ADAPTATIONS THAT PERMIT THE TERRESTRIAL SNAIL *Helicella virgata* (Da Costa) TO SURVIVE IN DRY PLACES

by

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DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma at any University, and, to the best of my knowledge contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

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SUMMARY

The terrestrial snail Helicella virgata (Da Costa) was introduced from Europe to South Australia where it has become established over a wide area. During the hot, dry summer in South Australia, snails have little opportunity of feeding or replenishing their supplies of water for a period of three to four months. This thesis contains the results of an investigation of the behavioural, structural and physiologic adaptations that enable H. virgata to survive in such dry places. The study was made during the years 1965 to 1968.

Field studies showed that with the onset of hot weather, snails moved from areas of short grass into places where they could climb off the surface of the ground. Favoured areas for climbing were seen to be those of long grass or plantations of trees. Having climbed from the ground the snails became dormant, remaining in this condition for considerable periods until aroused, usually by rain. Throughout the summer small scale movements were seen to occur, with snails moving a few inches up and down, or around an object on which they had climbed. It is suggested that these movements were made in response to changes in the microclimate surrounding particular snails. Snails that did not reach an area where they could climb had a greater probability of dying during the summer than those animals that did climb. At the end of summer the snail left the areas in which they had climbed and migrated away into the short grass where they fed and laid eggs.

Laboratory studies showed that water was lost very slowly from dormant snails. The shells were only very slightly permeable to water, and this waterproofing was found to be a property of the innermost layer of the shell. Much of the water that was lost from the dormant snail was lost through the aperture; the rate of water-loss per unit area of aperture was approximately 200 times as great as that from the shell. As with some other species of helicid snails that live in dry places, the area of the aperture in H. virgata appears to be small with respect to the total area of the surface.

The epiphragm was found to be relatively permeable to water (approximately 1000 times as permeable as the shell). The permeability of isolated epiphragms was found to be inversely proportional to their thickness. In still air, under conditions of extreme saturation deficit, snails with intact epiphragms lost less water than those from which epiphragms had been removed.

There was strong evidence to suggest that at least part of the living epidermis (the mantle-collar) was concerned with retarding the loss of water from the tissues of the snail.

No decrease in dry weight occurred in snails dormant at 20°C. Thus the loss in weight that was observed in living animals could be explained simply in terms of the weight of water lost. At 25°C it is possible that some slight decrease in dry weight took place, but the results were not statistically significant and it was concluded that the

changes in weight observed during dormancy in animals at 25°C were the result of water-loss. At 30°C, the loss in weight was caused partly by loss of water and partly by a decrease in the dry weight of the tissues of these snails.

Snails died sooner, and lost more water at 30°C than at 25°C or 20°C. However, just before death, the energy content per unit dry weight of the tissues was similar for snails at all three temperatures. The amount of energy expended during dormancy was similar regardless of temperature, but the rate of expenditure of energy was directly related to the temperature. This result indicated that the snails were dying of starvation and not desiccation.

Respiration during dormancy was found to be principally aerobic. Oxygen was taken in continuously at a low rate. The carbon dioxide produced from metabolism was bound within the snail at temperatures of 20°C and 25°C but at 30°C it is likely that some carbon dioxide was excreted. On arousal from protracted periods of dormancy, snails released a large volume of carbon dioxide. This release was usually observed just before the emergence of the snail from its shell.

The tissues of dormant snails contained approximately twice as much lactic acid per unit dry weight as those of active animals. Transport of calcium from the shell to the body was shown to occur in dormant snails. It is suggested that short periods of anaerobiosis may occur during dormancy, but it is likely that if such periods of

anaerobic metabolism occur they are of short duration.

The terminology used to describe dormant phases in the life-cycle of gastropods is discussed. It is concluded that this terminology is unsatisfactory and potentially misleading.

ACKNOWLEDGEMENTS

I should like to express my gratitude to my supervisor, Professor H. G. Andrewartha, for his continual advice and encouragement throughout the course of this study. I am also grateful to the Director of the South Australian Department of Agriculture for his permission to conduct the fieldwork at Northfield, and to Messrs. P. D. and P. G. Kempster who helped me fence and connect water and electricity to the site. Mr. S. H. Wheeler gave invaluable assistance in writing the computer programmes that were used to analyse the movements of snails. In the production of this thesis, I have been helped greatly by Miss S. Lawson, who typed the manuscript, and by my wife, Anne, who duplicated it.

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INTRODUCTION

Eight species of helicid snails have become established in South Australia during the last hundred years. Of these, six species are common (Helicella virgata, Helicella neglecta, Theba pisana, Helix aspersa, Cochlicella ventrosa and Cochlicella acuta) and two are rare (Helicella caperata and Helicella stolismena).

Of these snails, Helicella virgata is by far the most widespread. Its range extends over some 3,000 square miles of South Australia, and is centred on Adelaide (Pomeroy, 1966). This snail is most numerous in the agricultural areas of the state and relatively few are found in areas of natural vegetation. It has spread from the shipping ports along the railroads to the major regional railheads, and from these along roadsides and farm tracks. Thus the activities of man have been greatly responsible for the spread of Helicella virgata in South Australia (Pomeroy, 1966; Pomeroy and Laws, 1967).

The summer in South Australia is hot and dry. The hot weather may extend from October to March, after which the opening rains may be expected. The winters are generally mild, and it is during the period from late March to September that most of the rainfall occurs. During winter, snails are active on most nights and often during the days as well. During summer much less activity is observed and the snails become dormant for long periods during the hot weather.

Prior to the onset of dormancy, many snails climb on to vegetation,

fence posts and other upright objects. They may remain in such situations, exposed to sun and wind, until aroused from dormancy by rain. It is this aspect of the behaviour of Helicella virgata that causes it to become a problem in the cereal growing areas of South Australia. The densities encountered in such areas may become high in certain years, and when climbing takes place prior to harvesting it may be difficult to avoid including snails in the grain.

Helicella virgata has few predators in South Australia. Those that do exist probably take a trivial proportion of the snails within any given population. It seems likely that some control measures will have to be undertaken against the snail before long.

The ability of H. virgata to withstand the warm dry summer in South Australia attracted the attention of Pomeroy (1966), who undertook to study some of the more important aspects of the ecology of this snail.

Pomeroy concentrated on the problems of how long this animal could survive in warm dry places. He concluded that fundamental to its ability to live in such places "was its extraordinary impermeability, which, although remarked upon by previous workers, had never been measured critically, nor had its extent been generally acknowledged in the zoological literature".

Pomeroy's study contributed greatly to our understanding of the general ecology of Helicella virgata, and it seemed clear from his work that the most critical period in the life of a snail was that between

October and March when animals were exposed to hot weather and had very little opportunity of feeding or replenishing their water supplies. For this reason it seemed that a study of the snail during summer would be both interesting and profitable.

There have been few detailed studies of the ways in which gastropods survive under adverse conditions. There is considerable mention of the phenomena of hibernation and aestivation in the literature, but a great deal of this work, useful though it is, is neither quantitative nor experimental.

I decided to approach this study from three aspects, and the thesis falls into three major divisions as a result of this approach.

Section one considers the behaviour of H. virgata during spring and summer. Section two is concerned with the permeability of the dormant animals to water, and with the site and nature of the waterproofing mechanisms involved. Section three is devoted to a consideration of the physiological processes that take place within the dormant snail.

I have approached this study with the view to understanding the broad patterns of behaviour, structural adaptations and physiological mechanisms that enable Helicella virgata to survive during the summer. So I have not dwelt on details of these processes.

SECTION 1. THE BEHAVIOUR OF SNAILS IN NATURE DURING THE SUMMER

1.0 INTRODUCTION

With the exception of Mead's (1961) study of the giant African snail Achatina fulica there have been relatively few detailed ecological studies undertaken for single species of terrestrial gastropods.

Pomeroy (1966) undertook a study of the ecology of Helicella virgata, a snail that was introduced from Europe to South Australia in the latter part of the nineteenth century. In his study he placed particular emphasis on the problem of how long this snail could survive in the warm, dry conditions experienced during summer.

During the summer months many of these snails are to be seen clustering around the tops of fence-posts, on power poles and on taller stands of dried grass. At this time many of the snails are inactive and in the condition frequently referred to as "aestivation" although "dormancy" is probably a better term for this phenomenon.

Observations on a number of terrestrial gastropods have shown that the habit of climbing on vegetation to spend the hotter part of the year is relatively common. In South Australia such snails include Helicella virgata, Helicella neglecta and Theba pisana, all introduced species. In Europe this climbing habit is shown by Helicella itala and Cepaea hortensis, and in central America by Oxystyla undata. Morton (1958) has reported that, in Africa, Helix (= Otala) lactea and Helix (= Eremina) desertorum congregate in thousands on dry scrub in mid-day temperatures

as high as 43°C, becoming active after rain and emerging "in marauding swarms".

Pomeroy (1966, 1968) made a general study of the places in which Helicella virgata spent the summer and concluded that their behaviour, which he suggested presumably evolved in their native European range was less appropriate in South Australia. Despite this he found that high densities of snails occurred in some areas of the state.

In this present study it seemed wise to attempt an intensive study of certain aspects of the behaviour of snails on a small area, rather than to continue to study them in general over large areas of their range. In this way it was hoped to gain further insight into those aspects of the ecology of Helicella virgata that enable the animals to survive during the hot, dry summer in South Australia.

1.1 DESCRIPTION OF THE STUDY AREA AT NORTHFIELD

A study area was established on farming land belonging to the South Australian Department of Agriculture at Northfield, approximately 7 miles north-east of the city of Adelaide. This area supported a permanent population of snails and offered the advantages of mains water and electrical supply. A resident caretaker ensured that no interference took place in the study area, and equipment could thus safely be left at the site.

An area of grazing land, approximately 20 yards by 60 yards, immediately adjacent to the laboratories block was fenced in September 1966

to exclude cattle that were present initially on the pasture. Water and electricity were connected to this area.

With the exclusion of the cattle the vegetation, consisting of clover and annual grasses, grew rapidly. On 30.9.66 rather more than half of the area (see Fig. 1.01) was closely mown with a rotary lawn-mower. There was little subsequent growth in these areas of mown grass, and they remained very obviously different from the long, unmown grass even up to March 1968 when the field work was terminated.

Following the mowing, ten snail pens were constructed within the fenced area in the positions indicated in Figure 1.01. The method of constructing these cages is discussed in Appendix 1 (Section 4.1).

Daily records of humidity and temperature were kept after 29.11.66 with the aid of a thermohygrograph contained in a Stevenson Screen. The screen was supported at six inches above ground level.

Daily rainfall records were kept after 29.11.66. These were obtained from a rain gauge that recorded on a thermohygrograph drum, thus enabling the time, duration and amount of rainfall to be determined.

The field area was visited at approximately weekly intervals until 6.12.66. After this, and until May 1967, visits were usually made on two consecutive days separated by an interval of 6 days before the next two visits. During winter and spring, from June until October 1967 visits were made at monthly intervals. After the beginning of October until the end of field work in April 1968 visits were again made at approximately weekly intervals. At each visit wind direction was noted from the vane

above the laboratories.

During the field work the character of the surrounding paddock was changed. From pasture in 1966, the land was ploughed and sown to crop in 1967. Following this the cattle were reintroduced to graze on stubble and the land was not further ploughed before the field work was concluded at the beginning of April 1968.

1.2 THE DISTRIBUTION AND ABUNDANCE OF SNAILS ON THE STUDY AREA

Changes in the distribution and abundance of snails on the study area were followed from the end of September 1966 until early April 1968.

The abundance of snails was determined from randomly chosen quadrats In September 1966, numbered pegs were placed around the fence-line and across the study area in the manner shown in Figure 1.01. These pegs were spaced at three foot intervals.

For the initial determination of abundance on 30.9.66 pairs of random numbers were taken and the position of each quadrat determined as follows. From the first number of any pair of random numbers a peg on the east-west fence line was selected, and from the second a peg, on the north-south fence. The intercept of the projections from these pegs at right angles to the respective fences was taken as the point within the study area at which the centre of the quadrat was positioned.

A length of heavy gauge fencing wire was bent to form a square with sides of one meter. This was placed on the ground at the position indicate

from the appropriate pegs and at the corners small wire stakes were pushed into the ground. All living snails within this quadrat were counted but not disturbed, as it was felt that any disturbance might possibly increase the chance that a snail would move and that this might influence the subsequent survival of that snail. It is possible that some errors may have been introduced from this restriction as snails that had recently died might have been counted as living animals. In general, however, it is thought that if such errors occurred they were small.

Twenty such quadrats were taken on the study area. If any quadrat fell in such a position as to overlap the wire pegs of another, marked out on the same day, it was discarded and a further quadrat taken to replace it. This happened infrequently.

When all the quadrats had been sampled on 30.9.66 the wire pegs were removed from the area and approximately half of the area was mown to a height of approximately two inches with a rotary lawn-mower. It was intended that exactly half of the study area should have been mown, but an error in measurement along one fence line resulted in the mown area beir greater than the unmown.

For all subsequent determinations of abundance the two areas were examined as above, with ten quadrats being taken in the mown area and ten in the unmown area. These areas will be referred to as the short grass and long grass areas respectively for the remainder of this thesis.

When sampling was completed at the end of each visit the wire pegs,

FIGURE 1.01. Plan of the study area at Northfield.

Legend.

