11709.12	90467.872

Correction Factor, $\Sigma x^2/N$ = 88901.125

Batch S.S. $\Sigma(T^2/n) - C$ = 1566.75

Total S.S., $\Sigma y^2 - C$ = 20113.57

Total d.f. = 26, Batch d.f. = 4

S.S.		d.f.	M.S.	V ratio	F value	P
Batch	1566.75	4	391.69	0.46	2.82	5%
Residual	18546.82	22	843.04			
Total	20113.57	26				

The V ratio is lower than the tabled F value for the appropriate degrees of freedom.

The Null Hypothesis that the mean consumption of each batch is the same can thus be rejected.

FOOD CONSUMPTION WITH DIFFERENT BATCHES OF FULLER'S YEAST - BRAN CONSUMPTION. ANALYSIS OF VARIANCE TABLE I2

F2	F3	F4	F5	F6	TOTAL
120.8	50.1	18.3	88.2	105.1	
	29.7	21.7	104.3	97.1	
	25.2	13.8	52.2	74.8	
	82.5	49.0	62.3	52.0	
	24.9	30.7		32.1	
	6.9	43.2			
		20.7			
		49.0			
		87.0			
		44.3			
		33.1			
Sum T	120.8	219.3	410.85	307.0	361.1	1419.05
n	1	6	11	4	5	27
$\bar{y}$	120.8	36.55	37.35	76.75	72.22	52.56
T^2	14592.64	48092.49	168797.72	94249.0	130393.21	
T^2/N	14592.64	8015.42	15345.25	23562.25	26078.64	87594.2

Correction Factor, $\leq x^2/N$ = 74581.59

Batch S.S. $\leq (T^2/n) - C$ = 13012.61

Total S.S. $\leq y^2 - C$ = 26242.33

S.S.		d.f.	M.S.	V ratio	F value	P
Batch	13012.61	4	3253.15	5.41	2.82	5%
Residual	13229.72	22	601.35			
Total	26242.33	26				

The V ratio exceeds the tabled F value for the appropriate degrees of freedom.

The Null Hypothesis that the mean consumption of bran with each batch of yeast is the same can thus be rejected.

TEST TO COMPARE THE CONSUNMPTION OF W7 AND F3

t - test of the difference between the means of two sets of paired observations. SEE TABLE I5

Amount eaten mg	F3	W7	Difference, D
	59.9	61.2	- 1.3
	55.7	132.7	- 77.0
	115.0	19.3	+ 95.7
	55.4	50.0	+ 5.4
	35.5	3.4	+ 32.1
Total	321.5	266.6	54.9
Mean	64.3	53.32	10.98

Number of pairs, n = 5

Sum of squared differences, $\leq D^2$ = 16148.75

Correction Factor, $(\leq D)^2/n$ = 3014.01/n = 602.8

S.S. $\leq D^2 - C$ = 15545.95

d.f. = 4

Variance of the difference, SS/d.f. = 3886.49

Variance of the mean difference = 777.30

$S.E._d$ = 27.88

t ratio of the difference, $\bar{D} - S.E._d$ = 0.39

Students t for 4 d.f. at P = 0.05 is 2.78

The difference between the consumption of the two foods, W7 and F3 is therefore not significant.

APPENDIX 2

NUMBER OF Deroceras reticulatum (ADULTS AND JUVENILES) TRAPPED PER PLOT PER DAY.

DAY 1.

column / row	a	b	c	d	e	total
0	1	3	3	2	1	10
1	3	0	5	3	3	14
2	7	10	10	3	4	34
3	13	7	12	7	8	47
4	20	9	8	13	12	62
5	13	8	12	8	15	56
6	19	19	14	12	10	73
7	19	17	12	12	13	73
8	11	13	14	7	10	55
9	13	10	12	7	9	51
total	119	96	102	74	85	476

DAY 2.

column / row	a	b	c	d	e	total
0	5	3	7	2	3	20
1	2	3	11	8	4	28
2	8	19	15	5	8	55
3	18	18	15	13	11	75
4	14	7	16	8	12	57
5	24	19	13	9	14	79
6	39	39	17	15	14	124
7	35	24	19	15	18	111
8	13	24	10	14	11	72
9	20	12	16	11	10	69
total	178	168	139	100	105	690

DAY 3.

column / row	a	b	c	d	e	total
0	5	8	9	6	7	35
1	3	2	11	6	9	31
2	6	21	11	9	2	49
3	47	33	29	19	21	149
4	46	24	34	18	26	148
5	42	38	30	25	26	161
6	60	57	33	39	23	212
7	55	36	23	18	19	151
8	29	25	19	19	30	122
9	31	26	29	23	19	128
total	324	270	228	182	182	1186

DAY 4.