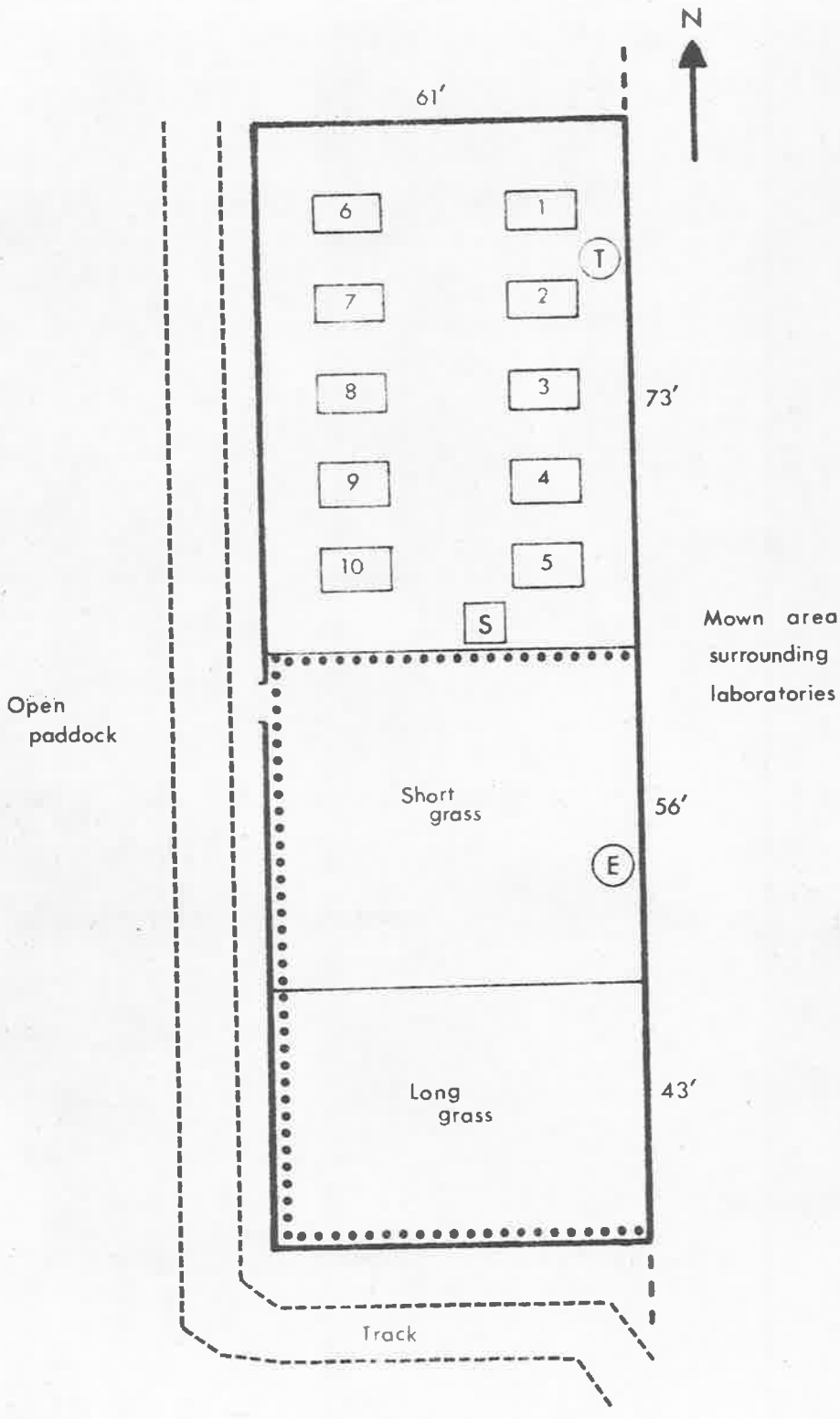
1 to 10 Snail pens

E Electrical supply point

S Stevenson screen and rain gauge

T Water supply

The pegs used to determine the positions of the quadrats are shown as dots in this diagram.


N
61′
6
1
T
7
2
8
3
73′
9
4
10
5
S
Mown area surrounding laboratories
Open paddock
Short grass
56′
E
Long grass
43′
Track

used to mark the quadrat boundaries, were removed.

At approximately monthly intervals five quadrats were marked out in each area for determinations of the number of snails that had died there. These quadrats were usually left for a month after being marked out. At the time of marking out all dead snails were removed from the body of the quadrat. When the count was made any dead snail found in a quadrat was presumed to have died there in the interval between marking out and counting.

The area of the short grass was 317 square meters, and that of the long grass 244 square meters, so that at each visit approximately 3.15 per cent of the short grass and 4.09 per cent of the long grass was examined for living snails.

In both areas, great care was taken to preserve the habitat. This was especially necessary in the long grass area after the grass had dried off. No excursions were made into either area unless these were absolutely necessary, and all general inspections of the areas were made from outside the fences.

1.21 The abundance of live snails in the long and short grass

The abundance of living snails in the two areas at each determination was expressed as the mean number of snails per square meter. The ninety five percent confidence limits were ascribed to each mean using the t-distribution. The means, together with their respective confidence limits, are plotted for both areas over the whole period of the investigation in the folding Figure 1.02 at the end of this thesis. A clear pattern

emerged with respect to the numbers of snails in each area in two consecutive years and it is proposed to discuss this briefly.

Initially, at the end of September 1966, the two areas were similar with respect to ground cover and snail densities. Following the mowing about half the snails in the mown area were killed and the density fell from about 30 to 15 snails per square meter.

During October and November, snails were active and moving overnight The numbers in the long grass increased only slightly during this period to about 36 snails per square meter, but in the short grass there was a greater increase with the density returning to about 32 snails per square meter at the end of November. These increases resulted from the movement of snails from the grazed pasture. There seemed to be a general tendency for the snails to move on to the mown area and to remain there. It is thought that this may reflect the fact that there was a large amount of decaying vegetation on this area as a result of the mowing. Butler (pers. com.) has suggested that the principal source of food for Helicella virgata may be associated with decaying vegetation.

The hot weather began in early December in 1966. During December there was a great increase in the number of snails present in the long grass, and by the end of that month the density had reached approximately 100 snails per square meter. During the same period there was a marked decline in the numbers of snails in the short grass, the density falling tc approximately 5 snails per square meter at the end of December.

Field observations indicated that individual snails on short grass tended to move greater distances overnight during the early part of December than they had previously. Snails on long grass tended not to move once they had climbed some distance from the ground. There was thus a steady increase in the numbers present on the long grass due to immigration from the surrounding short grass and grazed pasture.

Large scale horizontal movements of snails appeared to cease towards the end of December 1966. By this time many snails had died in the grazed pasture and relatively few living snails remained in the short grass. Snails in the long grass showed little lateral movement but some vertical movement occurred on the grass stems, with snails generally climbing upwards. The general impression was gained that the mean height above ground for the population of snails in the long grass was at a maximum during late January and early February. This impression was confirmed in a separate study of climbing (see Section 1.36).

The numbers in the short grass continued to fall until mid February, largely as a result of deaths. After this time it was difficult to find living snails in either the short grass or pasture areas.

Following rain at the end of February many snails emerged from the long grass and moved back into the short grass and paddock. The numbers in the long grass declined steadily but slowly during the period March to May 1967 and there was a similar steady but slow increase in the numbers of snails on the short grass areas.

The winter of 1967 was a particularly dry one, leading to widespread drought over a large portion of Australia. Breeding took place at Northfield during June and July. In both the long grass and short grass areas there was an increase in the numbers of snails, due almost entirely to the appearance of young animals. The numbers obtained in both areas were maximal in August with densities of approximately 30 and 120 snails per square meter in the long and short grass respectively. This result suggested that relatively few of the snails bred in the long grass. This observation was thought to be correct in spite of the difficulty with which the small snails could be seen in the long grass.

After August 1967, the numbers declined in both areas due to the death of young snails. There was little growth of the surviving young and of those adults that survived from the previous year. It seems probable that 1967 was quite atypical since there was so little rainfall during winter.

During September (spring in South Australia) the numbers remained fairly constant on the two areas, however with the onset of hot weather in October the numbers of snails in the long grass again increased. This increase resulted from an influx of adult snails from surrounding areas of shorter grass. The numbers declined rapidly in the short grass due partly to emigration of large snails and death of the young. The young snails appeared to lack the necessary powers of dispersal to enable them to leave the short grass areas. It is probable that in a normal winter earlier

breeding together with a rapid rate of growth during winter and spring would lead to the young of the year being very much larger, with much greater powers of dispersal at the onset of hot weather.

The summer of 1967 was also atypical in that no rain was recorded on the study area for a period of approximately two months. During this period from 13.10.67 to 11.12.67, there was considerable mortality amongst small snails on the short grass. Large snails continued to move slowly out of these areas, but this movement was gradual unlike that in October.

In the long grass a great increase in the numbers of snails took place, with most of the snails being present around the margins of the area This heterogeneous distribution of snails is reflected in Figure 1.02 by th wide confidence limits.

Following rain in December many snails emerged from the long grass. In general they did not move far out on to the short grass areas but remained around the edges of the long grass. This emergence was unlike that observed in the previous year. In 1966-67 the hottest part of the summer was over by the time the snails emerged; but in 1967-68 the emergence occurred prior to the onset of the hottest part of the summer, and most of the snails in the short grass area died during the latter part of November and December. So very few living snails could be found in the short grass after mid-December until about mid-February.

A very heavy rainstorm in January 1968 confused the records thereafter. The rain was responsible for the destruction of much of the

habitat. Water built up behind an earth bank along the eastern fence of the study area. This bank finally broke and water flooded over the area, flattening much of the tall grass.

Following this deluge few snails could be seen on the study area, but by the time of the following visit, 5 days later, snails were again apparent in the flattened long grass.

The numbers present on this flattened grass fluctuated about a mean of some 15 snails per square meter until the end of February 1968, when they declined and few snails could then be found in the area.

Towards the end of February an increase in the numbers of snails took place on the areas of short grass, reaching the surprisingly high density of some 30 snails to the square meter by late March. It is far from clear where these snails came from as there had been very few snails in the surrounding areas prior to this time. One possible explanation is that the increase in numbers was a result of the emergence of snails tha had burrowed underground earlier in the summer, and this suggestion will be considered again in Section 1.5.

1.22 Mortality amongst snails in the long and short grass

The number of snails that died in each area was determined from randomly placed quadrats in the manner previously described in Section 1.2. The method was similar to that used by Pomeroy (1966) in that it depended upon the removal of all dead snails in the quadrats at the time when they were marked out. When the time came to examine these quadrats, any

dead snails were assumed to have died in the interval.

The results are shown in Table 1.1 where the mean number of living snails found at the beginning and end of each period is given together with the mean number that died during the period. These numbers refer to one square meter of area.

It is difficult to make meaningful statements about mortality in a situation like that at Northfield. The reason for this is that while estimates may reasonably be made of the number of snails dying per unit area of surface in a given time, it is extremely difficult to assess the numbers of snails that were present on the surface for the whole period over which the determination was made. Such situations are clearly complicated when large scale migration of animals is taking place across an area.

To consider an actual example, in the period between 6.12.66 and 19.1.67 many snails were moving from the pasture and short grass. Initially the mean density of living snails was estimated to be approximately 31.9 animals per square meter on the short grass, but by the end this had decreased to approximately 7.1. In this period it was estimated that 44.2 snails had died per square meter, so that more snails had died on the area than were present initially.

Field observations clearly indicated that this result was obtained as a result of migration. Snails were crawling into and through the area from the surrounding paddock, some died on the way and others moved on. This lead to a build-up of dead animals in the short grass.

TABLE 1.1

The mean number of snails, living and dead, in the two grass areas at Northfield. (The numbers refer to one square meter of area.)

Period	SHORT GRASS			LONG GRASS		
	Initial No. alive	Final No. alive	No. dead	Initial No. alive	Final No. alive	No. dead
3.10.66) 22.10.66)	16.0	19.2	3.0	30.5	32.3	2.6
22.10.66) 6.12.66)	19.2	31.9	1.4	32.3	36.7	2.6
6.12.66) 19. 1.67)	31.9	7.1	44.2	36.7	103.6	10.4
19. 1.67) 23. 2.67)	7.1	0.5	8.0	103.6	89.9	28.2
23. 3.67) 29. 3.67)	0.5	17.9	0.6	89.9	26.6	7.6
29. 3.67) 27. 4.67)	17.9	26.4	0.2	26.6	12.7	1.0
27. 4.67) 31. 5.67)	26.4	19.8	0.0	12.7	10.7	0.4
31. 5.67) 10. 7.67)	19.8	70.4	0.8	10.7	20.3	0.2
10. 7.67) 10. 8.67)	70.4	120.2	1.2	20.3	29.8	0.6
10. 8.67) 10. 9.67)	120.2	76.1	many young	29.8	14.5	some young
10. 9.67) 2.10.67)	76.1	70.6	many young	14.5	11.7	some young
2.10.67) 18.10.67)	70.6	26.9	17.6	11.7	49.0	6.8
18.10.67) 1.11.67)	26.9	18.7	10.4	49.0	87.6	15.4
1.11.67) 29.11.67)	18.7	11.9	14.4	87.6	83.0	7.2
29.11.67) 28.12.67)	11.9	1.4	17.6	83.0	31.8	14.4
28.12.67) 24. 1.68)	1.4	0.6	4.6	31.8	18.8	6.8
24. 1.68) 26. 2.68)	0.6	4.0	0.4	18.8	26.7	8.6
26. 2.68) 27. 3.68)	4.0	39.4	0.4	26.7	7.2	2.6

In order that some relative estimate of mortality could be made the following procedure was adopted. The number of snails that were present on an area during any one period was taken to be the average of the number of living snails present plus the number that had died. Thus percentage mortality was derived from

$$\frac{100 \times \text{No. dead}}{\frac{(\text{initial no. alive} + \text{final no. alive})}{2} + \text{no. dead}}$$

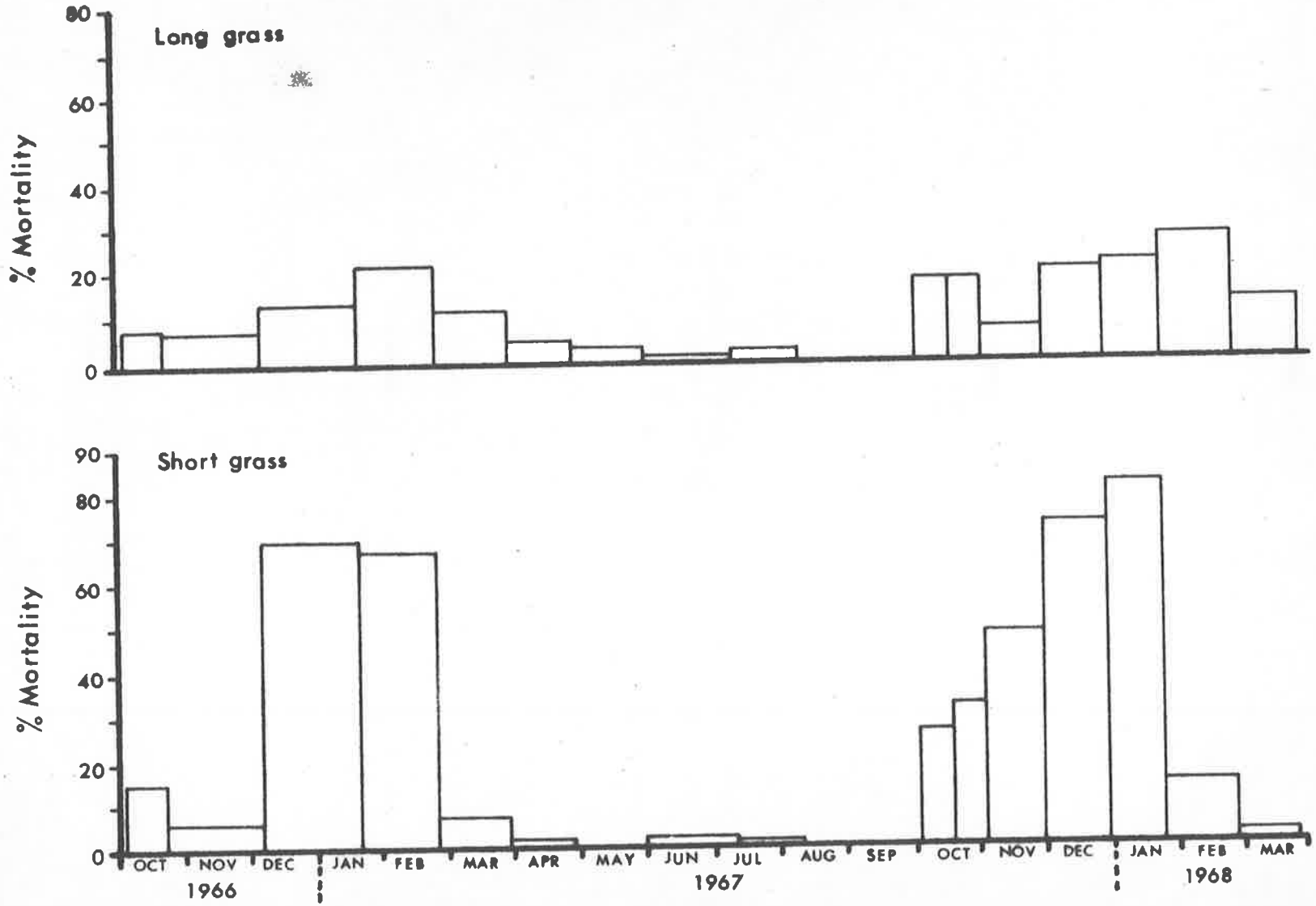
Thus in the example quoted above the percentage mortality was taken to be

$$\frac{100 \ (44.2)}{\frac{(31.9 + 7.1)}{2} + 44.2} \qquad \text{or} \quad 69.4\%$$

In spite of the obvious limitations of this method it was felt that it would allow a general comparison of mortality to be made for snails in the two areas of grass. The result of the calculations of the percentage mortality of snails in the two areas is shown in Figure 1.03.

It seems probable that the actual values of percentage mortality in Figure 1.03 should be viewed with caution. Perhaps the only valid conclusion that can be drawn from these results is that snails on areas of short grass have approximately three times as great a chance of dying during summer as those on long grass.

FIGURE 1.03. The percentage mortality amongst snails on the long and short grass.


Long grass
% Mortality
80
60
40
20
0
Short grass
% Mortality
90
80
60
40
20
0
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
1966
1967
1968

1.3 THE CLIMBING OF SNAILS

1.31 General remarks on climbing in H. virgata

The snails may be found climbed on trees, fence posts, power poles, buildings and almost any vertical surface. It is this aspect of their behaviour which causes them to become something of a problem in the wheat and barley growing areas of the state. In some years high densities of snails may occur in particular regions. If climbing occurs before the crop is harvested many snails may be harvested along with the grain. If snail densities are high this poses a considerable problem for the farmer as the inclusion of snails in the grain may raise the moisture content to a point where the bulk silos will not accept the grain for storage. Farmers in these areas may then resort to harvesting at night, and in those regions where this is done early in the period when snails are climbing it may be effective. In other areas where this is not possible the wheat farmers have found that a horizontal bar placed ahead of the harvester will knock many snails off the ears without damaging the crop, however this method cannot be used with barley (Birks pers. com.).

It is thought that climbing has been important in the spread of Helicella virgata in South Australia. Pomeroy and Laws (1967) found that the snails were carried along railways and roads, presumably by agricultural traffic. It is easy to see how this may come about. During the late spring and early summer, when snails were moving in the early mornings, many animals climbed on to the wheels and tyres of the motor vehicle used

to visit the Northfield site. Some snails would even climb on to the suspension and chassis members. Pomeroy (1966) records that a snail became attached to one wheel of his vehicle and remained there after a journey of some 30 miles at quite high speeds.

From the results of Section 1.22 it is clear that the probability that a snail will die during summer is greatly increased if that snail does not reach an area in which it can climb. Thus a place on which to climb constitutes an important component of the environment in any area which supports a permanent population of Helicella virgata, and it seemed of importance to investigate climbing and the behaviour of snails that had climbed.

1.32 Climbing and behaviour of snails on poles

An experiment was performed at Northfield to investigate the climbing and subsequent behaviour of Helicella virgata on wooden poles.

The poles were made from six foot jarrah garden stakes, they were square in section with sides of approximately one inch. Six heights of pole were used in this experiment, the heights were six inches, one foot, two feet, three feet, four feet and five feet above the surface of the ground. On each pole lines were drawn in paint at six inch intervals down from the top to the ground level. Six poles of each height were used.

The poles were placed into pen 6 at Northfield on 29.11.66 after the grass in the pen had been cut to about two inches in height with garden shears. The poles were driven in along four lines, each one foot apart,

such that there were nine poles in each line. Every pole on a line was one foot distant from its neighbours. The outer poles were one foot from the edge of the pen.

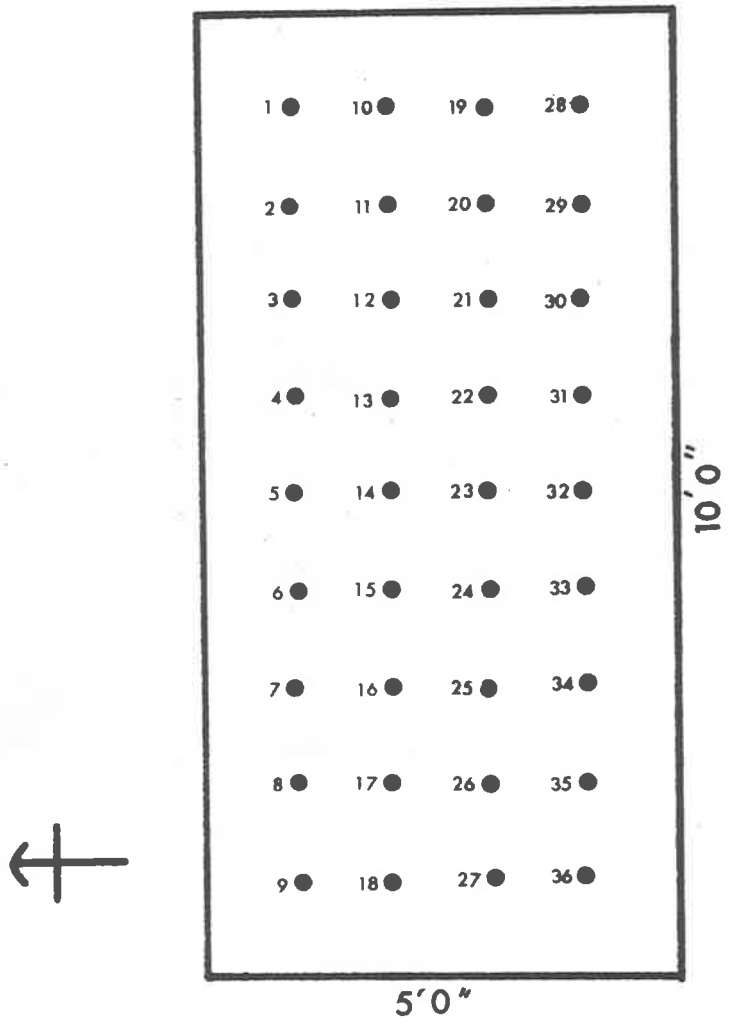
The position of the individual poles along the lines was determined from a table of random numbers, so that the poles were randomly placed with respect to height. The flat edges of the poles were orientated to face north, south, east and west. The arrangement of the poles is shown in Figure 1.04 and the height of each pole is given in Table 1.2.

TABLE 1.2

The height of each pole in Pen 6

Pole No.	Height (ft)	Pole No.	Height (ft)	Pole No.	Height (ft)
1	5.0	13	4.0	25	5.0
2	0.5	14	0.5	26	3.0
3	2.0	15	4.0	27	4.0
4	1.0	16	4.0	28	5.0
5	0.5	17	0.5	29	5.0
6	2.0	18	1.0	30	3.0
7	1.0	19	2.0	31	0.5
8	3.0	20	0.5	32	5.0
9	1.0	21	1.0	33	3.0
10	3.0	22	4.0	34	1.0
11	3.0	23	5.0	35	4.0
12	2.0	24	2.0	36	2.0

FIGURE 1.04. The arrangement and numbering of the poles in pen 6.



Two hundred and fifty individually marked snails were released into pen 6 following rain on 2.12.66.

The method of marking individuals consisted of painting a coloured band on the shell with a chloroform solution of "Marktex" marking pen ink. This dried rapidly and possessed good adhesive properties. So that individuals could be recognised, a number was placed on the shell, using black waterproof drawing ink, with a "Rapidograph" drawing pen. When this ink had dried the number was coated with a layer of clear nail varnish to protect it. This marking was most successful, it was still possible to read the numbers on many of the snails a year later.

Following the introduction of snails to the pen, the area was visited twice a week until May 1967. Visits were made on two consecutive days followed by an interval of six days before the next visits. In this way it was possible to obtain records of changes in the distribution of snails overnight for one night a week.

At each visit each pole was examined in turn for the presence of marked animals. When a marked snail was found its number was recorded together with the number of the pole, the height at which the snail was found and the face (i.e. north, south etc.) on which it occurred.

The height of a snail was measured in terms of "zones". Each pole was divided up into zones of six inches, from ground level to the top of the pole. Thus a snail that was between two feet six and three feet from the surface of the ground was in zone six. If any snail was found to be

present on the flat top of any pole, then its zone was taken to be that one immediately below, and instead of the "face" being a point of the compass it was called "top".

Fifty visits were made between 6.12.66 and 9.7.67. No snails climbed before 6.12.66 and none climbed after 22.5.67. In all 2,200 recaptures were made and the records were transferred to computer cards, one card for each recapture, for analysis on the CDC 6400 computer at the University of Adelaide.

1.33 The dates on which visits were made to record climbing

In order to simplify the analysis of these records and the production of tables in this section the numbers of snails present are given by visits, and comparisons have been made between visits rather than by actual dates. The relationship between the visit number and the date of that visit is presented in Table 1.3.

TABLE 1.3

The relationship between visit number and date of visit

Visit No.	Date	Visit No.	Date
1	6.12.66	26	2.3.67
2	8.12.66	27	8.3.67
3	14.12.66	28	9.3.67
4	15.12.66	29	15.3.67
5	21.12.66	30	16.3.67
6	22.12.66	31	22.3.67
7	28.12.66	32	23.3.67
8	4. 1.67	33	29.3.67
9	5. 1.67	34	30.3.67
10	11. 1.67	35	5.4.67
11	12. 1.67	36	6.4.67
12	18. 1.67	37	12.4.67
13	19. 1.67	38	13.4.67
14	25. 1.67	39	19.4.67
15	26. 1.67	40	20.4.67
16	1. 2.67	41	26.4.67
17	2. 2.67	42	27.4.67
18	8. 2.67	43	5.5.67
19	9. 2.67	44	10.5.67
20	15. 2.67	45	11.5.67
21	16. 2.67	46	17.5.67
22	22. 2.67	47	22.5.67
23	23. 2.67	48	22.5.67
24	24. 2.67	49	31.5.67
25	1. 3.67	50	9.6.67

1.34 The numbers of snails on poles

The numbers of marked snails present on the poles is shown together with the number of snails that appeared for the first time on poles in Table 1.4.

TABLE 1.4

Total number of snails and the number first appearing on poles at each visit

Visit No.	New Snails	Total Snails	Visit No.	New Snails	Total Snails
1	4	4	26	1	33
2	22	23	27	4	74
3	3	3	28	0	75
4	0	1	29	1	71
5	32	38	30	0	67
6	23	55	31	0	3
7	25	49	32	4	28
8	28	68	33	6	57
9	2	63	34	0	57
10	7	63	35	0	0
11	2	64	36	0	15
12	9	56	37	2	27
13	0	54	38	0	25
14	27	114	39	3	47
15	3	121	40	0	50
16	1	120	41	3	18
17	0	124	42	0	21
18	1	101	43	1	24
19	0	99	44	1	6
20	2	86	45	0	6
21	1	90	46	0	5
22	1	88	47	0	1
23	0	3	48	0	0
24	0	0	49	0	0
25	0	3	50	0	0

In the long grass area at Northfield, snails were climbing from early December 1966. The maximum density was achieved during January and early February. After a fall of ninety points of rain on 23.2.67, snails began to move out of the long grass.