column / row	a	b	c	d	e	total
0	1	5	4	8	5	23
1	3	8	29	18	6	64
2	15	36	23	20	10	104
3	38	23	50	26	28	165
4	40	33	41	26	34	174
5	53	43	35	29	16	176
6	50	45	20	25	34	174
7	64	43	33	24	22	186
8	56	53	25	24	36	194
9	53	41	39	31	19	183
total	373	330	299	231	210	1443

DAY 5.

column / row	a	b	c	d	e	total
0	1	4	4	3	4	16
1	1	3	14	9	5	32
2	10	19	15	15	8	67
3	12	15	29	9	18	83
4	27	19	27	17	23	113
5	24	24	20	24	21	113
6	27	35	19	23	22	126
7	25	23	17	19	17	101
8	38	33	17	17	20	125
9	17	31	22	20	14	104
total	182	206	184	156	152	880

DAY 6.

column / row	a	b	c	d	e	total
0	1	3	2	3	3	12
1	1	2	8	1	7	19
2	12	21	7	8	5	53
3	13	17	23	5	11	69
4	25	21	10	8	9	73
5	22	37	10	20	23	112
6	34	31	22	17	19	123
7	27	17	13	14	12	83
8	36	29	16	11	12	104
9	22	25	23	12	11	93
10	18	17	6	11	8	60
total	211	220	140	110	120	801

DAY 7.

column / row	a	b	c	d	e	total
1	3	3	10	6	10	32
2	11	17	6	6	11	51
3	12	11	7	6	5	41
4	26	16	15	6	2	65
5	16	10	20	27	29	102
6	17	32	8	25	13	95
7	25	16	9	4	12	66
8	35	36	11	15	6	103
9	19	20	23	10	8	80
10	21	14	25	24	23	107
total	185	175	134	129	119	742

NUMBER OF (Deroceras reticulatum)TRAPPED PER PLOT ON DAY 19
UNDER HESSIAN SACKS - UNBAITED AREAS.

DAY 19.

column / row	a	b	c	d	e	total
0	7	11	17	15	9	59
1	5	12	19	13	28	77
2	8	29	28	20	12	97
3	22	23	22	14	22	103
4	19	22	36	29	31	137
5	20	38	21	34	34	156
6	29	26	23	20	39	137
7	41	26	20	29	18	134
8	17	44	34	36	26	157
9	21	30	41	44	45	181
10	3	30	39	31	56	159
total	201	291	300	285	320	1397

STATISTICAL TREATMENT OF DATA

Analysis of variance

N = Total number of observations

y = Nature of observations

T = Number of treatments

R = Number of replicates or blocks

D = Number of days

S.S. = Sum of squares

M.S. = Mean squares = $\frac{\text{S.S.}}{\text{d.f.}}$

d.f. = Degrees of freedom

V ratio = Variance ratio = $\frac{\text{M.S.}}{\text{Residual M.S.}}$

F value = Value of variance ratio obtained from standard tables

P = Significance level achieved by data

S.E. = Standard error

$S.E._d$ = Standard error of the variance of two varietal means

$$= \left(\frac{2 \times \text{Residual M.S.}}{R}\right)$$

C = Correction Factor

$$= \frac{(\Sigma y)^2}{N}$$

Total S.S. $= \Sigma (y)^2 - C$

Treatment S.S. $= \frac{\Sigma (T^2)}{R} - C$

Replicate S.S. $= \frac{\Sigma (R^2)}{T} - C$

A Null Hypothesis is proposed that the means of the treatments and replicates do not differ more than by chance alone. If the F value exceeds the V ratio, the Null Hypothesis is rejected and it is concluded that the means differ more than expected on the basis of chance.

"t" test

The "t" test compares individual treatments with one another using the difference between the two means and the standard error of the variance of the mean

$$t = \frac{\text{Difference between treatment means}}{S.E._d}$$

A matrix is then constructed to compare values of t with values of p - probability of similarity. A table of percentage points of the t distribution provides a value of p for the appropriate d.f.

PREBAITING TRAPPING FIGURES FOR DAY 6 REARRANGED INTO TREATMENT GROUPS

The trapping figures for Day 6 have been rearranged from the rows 1 - 10 into the treatment groups shown in Table 32
This gives a true indication of the distribution of slugs in the randomised plots subsequently baited with each treatment.

Analysis of variance

Correction Factor, C $= \frac{622521}{50} = 12450.42$

Total Sum of Squares $= 16341 - C = 3890.58$

Treatment S.S. $= \frac{64973}{5} - C = 544.18$

Replicate S.S. $= \frac{135371}{10} - C = 1086.68$

S.S.		d.f.	M.S.	V ratio	F value	P
Replicate	1086.68	4	271.67	4.33	3.83	1%
Treatment	544.18	9	60.46	0.96	2.12	5%
Residual	2259.72	36	62.77			
Total	3890.58	49				

S.E. = 3.54

$S.E._d = 5.00$

The replicate or column effect is significant (P = 0.01) though the treatment or row effect is not significant (P= 0.05).