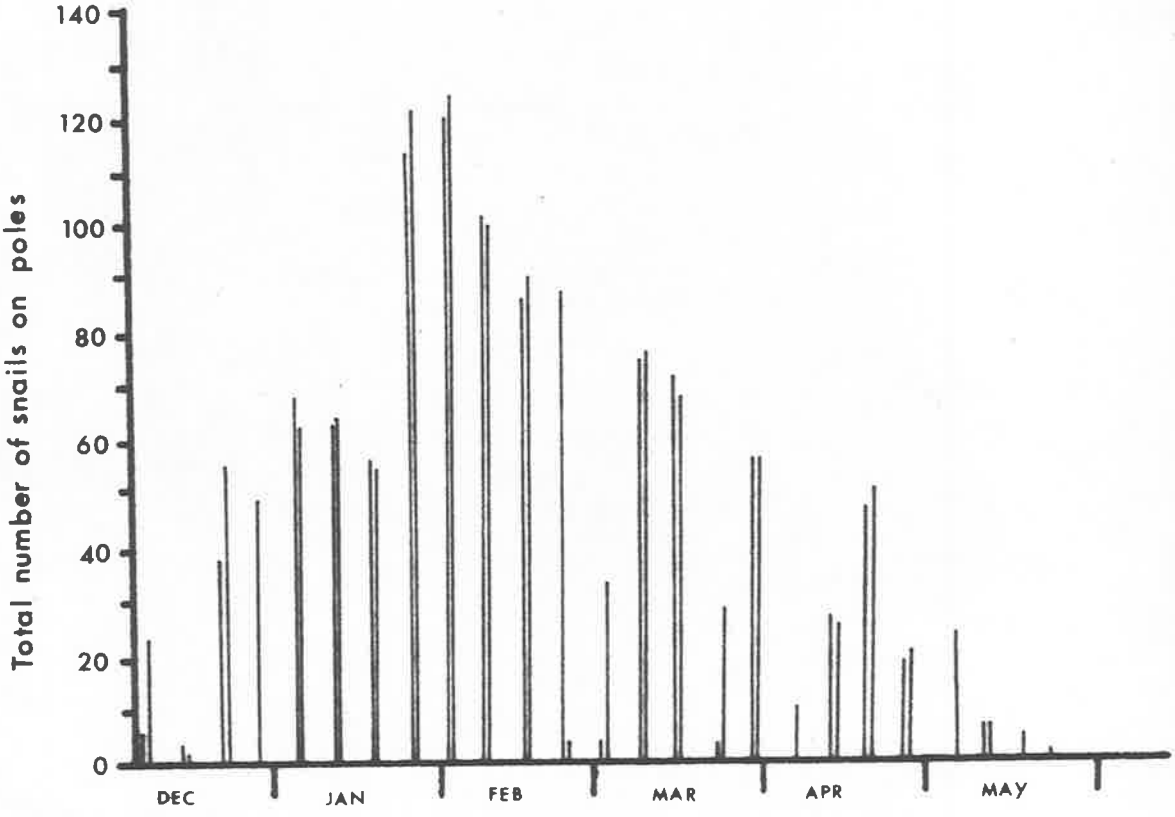
During the same period the number of snails on the poles was also at a maximum. From Table 1.4, the total number of snails appearing for the first time on poles was 193 up until visit 22 on 22.2.67. As 250 snails had been released into pen 6, this figure represents 77.2 per cent of the number released initially.

That some snails had survived the hot weather without having climbed poles is shown from the fact that after the twenty-second visit an additional 26 animals appeared for the first time on poles. It is thought that during the earlier period many of these snails climbed the walls of the pen, and that few actually remained on the short grass beneath the poles.

The total number of new snails that appeared on poles for the whole period of climbing was 219. This figure shows that 87.6 per cent of the marked snails, released in the pen on 2.12.66, had climbed a pole by the time that all climbing was finished in late May 1967.

The total number of snails present on poles at each visit is shown by date, for the whole period of climbing, in Figure 1.05. It is clear that the snails were most abundant on the poles during late January and early February.

FIGURE 1.05. The total number of snails climbed on poles at each visit (from December 1966 to June 1967).



1.35 Do snails tend to climb tall objects rather than short ones?

That such a large proportion of the total number of snails climbed poles in pen 6 suggested that one of two factors was operating to bring this about. Firstly, it could be that snails were able to perceive the poles and that they sought these out to climb on. Secondly, that a snail that had climbed a pole had a lessened probability of arousal, thus snails remaining in the grass would crawl more often and thus be likely to find a pole and climb it, while those on the pole remained dormant. Such a situation could be thought of as an orthokinesis, and would lead to a gradual accumulation whereby most of the snails were on poles and few remained in the grass.

Field observations suggested that both of these factors were probably operating. On several occasions when snails were observed to be active in the early mornings, it was seen that many snails would travel directly towards a pole. These snails crawled on the ground between the grass stalks until they reached the base of a pole, which they then climbed. There was no evidence that snails were moving along a temperature gradient at these times, nor did they appear to move down the shadowline cast by a pole on the ground. It seemed as though the snails had seen a pole and that their movements towards it were directed visually. The distance over which snails were observed to move in this fashion was rarely more than some six inches, and it seemed probable that for distances of six inches and less the snails were able to perceive the presence of a pole.

In order to determine whether more snails were found to have climbed on tall poles than short poles, the records were examined for each occasion in which a snail was found to have climbed a pole, regardless of whether this snail had previously been found on another pole. The numbers of climbs that were found to have taken place on each different height of pole was recorded for each interval between visits to Northfield. In all, 648 climbs were found to have taken place between 6.12.66 and 22.5.67.

The nature of the data derived from the inspection of snails on the poles at Northfield is such that no statistical tests can be made of the differences between the means of sets of observations. The conditions of randomness, normality and independence cannot be established with any degree of certainty. Nevertheless, certain comparisons are possible which enable the overall trends within these data to be expressed.

The number of climbs recorded for each of the six heights of poles is shown in Table 1.5, together with the percentage of the total number of climbs that were recorded on each size of pole for the period 6.12.66 to 22.5.67.

TABLE 1.5

The number and percentage of climbs on poles of different height from 6.12.66 to 22.5.67

Height of pole (ft.)	0.5	1	2	3	4	5	TOTAL
No. of climbs	41	132	109	117	144	105	648
% of total no. of climbs	6.33	20.37	16.82	18.06	22.22	16.20	

For the period from 6.12.66 to 22.2.67, when snails were present in the long grass at Northfield, a total of 336 climbs was recorded. The results for the numbers of snails found climbed and the percentage found climbed on each size of pole for this period are shown in Table 1.6.

TABLE 1.6

The number and percentage of climbs on poles of different height from 6.12.66 to 22.2.67

Height of pole (ft.)	0.5	1	2	3	4	5	TOTAL
No. of climbs	14	69	62	57	76	58	336
% of total no. of climbs	4.17	20.54	18.45	16.96	22.62	17.26	

The results for both the overall period of climbing, and the period during which snails were in the long grass, show that fewer snails were found to have climbed on the six inch poles than on any other size of pole. It appeared as though similar numbers were found to have climbed on all sizes of poles of one foot and over.

These results do not permit a distinction to be made between the suggestion that this phenomenon was the result of an orthokinesis on the one hand, or an active discrimination against short poles on the other. The fact that more snails were found to have climbed on the taller poles could have come about if approximately equal numbers of snails had climbed all the sizes of poles at the beginning of a period between visits.

The results presented in Section 1.37 indicated that snails on objects close to the ground tended to move away from that place more often than snails that were further from the ground, thus if a greater proportion of snails moved away from small poles and climbed again before the next visit then clearly the chance of finding snails climbed on tall poles rather than short poles is increased.

The number of climbs recorded is thus a minimum value and does not allow speculation on the actual number of climbs that took place between the times that visits to Northfield were made.

The study area was visited twice a week for the purpose of recording the positions of snails on the poles. These visits were made on two consecutive days followed by an interval of six days before the next two visits. Thus for each week data were collected for two periods, one of six days and the other that spanned only one night.

Field observations indicated that in the absence of rain, climbing and movement of snails on to objects from the ground was restricted to the early mornings. Movements from objects to the ground generally took place during the late evening and night. Thus it seems possible that if an orthokinesis was operating, it would have been unlikely to affect the number of climbs that were recorded on poles of various sizes from one day's visit to that of the following day, it would, however, have been expected to affect the results obtained over the six day intervals.

For this reason the overnight records were examined for the number of climbs recorded for each of the various heights of poles. The results

of this examination are presented in the form of the total number of climbs recorded on each size of pole in Table 1.7 for that period from 6.12.66 to 22.2.67, Table 1.8 for that period from 22.2.67 to 22.5.67, and in Table 1.9 for the overall period 6.12.66 to 22.5.67.

TABLE 1.7

Total No. of climbs recorded from each height of pole on overnight visits from 6.12.66 to 22.2.67

Pole Height (ft.)	0.5	1	2	3	4	5	TOTAL
No. climbs	3	13	15	10	14	2	57

TABLE 1.8

Total No. of climbs recorded from each height of pole on overnight visits from 22.2.67 to 22.5.67

Pole Height (ft.)	0.5	1	2	3	4	5	TOTAL
No. climbs	7	20	15	19	14	14	89

TABLE 1.9

Total No. of climbs recorded from each height of pole on overnight visits from 6.12.66 to 22.5.67

Pole Height (ft.)	0.5	1	2	3	4	5	TOTAL
No. climbs	10	33	30	29	28	16	146

These results are not amenable to statistical analysis for the reasons discussed in this section, however, the number of climbs recorded from the six inch poles was generally low when compared with the number of climbs recorded from one foot poles and over, although for the period 6.12.66 to 22.2.67 fewest climbs were recorded from the five foot poles.

Clearly the results presented in this section indicate that snails were found to have climbed tall poles rather than very short ones during the period of climbing 1966-7. The behavioural mechanisms that lead to this finding are not clear but it is suggested that an orthokinesis may operate to bring it about. While there is rather slender evidence for suggesting that snails may discriminate against very short objects as a place on which to climb, this suggestion is still regarded as tenable at the present state of our knowledge of Helicella virgata.

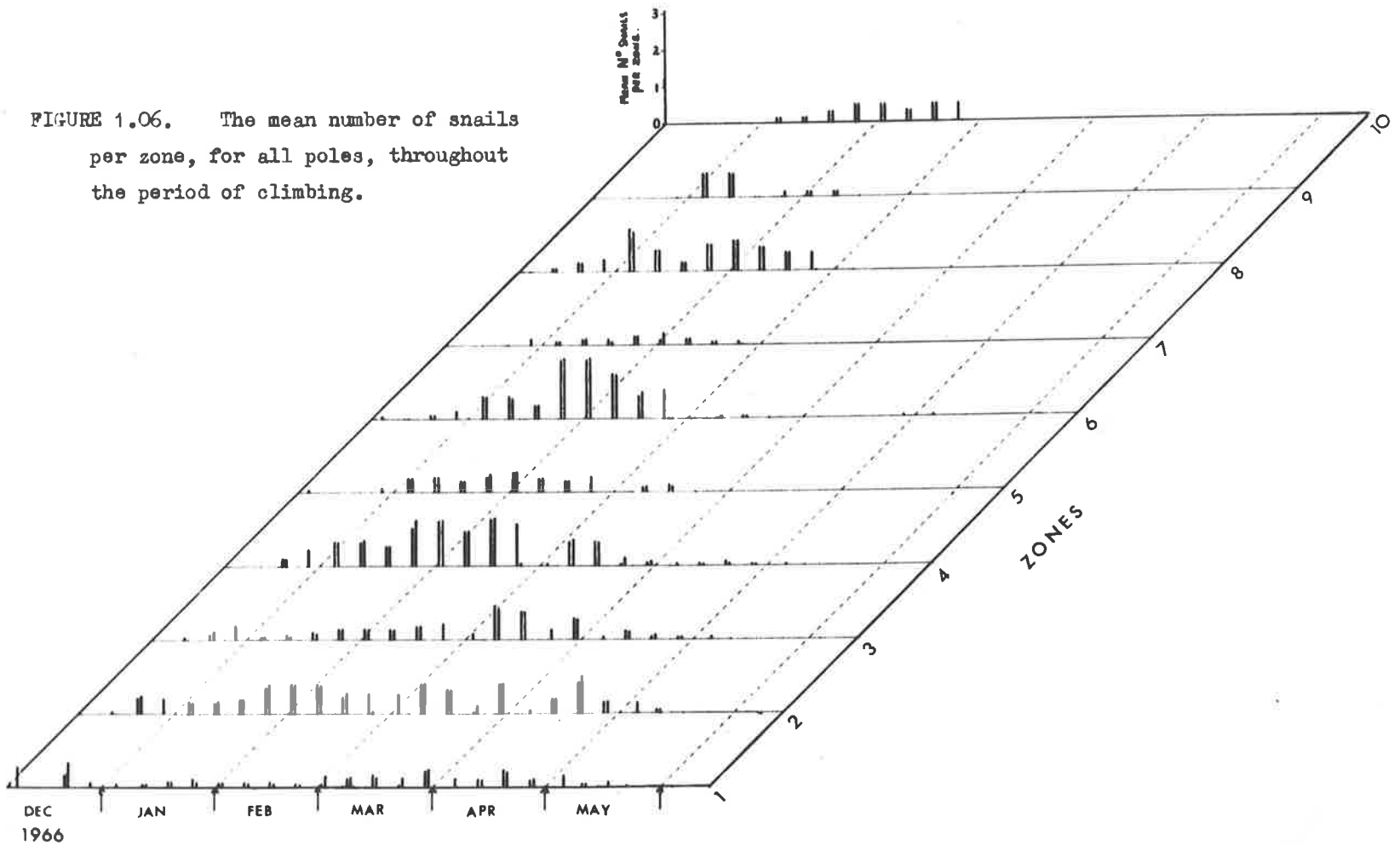
1.36 The distribution of snails with respect to height on poles

The records from all poles were analysed to determine the mean number of snails present in each zone at each visit. The mean number of snails per zone was used as the poles were of different heights and there were different numbers of the respective zones.

The results of this analysis are shown in Figure 1.06 where the mean number of snails in each zone is shown for all zones over the period of the investigation. From this figure it may be seen that while snails were present in the low zones throughout much of the period, the numbers there were small. In the high zones, snails were present for a much shorter period but the numbers found there were relatively large. Following rain in late February all the snails left the poles, and subsequently no snail climbed higher than zone 6.

Snails were climbing highest during January and February in 1967. This coincided with the hottest part of the summer. Figure 1.06 indicates that rather more snails were present in zones two, four, six, eight and ten than were present in zones three, five, seven and nine on any one visit. It was felt that the explanation for this result lay in the fact that zones two, four, six, eight and ten were all terminal on some poles. That is to say that there were six poles ending with each of these zones. Zones three, five, seven and nine were never terminal, and it seemed possible that snails were generally climbing to the top of poles and becoming congregated in terminal zones.

FIGURE 1.06. The mean number of snails per zone, for all poles, throughout the period of climbing.