The Null Hypothesis that the distribution of Deroceras reticulatum is the same for all columns can thus be rejected.

The Null Hypothesis that the distribution of Deroceras reticulatum is the same for all the rearranged (randomised) treatment rows can thus be accepted.

THE NUMBER OF POISONED Deroceras reticulatum FOUND ON EACH PLOT DAY 8.

column / bait	a	b	c	d	e	total	mean
A	0	0	0	0	2	2	0.4
B	6	7	0	2	0	15	3.0
C	2	2	1	1	0	6	1.2
D	5	4	1	0	0	10	2.0
E	0	2	7	0	2	11	2.2
F	7	1	2	0	2	12	2.4
G	1	0	0	0	2	3	0.6
H	4	0	0	0	0	4	0.8
J	0	5	5	0	5	15	3.0
K	20	0	1	3	2	26	5.2
total	45	21	17	6	15	104	2.08

Analysis of variance

Correction Factor, C $= \frac{10816}{50} = 216.32$

Total Sum of Squares $= 770 - C = 553.68$

Treatment S.S. $= \frac{1556}{5} - C = 94.88$

Replicate S.S. $= \frac{3016}{10} - C = 85.28$

S.S.		d.f.	M.S.	V ratio	F value	P
Replicate	85.28	4	21.32	2.05	2.61	5%
Treatment	94.88	9	10.54	1.02	2.12	5%
Residual	373.52	36	10.38			
Total	553.68	49				

S.E. = 1.44

$S.E._d$ = 2.04

The replicate or block effect is not significant (P = 0.05) and the treatment effect also not significant (P = 0.05).

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each replicate can thus be accepted.

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each treatment can thus be accepted.

THE NUMBER OF POISONED Deroceras reticulatum FOUND ON EACH PLOT DAY 9.

column / bait	a	b	c	d	e	total	mean
A	0	0	0	0	0	0	0.0
B	4	3	2	0	9	18	3.6
C	1	1	2	2	0	6	1.2
D	3	2	0	9	1	15	3.0
E	4	4	4	1	2	15	3.0
F	3	0	2	1	1	7	1.4
G	0	2	2	2	1	7	1.4
H	0	0	0	1	0	1	0.2
J	6	4	9	2	5	26	5.2
K	0	1	1	4	5	11	2.2
total	21	17	22	22	24	106	2.12

Analysis of variance

Correction Factor, C $= \frac{11236}{50} = 224.72$

Total Sum of Squares $= 502 - C = 277.28$

Treatment S.S. $= \frac{1706}{5} - C = 116.48$

Replicate S.S. $= \frac{2274}{10} - C = 2.68$

S.S.		d.f.	M.S.	V ratio	F value	P
Replicate	2.68	4	0.67	0.15	2.61	5%
Treatment	116.48	9	12.94	2.95	2.89	1%
Residual	158.12	36	4.39			
Total	277.28	49				

S.E. = 0.88

$S.E._d$ = 1.09

The replicate or block effect is not significant (P = 0.05) though the treatment effect is significant (P = 0.01).

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each replicate can thus be accepted.

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each treatment can thus be rejected.

THE NUMBER OF POISONED Deroceras reticulatum FOUND ON EACH PLOT DAY 10.

column / bait	a	b	c	d	e	total	mean
A	2	0	2	0	0	4	0.8
B	10	17	17	24	2	70	14.0
C	2	2	1	2	1	8	1.6
D	3	4	4	2	0	13	2.6
E	5	1	6	6	4	22	4.4
F	9	5	5	5	1	25	5.0
G	6	5	1	0	1	13	2.6
H	0	0	0	0	0	0	0.0
J	8	7	6	3	0	24	4.8
K	5	8	0	4	0	17	3.4
total	50	49	42	46	9	196	3.92

Analysis of variance

Correction Factor, C $= \frac{38416}{50} = 768.32$

Total Sum of Squares $= 1922 - C = 1153.68$

Treatment S.S. $= \frac{7292}{5} - C = 690.08$

Replicate S.S. $= \frac{8862}{10} - C = 117.88$

S.S.		d.f.	M.S.	V ratio	F value	P
Replicate	117.88	4	29.47	3.07	2.61	5%
Treatment	690.08	9	76.68	7.99	2.89	1%
Residual	345.72	36	9.60			
Total	1153.68					

S.E. = 1.39

$S.E._d$ = 1.97

The replicate or block effect is significant (P - 0.05) and the treatment effect is also significant (P = 0.01).

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each replicate can thus be rejected.

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each treatment can thus be rejected.

THE NUMBER OF POISONED Deroceras reticulatum FOUND ON EACH PLOT

DAY 11

column / bait	a	b	c	d	e	total	mean
A	0	0	1	2	0	3	0.6
B	8	25	13	18	16	80	16.0
C	2	2	0	1	6	11	2.2
D	0	3	3	1	5	12	2.4
E	0	4	7	3	2	16	3.2
F	6	6	0	8	7	27	5.4
G	17	5	1	8	7	38	7.6
H	0	0	0	0	0	0	0.0
J	5	4	4	3	3	19	3.8
K	6	2	2	10	6	26	5.2
total	44	51	31	54	52	232	4.74

Analysis of variance

Correction Factor, C $= \frac{53824}{50} = 1076.48$

Total Sum of Squares $= 2658 - C = 1572.22$

Treatment S.S. $= \frac{10140}{5} - C = 951.52$

Replicate S.S. $= \frac{11118}{10} - C = 35.32$

S.S.		d.f.	M.S.	V ratio	F value	P
Replicate	35.32	4	8.32	0.52	2.61	5%
Treatment	951.52	9	105.72	6.50	2.89	1%
Residual	585.38	36	16.26			
Total	1572.22	49				

S.E. = 1.80

$S.E._d$ = 2.54

The replicate of block effect is not significant (P = 0.05)though the treatment effect is significant (P = 0.01).

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each replicate can thus be accepted.

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each treatment can thus be rejected.

THE NUMBER OF POISONED Deroceras reticulatum FOUND ON EACH PLOT DAY 12.

column / bait	a	b	c	d	e	total	mean
A	1	0	0	0	0	1	0.2
B	20	22	16	17	15	90	18.0
C	0	1	1	2	0	4	0.8
D	4	2	3	0	2	11	2.2
E	1	2	7	15	4	29	5.8
F	12	6	2	1	1	22	4.4
G	7	5	0	1	4	17	3.4
H	0	0	0	0	0	0	0.0
J	11	3	6	1	4	25	5.0
K	7	3	3	0	2	15	3.0
total	63	44	38	37	32	214	3.92

Analysis of variance

Correction Factor, C $= \frac{45796}{50} = 915.92$

Total Sum of Squares $= 2242 - C = 1326.08$

Treatment S.S. $= \frac{9702}{5} - C = 1024.48$

Replicate S.S. $= \frac{9742}{10} - C = 58.28$

S.S.		d.f.	M.S.	V ratio	F value	P
Replicate	58.28	4	14.57	2.16	2.61	5%
Treatment	1024.48	9	113.83	16.84	2.89	1%
Residual	243.32	36	6.76			
Total	1326.08	49				

S.E. = 1.16

$S.E._d$ = 1.64

The replicate or block effect is not significant (P = 0.05) though the treatment effect is significant (P = 0.01).

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each replicate can thus be accepted.

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each treatment can thus be rejected.

THE NUMBER OF POISONED Deroceras reticulatum FOUND ON EACH PLOT

DAY 13.

column / bait	a	b	c	d	e	total	mean
A	0	0	0	0	0	0	0.0
B	1	5	4	0	0	10	2.0
C	0	0	0	1	4	5	1.0
D	0	1	2	0	1	4	0.8
E	0	0	4	1	3	8	1.6
F	2	1	1	0	1	5	1.0
G	1	9	1	3	4	18	3.6
H	0	0	0	0	0	0	0.0
J	1	1	0	0	2	4	0.8
K	2	0	0	0	0	2	0.4
total	7	17	12	5	15	56	1.12

Analysis of variance

Correction Factor, C $= \frac{3136}{50} = 62.72$

Total Sum of Squares $= 216 - C = 153.28$

Treatment S.S. $= \frac{574}{5} - C = 52.08$

Replicate S.S. $= \frac{732}{10} - C = 10.48$

S.S.		d.f.	M.S.	V ratio	F value	P
Replicate	10.48	4	2.62	1.06.	2.61	5%
Treatment	52.08	9	5.79	2.18	2.12	5%
Residual	90.72	36	2.52			
Total	153.28	49				

S.E. = 0.71

$S.E._d$ = 1.00

The replicate or block effect is not significant (P = 0.05) though the treatment effect is significant (P = 0.05).

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each replicate can thus be accepted.

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each treatment can thus be rejected.

THE NUMBER OF POISONED Deroceras reticulatum FOUND ON EACH PLOT

DAY 14.

column / bait	a	b	c	d	e	total	mean
A	0	0	0	0	0	0	0.0
B	1	1	3	4	2	11	2.2
C	0	0	0	0	1	1	0.2
D	0	0	0	0	0	0	0.0
E	0	0	2	0	1	3	0.6
F	1	1	3	0	1	6	1.2
G	5	1	0	5	4	15	3.0
H	0	0	0	0	0	0	0.0
J	1	2	3	1	1	8	1.6
K	0	1	0	0	0	1	0.2
total	8	6	11	10	10	45	0.90

Analysis of variance

Correction Factor, C $= \frac{2025}{50} = 40.5$

Total Sum of Squares $= 133 - C = 92.5$

Treatment S.S. $= \frac{457}{5} - C = 50.9$

Replicate S.S. $= \frac{421}{10} - C = 1.6$

S.S.		d.f.	M.S.	V ratio	F value	P
Replicate	1.6	4	0.4	0.36	2.61	5%
Treatment	50.9	9	5.66	5.10	2.89	1%
Residual	40.03	36	1.11			
Total	92.53	49				

S.E. = 0.47

$S.E._d$ = 0.66

The replicate or block effect is not significant (P = 0.05) though the treatment effect is significant (P = 0.01).