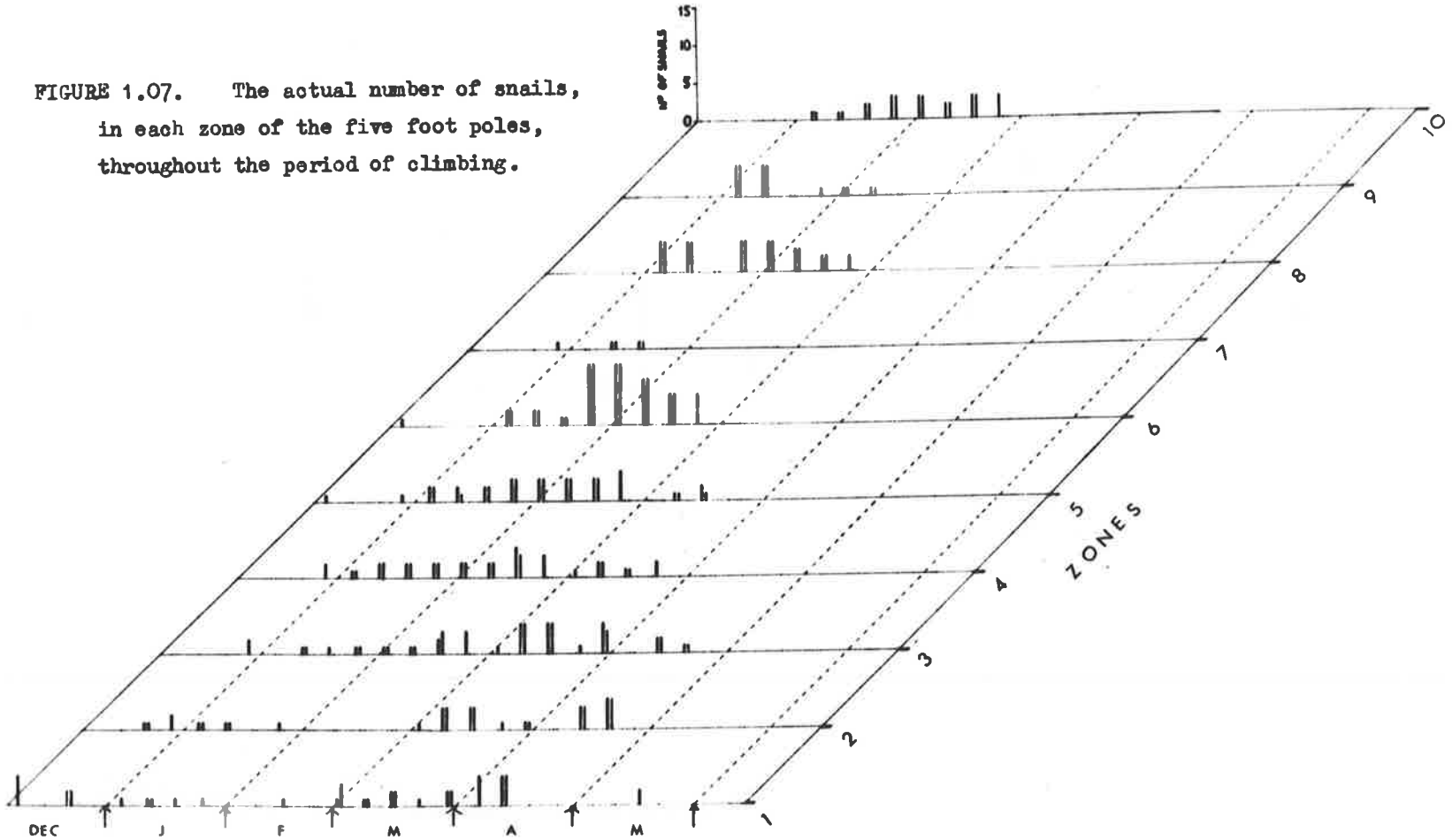
For this reason it was felt that to compare the mean number of snails on the various zones of all the poles was unlikely to provide a valid measurement of the height to which snails would have climbed had they not been restricted.

The analysis was repeated, and only snails in the various zones of the six five-foot poles were considered. As there was the same number of zones of all kinds on these poles the actual number of snails present per zone was used instead of the mean number per zone. The results are shown in Figure 1.07 where the number of snails in each zone on the five foot poles has been plotted for the whole period of the determination. A similar distribution of snails is shown to that in Figure 1.06.

It is clear that snails will climb very much higher than five feet. It is not uncommon to find large numbers of snails clustering around the insulators and cross-bars on telegraph poles in many parts of South Australia during summer. Many of these snails may be in excess of thirty feet above the surface of the ground.

On only a few visits were snails present in zone 10 on the five foot poles at Northfield. On these occasions the number in this zone was only a small fraction of the total number of snails that was present on these poles. It is therefore unlikely that the restriction imposed on climbing by the height of these poles was of importance in considering the height to which most of the snails climbed at Northfield during the summer of 1967.

FIGURE 1.07. The actual number of snails, in each zone of the five foot poles, throughout the period of climbing.



1.37 Movements of snails at different heights above the ground

Pomeroy (1966) considered the activity shown during summer by snails on a tree in the North Parklands near Adelaide. He found that snails were present on the trunk from near ground level to heights of more than five metres. He considered the numbers of snails that moved within three arbitrary zones on the trunk, the lowest zone was from 0 to 0.9 metres from the ground, the middle zone 0.9 to 1.5 metres and the upper zone 1.5 to 2.5 metres. He found a greater proportion of snails moving in the lowest zone than in the middle zone, and that moving in the middle zone greater than the upper zone.

The records from all poles in the Northfield experiment were examined for movements of snails over the period from 6.12.66 to 22.2.67 to see whether similar trends were shown with regard to the movements of snails at different heights off the ground. The proportions (percentages) of snails moving away from each zone was calculated for all zones over each period between counts.

The records were examined for periods in which rain had fallen, and these periods were excluded initially. The percentage of the snails moving in each zone was summed for each of the periods when no rain fell and for which snails had been present in that zone at the start of the period. There were 14 such periods for zone 1 and 11 for zone 10, The "mean percentage" of snails moving in each zone was therefore determined by dividing the sum of the percentages moving by the number of observations.

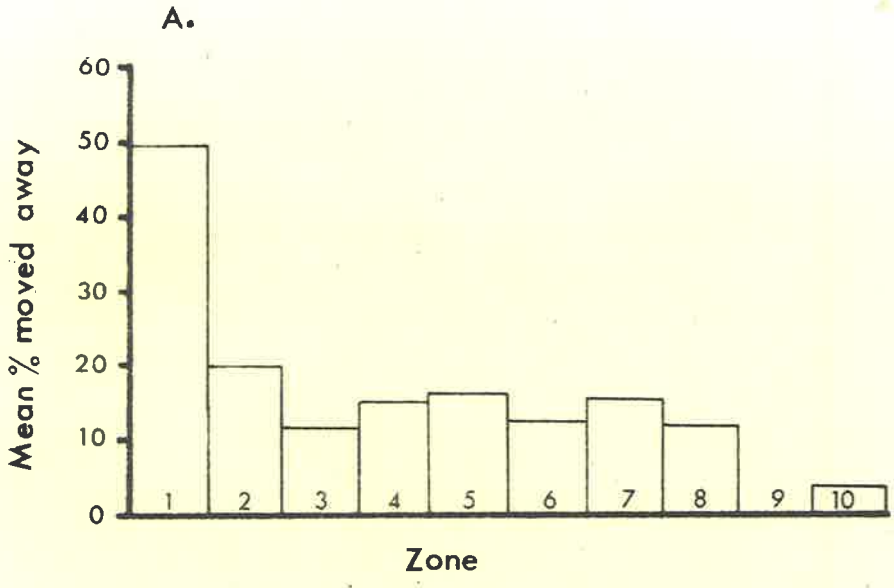
The results of these calculations are presented in Figure 1.08A for periods when no rain fell, and for the whole interval including periods of rain, in Figure 1.08B. Clearly this is an extremely crude method of analysis, nevertheless, the results indicate that for periods when no rain fell, and for the whole interval between 6.12.66 and 22.2.67 the probability that a snail would move away from a zone was greater for snails near to the ground than for snails that had climbed some distance up the poles.

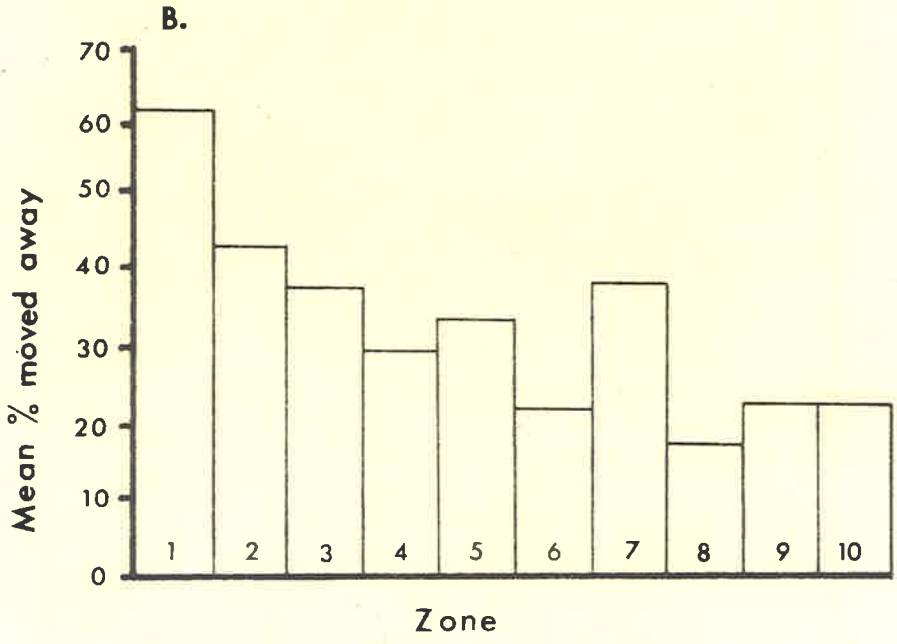
This result is in general agreement with that of Pomeroy, who suggested that snails nearest the ground would tend to move more often, as in such places the humidity would tend to remain higher after rain. The results shown in Figure 1.08A suggest that even when no rain fell, snails in the lowest zone at Northfield (between 0 and 6 inches above the ground) tended to move more often than snails in any other zone. Field observations suggested that while some of this movement no doubt took place when the nightly relative humidity was high, much of it took place in the early mornings, just after the sun had risen. It seemed clear that snails tended to wake when a sudden rise in temperature occurred. During the night one might expect temperatures on the surface of the ground to drop below that of air temperature, so that the temperature differential between the early hours of the morning and that on the ground after sun-up might be generally greater in situations close to the ground than in would be in places some distance above the ground. Thus the changes in, and the rate of change in temperature experienced by snails in the lower zones

FIGURE 1.08. The means of the percentages of snails that moved away from a zone during the period from 6.12.66 to 22.2.67.

A. (above) Movements not associated with rain

B. (below) Overall movements


A.
Mean % moved away
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
Zone


B.
Mean % moved away
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
Zone

would be likely to be considerably greater than those experienced by snails further up the poles. It is thought that this might have tended to promote activity in those lower animals.

Dainton (1954a) has shown that in the slug Agriolimax reticulatus, activity can be induced by changing temperatures. In fact, Dainton showed that atmospheric moisture had no direct effect on activity in these slugs, though it probably limited the duration of activity by influencing the water content of the body. She found that between 4°C and 20°C activity was induced by falling temperatures and suppressed by rising temperatures, further, the animals were able to "perceive" (respond to) temperature changes as slight as 0.1°C per hour.

Between 20°C and 30°C, Dainton showed that slug activity was induced by rising temperatures and suppressed by falling temperatures. The response to temperature changes under these conditions was far less sensitive than that for temperature changes below 20°C. Dainton suggested that this response enabled the animals to escape from a situation of rapidly rising temperature which might assume damaging proportions. She found that, once active under these conditions, animals would move rapidly down a temperature gradient towards a cooler place, and the resulting fall in temperature would soon cause them to become inactive again.

While it would be unwise to assume that the behaviour of Helicella virgata was of necessity like that of Agriolimax reticulatus, there are indications, based on observations in the field and the laboratory, that at least some close parallels exist between these two species.

In attempts to induce activity in dormant H. virgata it was early found that to place snails on a wet substratum in the laboratory under approximately constant conditions of temperature was only partially successful. Many snails would remain inactive inspite of the presence of water. It was found that, on placing these snails so that they could be illuminated by a heat-lamp, an increased proportion of the animals became active. The most efficient method that was finally adopted to arouse snails consisted of placing the animals into a 5°C temperature cabinet for ten minutes and then placing them under a heat lamp at approximately 30°C. All snails became active when treated in this way.

In the field, during summer, it occasionally happened that a snail would become dislodged from the position to which it had climbed. On several such occasions the temperature of the dry ground was measured and found to exceed 50°C (the maximum being 52°C). A snail on the ground under such conditions would invariably become active within a few seconds, and their behaviour at such times was very characteristic. Rapid crawling was a feature that was noticeable amongst such animals. They often appeared to be searching around for some object on which to climb. The anterior portion of the foot would frequently be raised off the ground. If an object (even a grass-stalk) was within about six inches of the snail, then the animal would move directly towards this object and climb, often no more than three or four inches off the ground, before becoming inactive again.

If, on such hot days, snails were further than about eight inches from an object, then they would almost always die on the ground before reaching it. Such snails always died extended, they simply continued crawling, getting drier all the time. Just before death the snails often possessed a shrunken, stiff foot from which eyestalks still protruded. The main source of water-loss was seen to be via the hot dry earth, which acted like a very efficient piece of blotting paper.

As a result of these observations it seems clear that,

(1) snails will become active in response to increasing temperatures, and

(2) that once active they will move down a temperature gradient before becoming inactive again, and

(3) that H. virgata can perceive objects from at least six inches.

Behaviour of the sort described would explain the movements of snails on the poles at Northfield during those periods in which rain did not fall. Clearly, for a snail to move down a temperature gradient it would have to climb further up a pole, or move around the pole to a cooler position.

1.38 The orientation of snails around poles

The poles of Northfield were placed so that the flat sides faced north, south, east and west. These sides were referred to as faces. In addition to these sides the small area at the top of a pole was also

considered as a face, so that for each individual recapture one of a possible five faces was recorded.