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each replicate can thus be accepted.

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each treatment can thus be rejected.

THE NUMBER OF POISONED Deroceras reticulatum FOUND ON EACH PLOT DAY 15.

column / bait	a	b	c	d	e	total	mean
A	0	0	0	0	0	0	0.0
B	0	2	0	3	5	10	2.0
C	1	0	1	0	0	2	0.4
D	1	0	0	2.	4	7	1.4
E	0	0	1	2	2	5	1.0
F	1	6	1	0	0	8	1.6
G	1	0	0	2	4	7	1.4
H	0	1	0	1	0	2	0.4
J	0	0	0	2	0	2	0.4
K	0	0	0	1	0	1	0.2
total	3	9	3	11	12	38	0.76

Analysis of variance

Correction Factor, C $= \frac{1444}{50} = 28.88$

Total Sum of Squares $= 116 - C = 87.12$

Treatment S.S. $= \frac{252}{5} - C = 21.52$

Replicate S.S. $= \frac{364}{10} - C = 7.52$

S.S.		d.f.	M.S.	V ratio	F value	P
Replicate	7.52	4	1.88	1.17	2.61	5%
Treatment	21.52	9	2.39	1.48	2.12	5%
Residual	58.08	36	1.61			
Total	87.12	49				

S.E. = 0.57

$S.E._d = 0.81$

The replicate or block effect is not significant (P = 0.05) and the treatment effect is also not significant (P = 0.05).

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each replicate can thus be accepted.

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each treatment can thus be accepted.

THE NUMBER OF POISONED Deroceras reticulatum FOUND ON EACH PLOT DAY 16.

column / bait	a	b	c	d	e	total	mean
A	1	0	0	0	0	1	0.2
B	1	5	11	3	2	22	4.4
C	2	0	2	1	1	6	1.2
D	0	1	1	0	0	2	0.4
E	0	0	8	1	1	10	2.0
F	0	0	4	2	0	6	1.2
G	4	2	1	0	1	8	1.6
H	0	0	0	0	0	0	0.0
J	2	1	3	1	0	7	1.4
K	1	1	1	1	2	6	1.2
total	11	10	31	9	7	68	1.34

Analysis of variance

Correction Factor, C $= \frac{4624}{50} = 92.48$

Total Sum of Squares $= 304 - C = 211.52$

Treatment S.S. $= \frac{810}{5} - C = 69.52$

Replicate S.S. $= \frac{1312}{10} - C = 38.72$

S.S.		d.f.	M.S.	V ratio	F value	P
Replicate	38.72	4	9.68	3.21	2.61	5%
Treatment	69.52	9	7.72	2.56	2.12	5%
Residual	108.80	36	3.02			
Total	211.52	49				

S.E. = 0.78

$S.E._d$ = 1.10

The replicate or block effect is significant (P = 0.05) and the tretament effect is also significant (P = 0.05).

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each replicate can thus be rejected.

The Null Hypothesis that the number of Deroceras reticulatum poisoned is the same for each treatment can thus be rejected.

PROBABILITY OF SIMILARITY IN THE NUMBER OF POISONED SLUGS TRAPPED WITH EACH TREATMENT - Percentage similarity

day 8

BAIT	A	B	C	D	E	F	G	H	J	K
A										
B	I0-25									
C	+50	25-50								
D	25-50	+50	+50							
E	25-50	+50	+50	+50						
F	25-50	+50	+50	+50	+50					
G	50	25-50	+50	50	25-50	25-50				
H	+50	25-50	+50	+50	+50	25-50	25-50			
J	I0-25	I00	25-50	25-50	+50	+50	25-50	25-50		
K	2.5-5	25-50	5-I0	I0-25	I0-25	2.5-5	5-I0	5-I0	25-50	

day 9

BAIT	A	B	C	D	E	F	G	H	J	K
A										
B	I-2.5									
C	25-50	5-I0								
D	2.5	+50	I0-25							
E	2.5	+50	I0-25	I00						
F	25	5-I0	+50	I0-25	I0-25					
G	25	5-I0	+50	I0-25	I0-25	I00				
H	+50	2.5-5	25-50	2.5-5	2.5-5	25-50	25-50			
J	-0.5	I0-25	0.5-I	5-I0	5-I0	0.5-I	0.5-I	0.5		
K	2.5-5	I0-25	25-50	25-50	25-50	25-50	25-50	I0-25	2.5	

day I0

BAIT	A	B	C	D	E	F	G	H	J	K
A										
B	-0.5									
C	+50	-0.5								
D	+50	-0.5	+50							
E	I0	-0.5	I0-25	25-50						
F	5-I0	-0.5	I0-25	25	+50					
G	25-50	-0.5	+50	I00	25-50	25				
H	+50	-0.5	25-50	I0-25	5-I0	2.5-5	I0-25			
J	5-I0	-0.5	I0-25	25-50	+50	+50	I0-25	2.5-5		
K	I0-25	-0.5	25-50	+50	+50	25-50	+50	I0-25	+50	

day II

BAIT	A	B	C	D	E	F	G	H	J	K
A										
B	-0.5									
C	+50	-0.5								
D	25-50	-0.5	+50							
E	25-50	-0.5	+50	+50						
F	5-I0	-0.5	I0-25	25-50	25-50					
G	2.5	I-2.5	I0-25	25	I0-25	25-50				
H	+50	-0.5	25-50	25-50	I0-25	5-I0	I-2.5			
J	I0-25	-0.5	25-50	25-50	+50	+50	25-50	5-I0		
K	I0-25	-0.5	25-50	25-50	50	+50	25-50	25-50	5-I0	

day I2

BAIT	A	B	C	D	E	F	G	H	J	K
A										
B	-0.5									
C	+50	-0.5								
D	25-50	-0.5	25.50							
E	0.5-I	-0.5	I-2.5	5-I0						
F	2.5-5	-0.5	5-I0	I0-25	25-50					
G	5-I0	-0.5	I0-25	25-50	I0-25	+50				
H	+50	-0.5	+50	I0-25	I	2.5-5	5-I0			
J	I-2.5	0.5	2.5-5	I0-25	+50	+50	25-50	I-2.5		
K	I0-25	-0.5	I0-25	+50	I0-25	25-50	+50	I0	25	

day I3

BAIT	A	B	C	D	E	F	G	H	J	K
A										
B	5-I0									
C	25-50	25-50								
D	25-50	25	+50							
E	I0-25	+50	+50	25-50						
F	25-50	25-50	I00	+50	+50					
G	0.5-I	I0-25	2.5-5	2.5-5	5-I0	2.5-5				
H	I00	5-I0	25-50	25-50	I0-25	25-50	0.5-I			
J	25-50	25	+50	I00	25-50	+50	2.5-5	25-50		
K	+50	I0-25	+50	+50	25	+50	I-2.5	+50	+50	

day I4

BAIT	A	B	C	D	E	F	G	H	J	K
A										
B	I									
C	+50	I-2.5								
D	I00	I-2.5	+50							
E	25-50	2.5-5	+50	25-50						
F	I0-25	I0-25	I0-25	I0-25	25-50					
G	-0.5	25-50	-0.5	-0.5	0.5-I	2.5-5				
H	I00	I-2.5	+50	I00	25-50	I0-25	-0.5			
J	2.5-5	25-50	5-I0	2.5-5	I0-25	+50	5-I0	2.5-5		
K	+50	I-2.5	I00	+50	+50	I0-25	-0.5	+50	5-I0	

day I5

BAIT	A	B	C	D	E	F	G	H	J	K
A										
B	2.5-5									
C	+50	5-I0								
D	+50	5-I0	+50							
E	25	25	25-50	25-50						
F	5-I0	+50	I0-25	I0-25	25-50					
G	I0-25	25-50	25	I0-25	+50	+50				
H	+50	5-I0	I00	+50	25-50	I0-25	25			
J	+50	5-I0	I00	+50	25-50	I0-25	25	I00		
K	+50	5-I0	+50	I00	25-50	I0-25	I0-25	+50	+50	

day I6

BAIT	A	B	C	D	E	F	G	H	J	K
A										
B	-0.5									
C	25-50	I-2.5								
D	+50	0.5-I	25-50							
E	I0-25	5-I0	+50	I0-25						
F	25-50	I-2.5	25-50	25-50	25-50					
G	I0-25	2.5-5	+50	25-50	+50	+50				
H	I00	-0.5	I0-25	+50	I0-25	25-50	I0-25			
J	I0-25	2.5-5	+50	+50	25-50	+50	+50	I0-25		
K	25-50	I-2.5	I00	25-50	25-50	I00	+50	25-50	+50	

TOTAL NUMBER OF SLUGS - POISONED AND UNPOISONED - TRAPPED ON DAYS 8 - 16

Analysis of variance - Data from Table 34

Correction Factor, C = $\frac{10640644}{90}$ = 118229.37

Total Sum of Squares = 159132 - C = 40902.63

Treatment S.S. = $\frac{1117916}{9}$ - C = 5983.51

Day S.S. = $\frac{1424548}{10}$ - C = 24225.43

S.S.		d.f.	M.S.	V ratio	F value	P
Day	24225.43	8	3028.17	20.39	2.66	1%
Treatment	5983.51	9	664.83	4.48	2.56	1
Residual	10693.69	72	148.52			
Total	40902.63					

Both day and treatment effects are highly significant (P = 0.01).