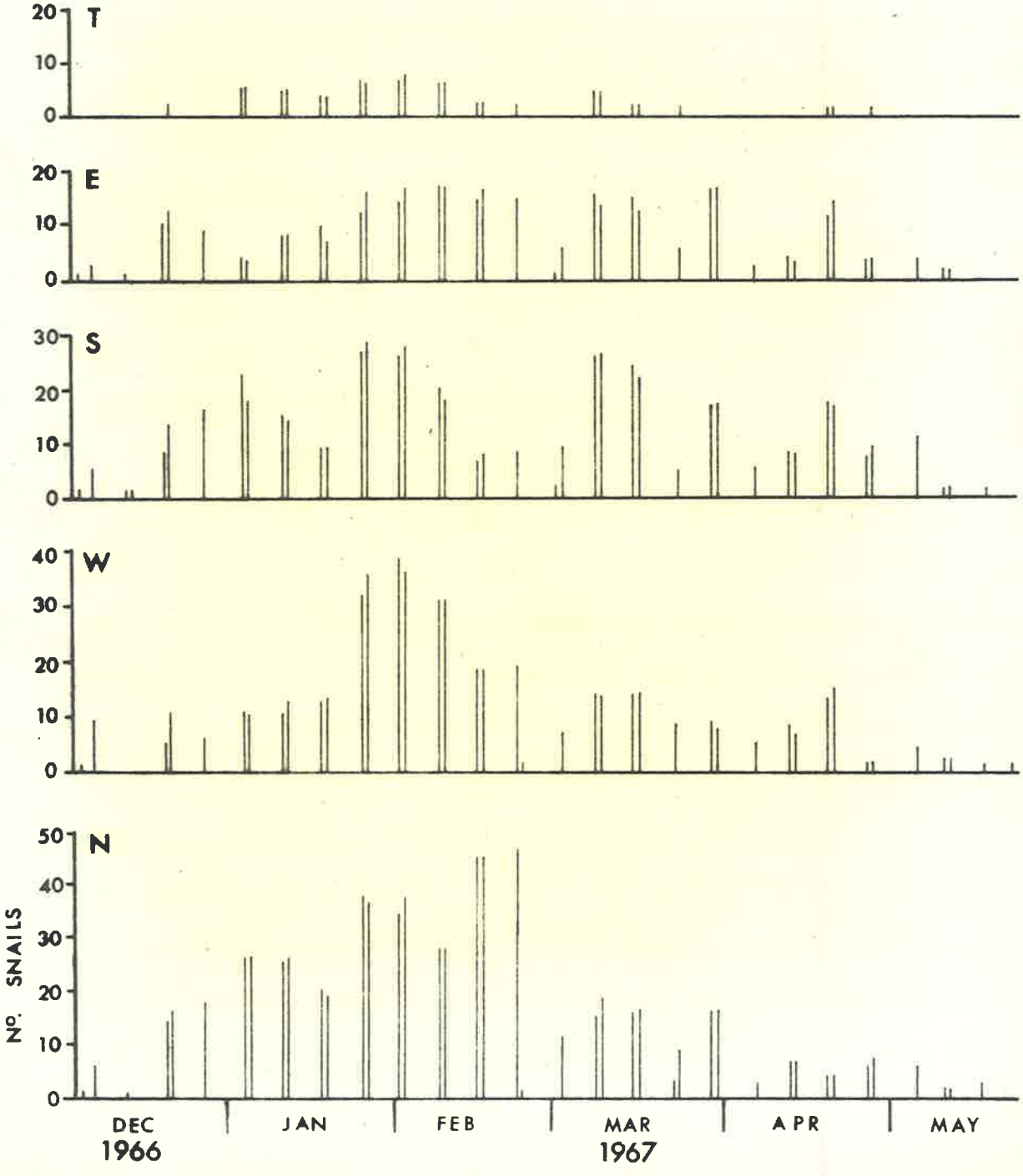
The records from all poles were examined for the total number of snails on each of the faces at each visit for the whole period of the investigation. The results of this examination are shown in Figure 1.09.

Pomeroy (1966, 1968) recorded the positions in which he found snails on three telegraph poles. His counts were made in February 1965 and February 1966, and were based on one visit only at each of those dates. His results showed that there was a preponderance of snails on the northern sides of poles. He found also that a similar situation occurred on a tree in the North Parklands in Adelaide.

With regard to the numbers counted on the telegraph poles, Pomeroy stated that it was puzzling to find "that the snails consistently chose to settle on the hottest side". He stated that the number of snails on the different sides ranked in the same order as the heat-loads on the snails. Further, he stated (Pomeroy 1966) that "since the relative numbers on the different sides of poles are too unequal to be accounted for by chance, it follows that the snails must have selected the side which they climbed and, therefore, that they could distinguish between the sides".

Pomeroy (1966) considered as an alternative hypothesis, that the high proportion of snails present on the north and west sides of poles resulted from an orthokinesis. Snails on the wetter side (which he concluded was the west side) would be woken whenever it rained, whilst

FIGURE 1.09. The number of snails on each face at each visit. The faces are identified as - T, top; E, east; S, south; W, west; N, north.



those on the dry side would not. This, he suggested, would lead to most of the snails being present on the north side. He objected to this hypothesis on the grounds that during the summer of 1964-5 "the wind was predominantly westerly or south-westerly whenever it rained, whereas the lowest number of snails were on the south and south-easterly sides."

In a later publication (Pomeroy 1968) he apparently rejected this hypothesis, presumably for the reason stated above. In this he stated that "the preference of aestivating H. virgata for the north and west sides of poles is puzzling since these are undoubtedly the hottest sides. Such a distribution could result from the movement of snails towards or away from some stimulus. It seems improbable that they would move to the north and west sides because these are the hottest, and no other obvious stimulus would account for the observed distribution. However in Europe the north and west sides would be the most appropriate. It is usually in the early morning that snails can be seen climbing, whilst the temperature is still low."

Pomeroy proposed that "if snails possessed a light-compass reaction of the sort described by Newell (1958 a, b) for Littorina littorea and by von Buddenbrock (1919) for Helix pomatia then they would have a simple means by which to identify the north side. Thus, behaviour evolved as a simple adaptive response in the northern hemisphere would produce a non-adaptive response in Australia."

This reasoning lead Pomeroy (1968) to state in his summary that "their behaviour, which presumably evolved in their native European range,

is less appropriate in the climate of South Australia."

Pomeroy's conclusions have been presented at some length for two reasons. Firstly, they appear to be amongst the first that have appeared in the literature attempting to describe the orientation in climbing terrestrial molluscs. Secondly, I believe them to be misleading in that as a result of counting snails on three telegraph poles, and having obtained a similar distribution on two single visits a year apart, Pomeroy has assumed this distribution to reflect the way in which snails choose to climb objects. Further, he speculated on the adaptive significance of the climbing response that led to this distribution with remarkably little evidence for much of his discussion which I find unacceptable.

It is clear from the results presented in Figure 1.09 that during the hottest part of the summer more snails were recorded from the north and west faces of poles at Northfield than from the south and east faces. In mid February 1967, an isolated visit would have shown that snails were distributed around the poles in a manner essentially similar to that described by Pomeroy, but that at other times the result would have been far less clear-cut, or positively different from his finding.

In attempting to explain the observed distribution it is necessary to consider the way in which snails move in the field. During the course of this study many visits were made to Northfield and snails were observed at all times of the day and night.

Shortly after sun-up on summer mornings, in the absence of rain

or wind, it was observed that many snails tended to climb on the shaded sides of objects. Others that did not initially would often tend to climb in a spiral path around an object, usually remaining on the darker, shaded face once there. It seemed that the snails were climbing on what was the darkest, or coolest place at the time. If any significant wind was blowing, snails would invariably move directly downwind to the most protected, though not necessarily shaded side of an object. In this Helicella virgata appears to resemble Helix aspersa (Machin 1964b). (Machin has shown that by moving downwind the evaporative loss of water from the extended foot of Helix aspersa is reduced to a minimum.)

During the course of the field observations it was seen clearly that the face on which a snail came to rest after a period of movement was not necessarily that on which it climbed initially, therefore it cannot be assumed, as Pomeroy has done, that the presence of a large proportion of snails on a particular face indicates that these snails have "chosen" to climb on that face.

The situation is complicated by rain. At Northfield, during 1967, several light falls of rain occurred during the day in association with moderately high temperatures and gusty winds. Thesewinds were usually from the south-easterly to south-westerly direction through south. On one such occasion (28.12.66) it was strikingly obvious that the east, south and west faces of the poles were wetted, while the north remained dry. Many snails on the three wet faces became active, and some on the dry north face. It was observed that on the south and west faces, which

were the wettest by far, most snails began to crawl downwards and many slipped, falling to the ground. Some snails fell from the other wet face, but many turned downwind and this movement brought them to the north face where most of them became inactive again. Most of the snails that aroused on the drier north face moved down a few inches and then came to rest again on the same face.

Many of the snails that fell from the poles began climbing, these snails were not observed to climb any particular face rather than another but once these animals had climbed about six inches off the ground they were no longer protected from the wind by the walls of pen 6. Many of these snails turned downwind, some coming to rest on the more sheltered north face, although many still crawled slowly without settling down.

A further fall of rain occurred shortly before dusk. However little wind accompanied this fall and many snails crawled from all faces.

The observations described above appeared to lend slight support to Pomeroy's "orthokinesis" hypothesis. In order to decide whether a snail on a particular face had a greater probability of moving than a snail on any other face, the records from the Northfield experiment were examined for the proportion of snails that moved away overnight on the various faces. Comparisons were made only between visits a day apart as these were the only periods for which it was at all possible to define the weather that accompanied movements. Only those overnight records were considered that fell between the beginning of the period of climbing and late February, when the hottest part of the summer was over and snails had begun to leave

FIGURE 1.10. Percentage of snails present on each face that moved away overnight.

(The numbers above the histograms refer to the number of snails present on that face initially. The percentages are the proportions of those snails that moved from a face overnight).

A.	21.12.66 to 22.12.66	Breeze S-W.	Warm day.
		Breeze S-W.	Hot day.
		No rain.	
B.	4.1.67 to 5.1.67	Wind S-SW.	Warm day.
		Wind N.	Hot day.
		No rain.	
C.	11.1.67 to 12.1.67	Wind S.	Cloudy warm day.
		Wind W.	Cloudy warm day.
		No rain.	
D.	18.1.67 to 19.1.67	Wind S-W.	Warm day.
		Wind N.	Hot day.
		No rain.	

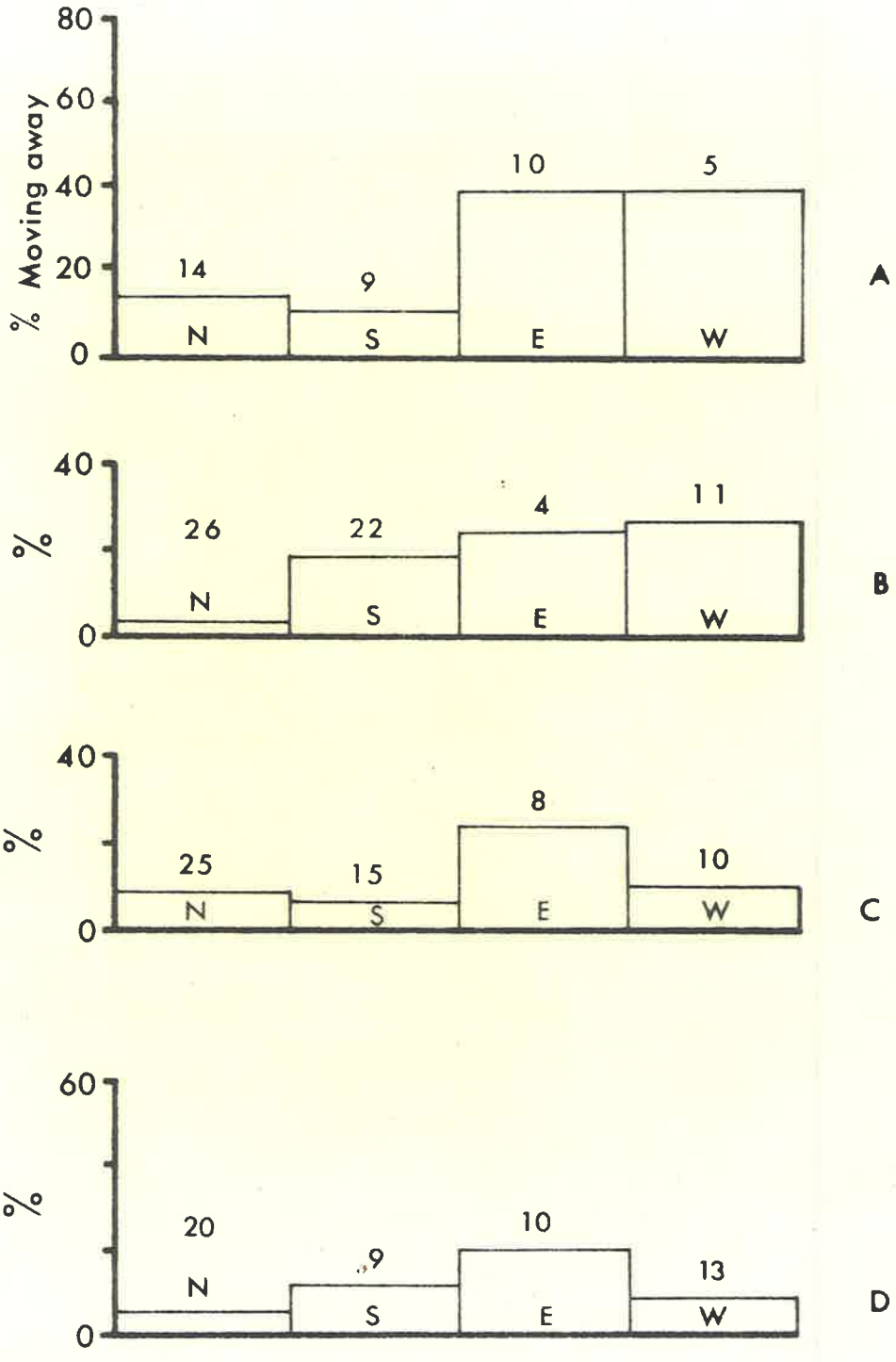
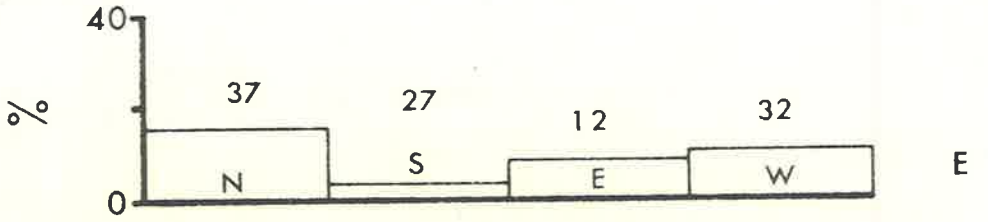
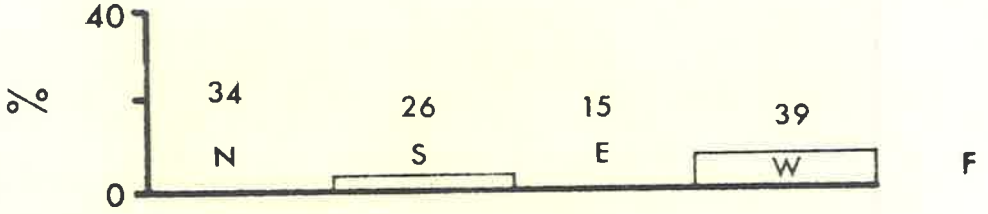

80
60
40
20
0
% Moving away
14
9
10
5
N
S
E
W
A
40
%
26
22
4
11
N
S
E
W
B
40
%
25
15
8
10
N
S
E
W
C
60
%
20
9
10
13
N
S
E
W
D

FIGURE 1.10 (cont.)