The Null Hypothesis that the total number of slugs trapped is the same on each day and for each treatment oan thus be rejected.

TOTAL NUMBER OF POISONED Deroceras TRAPPED ON EACH PLOT IN THE NINE DAY POST BAITING PERIOD

Analysis of variance - Data from Table 35

Correction Factor, C = $\frac{1121481}{50}$ = 22429.62

Total Sum of Squares = 41017 - C = 18587.38

Treatment S.S. = $\frac{185357}{5}$ - C = 14641.78

Replicate S.S. = $\frac{227920}{10}$ - C = 362.38

S.S.		d.f.	M.S.	V ratio	F value	P
Replicate	362.38	4	90.60	0.91	2.61	5%
Treatment	14641.78	9	1626.86	16.35	2.89	1%
Residual	3583.22	36	99.53			
Total	18587.38	49				

S.E. = 4.46

$S.E._d$ = 6.29

The block or column differences are not significant (P = 0.05) though the treatment differences are highly significant (P = 0.01).

The Null Hypothesis that the randomisation of treatments within the columns or replicates ensures a uniform population is accepted.

NUMBER OF DEAD Deroceras TRAPPED ON DAYS 8 - 16 WITH EACH BAIT

Analysis of variance

Data from Table 36

Correction Factor, C $= \frac{1121481}{90} = 12460.9$

Total Sum of Squares $= 33539 - C = 21078.1$

Treatment S.S. $= \frac{185357}{9} - C = 8134.32$

Day S.S. $= \frac{171317}{10} - C = 4670.80$

S.S.		d.f.	M.S.	V ratio	F value	P
Day	4670.80	8	583.85	5.08	2.82	1%
Treatment	8134.32	9	903.80	7.87	2.72	1%
Residual	8272.98	72	114.90			
Total	21078.10	89				

S.E. = 3.57

$S.E._d = 5.04$

Both treatment and day effects are therefore highly significant (P = 0.01).

The Null Hypothesis that the number of Deroceras reticulatum found poisoned on each treatment is the same can thus be rejected.

NUMBER OF UNPOISONED Deroceras FOUND ON DAYS 8 - 16 WITH EACH BAIT

Analysis of variance - Data from Table 40

Correction Factor, C $= \frac{4452100}{90} = 49467.78$

Total Sum of Squares $= 73830 - C = 24362.22$

Treatment S.S. $= \frac{523220}{9} - C = 8667.78$

Day S.S. $= \frac{585550}{10} - C = 9087.22$

S.S.		d.f.	M.S.	V ratio	F value	P
Day	9087.22	8	1135.90	12.38	2.66	1%
Treatment	8667.78	9	963.09	10.50	2.56	1%
Residual	6607.22	72	91.76			
Total	24362.22	89				

S.E. = 3.19

$S.E._d = 4.50$

Both treatment and day effects are highly significant (P = 0.01).

The Null Hypothesis that the number of Deroeceras reticulatum found on each treatment is the same can thus be rejected.

SQUARE ROOT TRANSFORMATION OF DATA FOR NUMBERS OF POISONED *Deroceras reticulatum* TRAPPED ON DAYS 8 - 16. (see Table 36)

Since the trapping results were very variable, square root transformation of the data was carried out, to improve the uniformity of the data and ensure that it conformed to the mathematical model used for analysis of variance.

DAY / BAIT	8	9	10	11	12	13	14	15	16	Total	Mean
A	1.41	0.00	2.00	1.73	1.00	0.00	0.00	0.00	1.00	7.14	0.79
B	3.87	4.24	8.37	8.94	9.49	3.16	3.32	3.16	4.69	49.24	5.47
C	2.45	2.45	2.83	3.32	2.00	2.24	1.00	1.41	2.45	20.15	2.24
D	3.16	3.87	3.61	3.46	3.32	2.00	0.00	1.00	1.41	21.83	2.43
E	3.32	3.87	4.69	4.00	5.39	2.83	1.73	2.24	3.16	31.23	3.47
F	3.46	2.65	5.00	5.20	4.69	2.24	2.45	2.83	2.45	30.97	3.44
G	1.73	2.65	3.61	6.16	4.12	4.24	3.87	2.65	2.83	31.86	3.54
H	2.00	1.00	0.00	0.00	0.00	0.00	0.00	1.41	0.00	4.41	0.49
J	3.87	5.10	4.90	4.36	5.00	2.00	2.83	1.41	2.65	32.12	3.57
K	5.10	3.32	4.12	5.10	3.87	1.41	1.00	1.00	2.45	27.37	3.04
total	30.4	29.2	39.1	42.3	38.9	20.0	16.2	17.1	23.1	256.2	

Analysis of variance

Correction Factor, C $= \frac{65648.69}{90} = 729.43$

Total Sum of Squares $= 1059 - C = 329.57$

Treatment S.S. $= \frac{8107.9}{9} - C = 171.45$

Day S.S. $= \frac{8090.76}{10} - C = 79.65$

S.S.		d.f.	M.S.	V ratio	F value	P
Day	79.65	8	9.96	9.13	2.82	1%
Treatment	171.45	9	19.05	17.48	2.72	1%
Residual	78.47	72	1.09			
Total	329.57	89				

S.E. = 0.35

$S.E._d$ = 0.49

The daily differences are significant (P = 0.01) and the treatment differences are also significant.