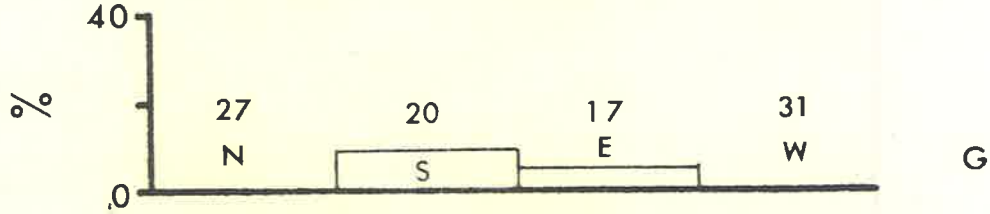
E.	25.1.67 to 26.1.67	Wind S-SW.	Warm day.
		Breeze S-SE.	Hot day.
		No rain.	
F.	1.2.67 to 2.2.67	Breeze SE-E.	Warm day.
		Wind S-W.	Hot day.
		No rain.	
G.	8.2.67 to 9.2.67	Breeze E.	Warm after hot period.
		No wind.	Warm day.
		Possibly a few spots of overnight rain.	
H.	15.2.67 to 16.2.67	Wind S.	Warm day.
		Wind E.	Overcast.
		No rain.	
I.	22.2.67 to 23.2.67	Breeze S-W.	Heavily overcast.
		Winds variable.	Cool day.
		Heavy overnight rain continuing until 1600 hours on 23.2.67.	


40
%
37
N
27
S
12
E
32
W
0

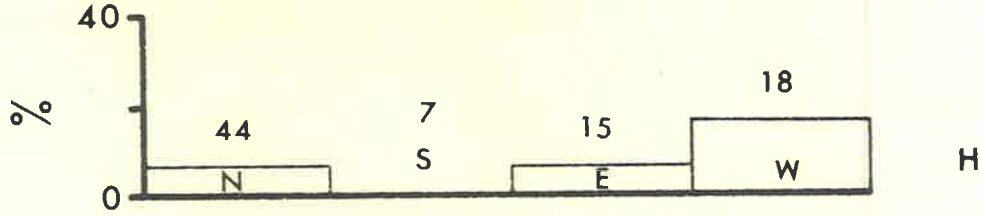
E


40
%
34
N
26
S
15
E
39
W
0

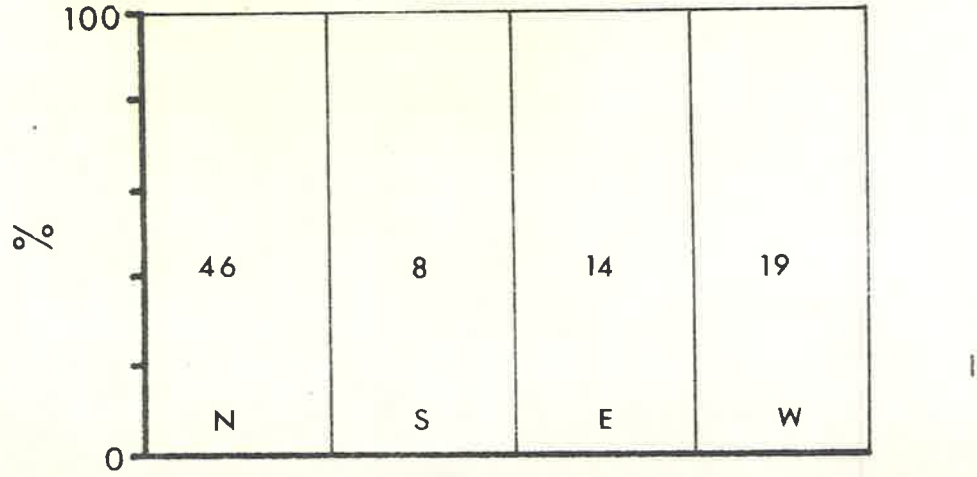
F


40
%
27
N
20
S
17
E
31
W
0

G


40
%
44
N
7
S
15
E
18
W
0

H


100
%
46
N
8
S
14
E
19
W
0

I

the long grass area.

The results of this examination are shown in Figure 1.10. The numbers of snails present on each face on a first count is shown together with the percentage of that number moving from a face, as determined from the count of the following day. Brief notes on the weather at the time of each determination have been included.

No obvious trend was shown with respect to the faces from which snails moved during these overnight determinations. Nevertheless it is clear that even when rain did not fall some snails moved, often on all faces.

The records from 6.12.66 to 23.2.67 were examined for periods in which the movements of snails had been associated with rain. There were seven such occasions of rain, however at the time of the first two falls, (14.12.66 and 19.12.66) so few snails had climbed any poles that these records have been ignored. In Figure 1.11 the percentage of snails moving from the various faces is shown for the five remaining determinations. Discounting the last determination where heavy rain fell for some hours, it may be seen from the figure that for three of the four other occasions fewer snails moved away from the north faces than from any others.

In order to make some sort of general statement about the relative chance that a snail on a particular face would move away from that face, the whole record was examined for the proportions of snails that moved from all the various faces from 6.12.66 to 22.2.67.

FIGURE 1.11. Percentage of snails moving from each face during periods when rain fell.

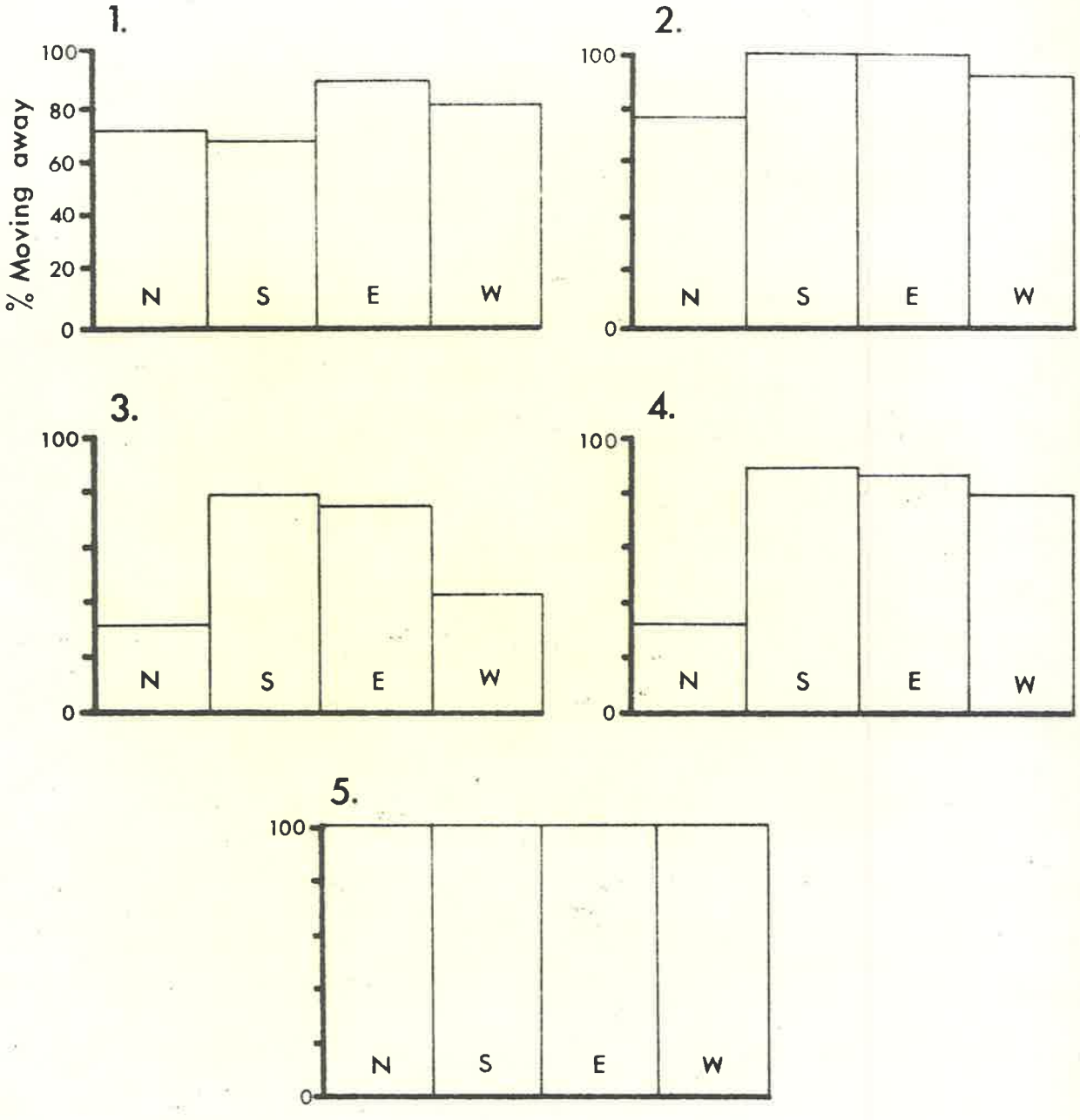
1. 28.12.66 to 4.1.67
(9 pts. on 28.12.66)

2. 12.1.67 to 18.1.67
(40 pts. on 15.1.57)

3. 19.1.67 to 25.1.67
(A few spots only)

4. 9.2.67 to 15.2.67
(13 pts. on 13.2.67)

5. 22.2.67 to 23.2.67
(approx. 100 pts.)


1.
% Moving away
100
80
60
40
20
0
N
S
E
W
2.
100
0
N
S
E
W
3.
100
0
N
S
E
W
4.
100
0
N
S
E
W
5.
100
0
N
S
E
W

Those periods in which rain fell were excluded from the record and the sum of the percentages moving from each face was divided by the number of times in which snails had initially been present on each of the faces. A mean figure for the percentage of snails that moved away from a particular face over the whole period was thus obtained. These "mean percentages" have been used to draw Figure 1.12A. From which it is suggested that, in the absence of rain, the probability that a snail will move away is about the same for snails on north, south or west faces, but slightly higher for snails that are on east faces.

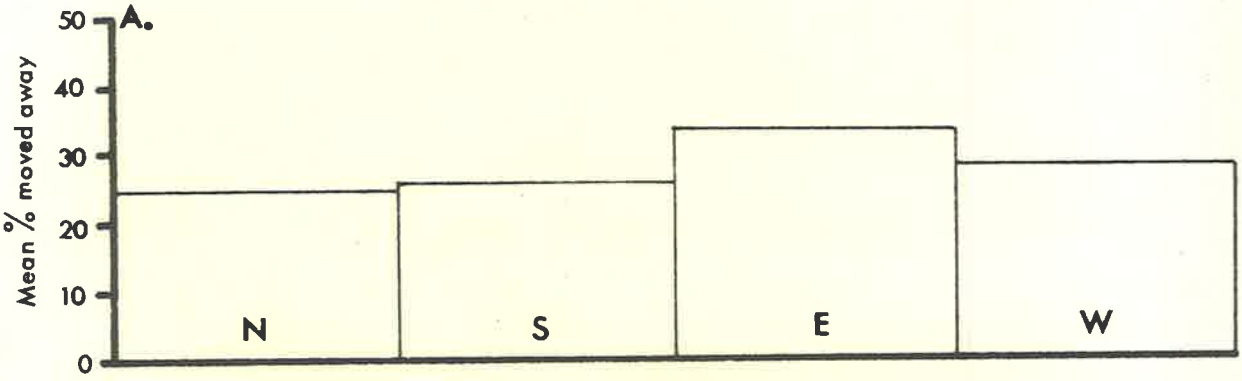
The overall "mean percentages" have been used to draw Figure 1.12B. These were derived from all the records of movement, including those associated with rain, for the period 6.12.66 to 22.2.67. The conclusion drawn from this figure is essentially the same as that from Figure 1.12A, in that the probability of snails moving from north, south and west faces is similar but that for snails on east faces rather greater.