The Null Hypothesis that the number of poisoned Deroceras trapped is the same on each day and on each treatment can thus be rejected.

RESIDUAL POPULATION ANALYSIS - DAYS 20 AND 21

Number of Deroceras reticulatum (adults and juveniles) trapped under straw traps. (combined data from Tables 46 & 47)

Analysis of variance

Correction Factor, C $= \frac{5216656}{50} = 104333.12$

Total Sum of Squares $= 137890 - C = 33556.88$

Treatment S.S. $= \frac{535150}{5} - C = 2696.88$

Replicate S.S. $= \frac{1242434}{10} - C = 19910.28$

S.S.		d.f.	M.S.	V ratio	F value	P
Replicate	19910.28	4	4977.57	16.36	3.83	1%
Treatment	2696.88	9	299.65	0.99	2.12	5%
Residual	10949.72	36	304.16			
Total	33556.88	49				

Number of Deroceras reticulatum (adults and juveniles) trapped under straw traps on day 21 only (columns 'b' - 'e' only).

Analysis of variance

Correction Factor, C $= \frac{2090916}{40} = 52272.9$

Total Sum of Squares $= 59878 - C = 9351.2$

Treatment S.S. $= \frac{214650}{4} - C = 1389.6$

Replicate S.S. $= \frac{540190}{10} - C = 1746.1$

S.S.		d.f.	M.S.	V ratio	F value	P
Replicate	1746.1	3	582.03	2.53	2.96	5%
Treatment	1389.6	9	154.40	0.67	0.67	5%
Residual	6215.5	27	230.20			
Total	9351.2	39				

RESIDUAL POPULATION ANALYSIS - TILE TRAPPING, DAY 22

Juvenile Deroceras reticulatum - see Table 48

Analysis of variance

Correction Factor, C = $\frac{107584}{50}$ = 2151.68

Total Sum of Squares = 2704 - C = 552.32

Treatment S.S. = $\frac{11064}{5}$ - C = 61.12

Replicate S.S. = $\frac{22116}{10}$ - C = 59.92

S.S.		d.f.	M.S.	V ratio	F value	P
Replicate	59.92	4	14.98	1.25	2.61	5%
Treatment	61.12	9	6.79	0.57	2.12	5%
Residual	431.28	36	11.98			
Total	552.32	49				

Adult Deroceras reticulatum - see Table 49

Analysis of variance

Correction Factor, C = $\frac{8100}{50}$ = 162.0

Total Sum of Squares = 290 - 162 = 128.0

Treatment S.S. = $\frac{972}{5}$ - C = 32.4

Replicate S.S. = $\frac{2044}{10}$ - C = 42.4

S.S.		d.f.	M.S.	V ratio	F value	P
Replicate	42.4	4	10.6	7.16	3.83	1%
Treatment	32.4	9	3.6	2.43	2.12	5%
Residual	53.2	36	1.48			
Total	128.0	49				

RESIDUAL POPULATION ANALYSIS - TILE TRAPPING, DAY 22

The number of adult Deroceras reticulatum trapped on plots baited with poison treatments only (baits B,C,D,E,F,G,J,K)

column bait	a	b	c	d	e	total
B	3	4	0	0	0	7
C	4	3	1	1	3	12
D	0	0	2	0	1	3
E	0	4	2	1	1	8
F	1	3	0	1	1	6
G	4	2	0	2	0	8
J	2	1	3	0	2	8
K	2	3	2	0	2	9
total	16	20	10	5	10	61

Analysis of variance

Correction Factor, C $= \frac{3721}{40} = 93.025$

Total Sum of Squares $= 163 - C = 69.975$

Replicate S.S. $= \frac{881}{8} - C = 17.10$

Treatment S.S. $= \frac{511}{5} - C = 9.175$

S.S.		d.f.	M.S.	V ratio	F value	P
Replicate	17.100	4	4.275	2.74	2.71	5%
Treatment	9.175	7	1.310	0.84	2.36	5%
Residual	47.700	28	1.560			
Total	69.975	39				

S.E. = 0.56

$S.E._d = 0.79$

The block or replicate effect is significant (P = 0.05) and the treatment effect is not significant. (P = 0.05).

The Null Hypothesis that the number of Deroceras reticulatum found in each column after baiting is the same can thus be rejected.

The Null Hypothesis that the number of Deroceras reticulatum found on each treatment plot after baiting is the same for each poison bait can thus be accepted.