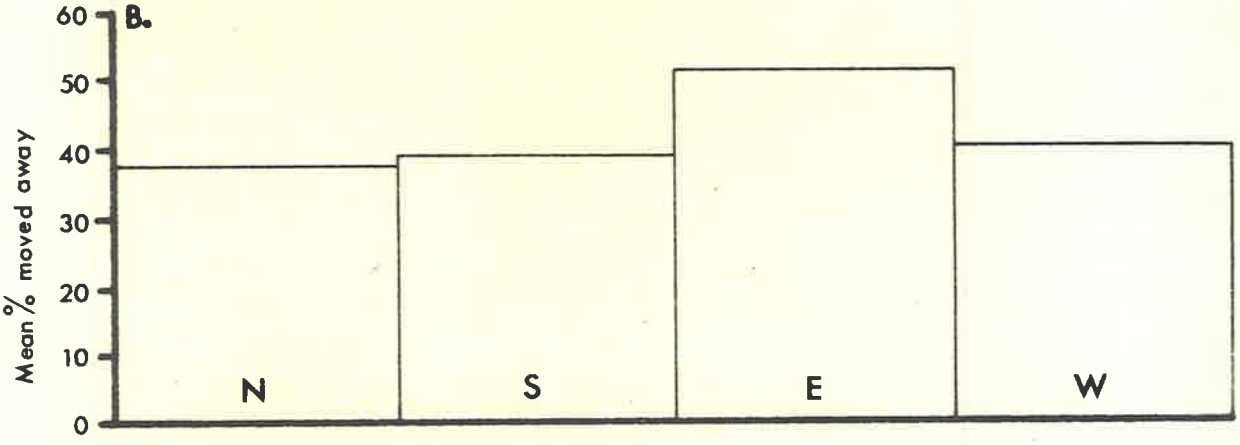
While this is an extremely crude method of analysis, it is possible that it does reflect the situation in the field. It was mentioned earlier that snails climbing in the early mornings, as the sun rose, generally tended to move around the objects on which they climbed so as to come to a cooler or darker face. It is clear that some snails became active on poles even when there had been no rain. If these snails too, made most of their movements in the early mornings (and it is suggested that they did), then snails that became active on an east face might be more likely to change faces than those which became active on the other, possibly cooler

FIGURE 1.12. The means of the percentages of snails that moved away from a face during the period from 6.12.66 to 22.2.67.

A. (above) Movements not associated with rain

B. (below) Overall movements


A.
50
40
30
20
10
0
Mean % moved away
N
S
E
W


B.
60
50
40
30
20
10
0
Mean % moved away
N
S
E
W

and certainly more shaded, faces. Further, from the discussion in Section 1.37 it is thought that snails on an east face may also wake more often as a result of a rapid rise in temperature.

It is clear, I think, that any further attempts to study the behaviour of snails in the field will need to be accompanied by accurate and detailed measurements of the various components of weather. These measurements will be vital for the understanding of the changes in distribution of snails around objects on which they have climbed.

There is, to my knowledge, no study dealing with the movement of snails in nature under conditions like those found in South Australia during summer. Nevertheless, I consider that the distribution of snails around objects like poles can be explained simply in terms of the sorts of behaviour described in this section. It is unlikely, I think, that the snails have evolved a specific type of climbing behaviour that enables them to become dormant in situations that are optimal from the point of decreased heat-load. I believe that they settle down to become dormant in response to an immediate set of stimuli, of which rain, wind, temperature and/or light intensity are probably of particular importance.

One proviso must be mentioned in connection with the statement that no specific climbing behaviour was noted which would tend to prevent snails from becoming dormant in positions of high heat-load. Relatively few snails ever became dormant on the flat tops of the poles. Even when, as was thought, snails were crowding into terminal zones on the lower poles, few

were found on the flat tops of the poles.

This observation appears to be borne out generally, as in areas where Helicella virgata reaches high densities it is not uncommon to find snails "capping" the fence posts in that area. Under such conditions some snails do become fixed to the horizontal tops of these posts, but the actual numbers present there appear to be only a small proportion of the total number forming the "cap". No data have been collected on this point however.

1.4 IS DORMANCY OBLIGATORY DURING SUMMER?

It is pointed out in Section 3 of this thesis that certain authors (among them Pelseneer, 1935; Comfort, 1957; Hunter, 1964; and Owen, 1965) have used the term diapause to describe the phenomenon of dormancy in gastropods. Indeed Kühn (1914) and Künkel (1916) both stated that they had been unable to prevent Helix pomatia from entering dormancy (hibernation) during autumn although the animals had been kept warm and moist and provided with food.

Helicella virgata will generally become inactive at any time of the year when placed under conditions of constant temperature for a wide range of temperature. This is observed even under conditions of moisture and in the presence of food.

Dainton (1954a) has shown for the slug Agriolimax reticulatus that when these animals are placed under different but constant temperature,

the slugs will exhibit a persistent, but steadily deteriorating diurnal rhythm of activity and rest. As Dainton pointed out, such conditions are not generally experienced by animals in the field, where temperatures fluctuate daily throughout the year. Dainton stated that slugs would only experience conditions of constant temperature during periods of heavy frost and at such times the slugs are not active, having retreated to warmer crevices in the soil.

It is clear from these results that any attempt to relate the behaviour of terrestrial molluscs under laboratory conditions to the situation in the field must be approached with caution. For this reason I decided to conduct the following experiment in the field to determine whether, given "rain" during summer, snails could be kept active without becoming dormant.

Three pens at Northfield were used for this experiment. They were pens 1, 2 and 3, shown in Figure 1.01. The grass in each pen was cut to approximately two inches in height. In pens 2 and 3 spraying nozzles were set up so that the outlet of each nozzle was some four inches above the surface of the ground. Three spray nozzles were placed in each pen, such that when these were operating a fine mist spray was produced, covering the ground area within the pen. The sprays used were made by Spraying Systems Co. (Tee Jet TN-3W).

The sprays in each pen were controlled from a solenoid tap connected to the mains water supply. Each solenoid tap was controlled by a separate electric time-clock. In pen 2 the clock was set so that the sprays were

turned on for 30 minutes every night at midnight. In pen 3 the sprays operated for 30 minutes at midnight and for a further 30 minutes at midday.

On 8.12.66, one hundred and fifty large snails were collected from a roadside near the study area at Northfield. These snails were divided at random into three groups of fifty animals. These were marked with spots of yellow and orange marking paint, numbered with waterproof drawing ink and released into the pens, one group to a pen.

At approximately weekly intervals each pen was searched for two hours, or until all the snails had been recaptured, whichever took the lesser time. As each snail was found its number was recorded. All those found to be dead were discarded and the living animals were returned to the pens in the late evening when it was cooler.

Observations on the snails in the pens showed that in both watered treatments, the snails became active and crawled around in the pens. It was seen that these snails grew during the summer, adding to the shell margin. There were differences between times of activity in the two watered pens. In pen 2, where the sprays operated at midnight, the snails were active thereafter throughout the night, They ceased activity in the early mornings and were usually found to have crawled on the walls of pen 2 or on the vegetation within this pen. The nightly activity was similar in pen 3, but there was usually a further period of movement associated with the spraying at midday. The activity associated with this second spraying was variable in character, depending on the weather

conditions of the particular day. At midday on cool days, the snails in pen 3 would show similar activity to snails during winter, however, on hot clear days the snails tended to move off any objects that they had climbed and to creep about very slowly on the wet ground. Their behaviour under these conditions was quite unlike that observed for snails at any other time of the year. It is clear that conditions of precipitation do not occur naturally on days with no cloud cover, nor are they associated with temperatures as high as these snails were experiencing. It appeared from observations made on these animals that two different behavioural responses were operating at the same time, leading to quite abnormal behaviour.

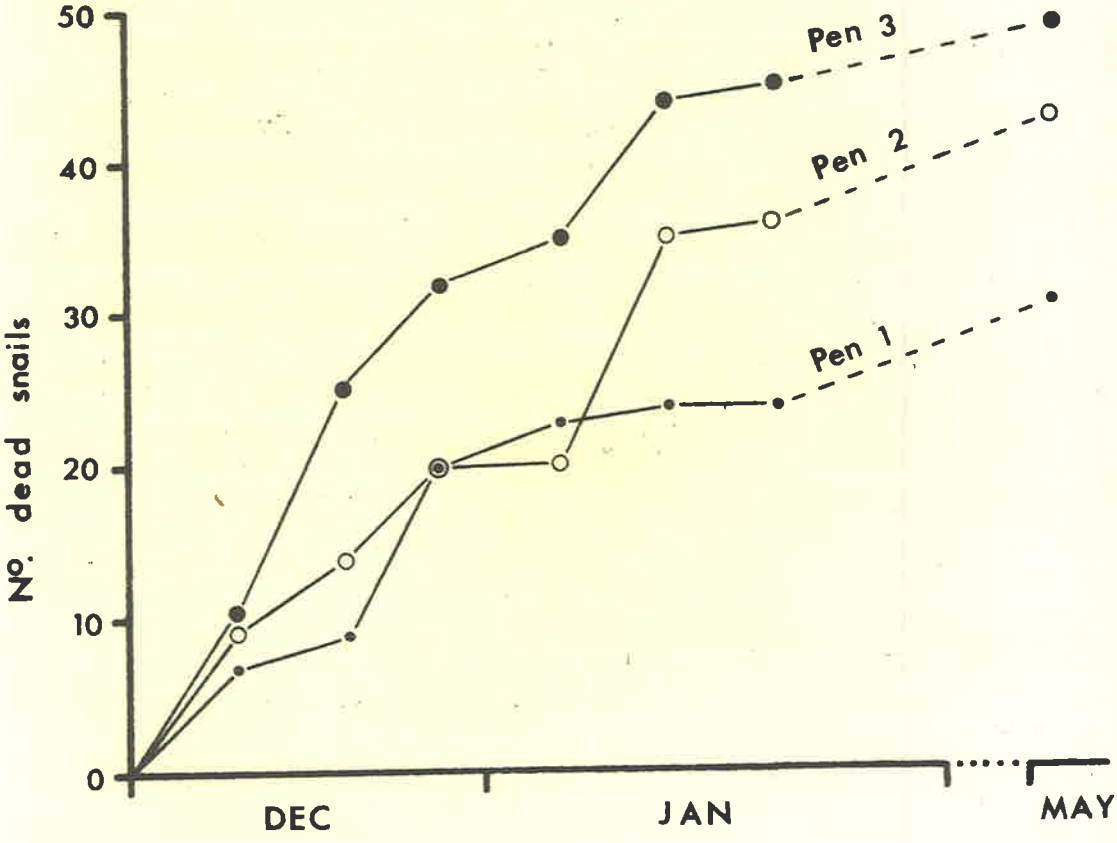
It was clear from observations on many animals that on hot days, a few snails only would be active in the early mornings (such activity was always associated with climbing), however, most of the animals were not active. Those snails in pen 3 became active at midday, following the spraying. At such times the wet ground surface in the pen was quite hot. The snails would emerge from the walls of the pen or the vegetation and descend to the ground, they would then move slowly to another object, climb it for a few inches, and return to the ground. Thus continual climbing and descending was observed throughout the period when the sprays were operating. The movements became progressively slower during this period of activity. Following the cessation of spraying most snails would climb and not return to the ground. It was seen however that many snails climbed very short objects that were close by rather than return to the taller vegetation or pen walls.

It is thought that the explanation of this curious behaviour lies in the fact that snails were responding to the stimulus of heat in the normal fashion, and as a result were climbing. This climbing brought them out of the regions of high ground heat and under the cool sprays. The combination of cooling and wetting caused them to climb downwards again to the hot ground. Thus the observed behaviour was the result of two stimuli, which under natural conditions would never be expected to operate simultaneously.

The snails in the unwatered pen usually moved to the walls of that pen or to the few remaining stalks of grass. This movement seemed always to occur during the night that the animals were released. Unless rain fell, few of these animals moved again before the next census. They all produced thick white epiphragms with which they attached themselves to the objects on which they had climbed. No shell growth was observed among these snails. No thickened epiphragms were ever seen amongst snails in the watered pens, these snails produced only thin, uncalcified structures when they became inactive.

The results of the number of animals that died in each pen are shown in Figure 1.13 for the whole period of the investigation. It is clear from these results that while snails were active in the watered pens, more of them died in these treatments than in the unwatered treatment where many snails entered dormancy. Further, more snails died under the heavier watering regime in pen 3 than died in the less watered pen 2. Observations showed that the most likely cause of death in both pens 2 and 3 was that

FIGURE 1.13. The number of snails that died in each of the pens. The points are derived from the number of empty shells found from 8.12.66 to 25.5.67.



snails were caught out in hot places on the ground after periods of activity. They did not seem to be able to escape from situations of high heat-load.

From the results of this experiment it seems that dormancy can be prevented, and snails can be kept active by "rain" even during the hottest part of the summer. It would be wrong, therefore, to conclude that dormancy was obligatory in Helicella virgata during the summer, and the observation that in the laboratory, H. virgata cannot be prevented from entering dormancy under conditions of moisture, constant temperature and in the presence of food is not relevant to the situation in the field during summer. To conclude from it that dormancy was obligatory in this species would, I suggest, be to fall into serious error.

1.5 BURROWING OF Helicella virgata

In Section 1.21 it was suggested that one explanation for the apparently rapid increase in the numbers of snails, found at the end of February on the short grass area at Northfield, was that snails had emerged from burrows in the ground.

That H. virgata burrows during winter in Britain is known, and Pomeroy (1966) found during his study that some snails burrowed such that they were just beneath the surface of the earth, the shells could be exposed by searching through the litter on the surface of the ground. Pomeroy found that after rain or heavy dew these snails emerged again.

He was not able to estimate the proportion of snails that burrowed, but the numbers were low.

From Section 1.34 it is clear that most of the snails released into pen 6 at the start of summer climbed poles during the hot weather (77.2 per cent), before snails left the long grass area at Northfield. That some of the marked snails did not climb poles during this period was shown by the fact that after 22.2.67 an additional 26 animals appeared for the first time on poles. It was suggested in Section 1.34 that most of these animals climbed the walls of the pen during the earlier part of summer, but it is possible that some may have burrowed or gone down cracks in the soil during this period.

It is of interest to speculate on the effectiveness of burrowing as a means of avoiding the heat. It seems clear that if a snail were to burrow in the relatively open and unshaded areas of soil at Northfield, it would have to go deeper than the immediate surface layer. This top layer may become extremely hot during a clear summer's day, and temperatures as high as 52°C have been measured on the surface of the ground. There is almost no litter on the surface of much of the ground at Northfield. Although one would expect the temperature to decrease rapidly for a relatively small decrease in depth burrowed, it is probable that similar decreases in temperature could be achieved, for a smaller energy expenditure, by climbing.

Baverstock (pers. com.) has reported seeing few snails in a large open paddock near Adelaide. He visited this area at the end of summer

and was impressed by the scarcity of snails. Those animals that were present were confined to a few dry bushes, and were present in great numbers upon these. Following heavy rain he returned to the area and found large numbers of adult snails present on the ground. The numbers appeared to be such that they could not be explained simply by the number of animals that had been present on the bushes. There was no question of breeding, and it seemed unlikely that snails had entered the area from surrounding areas. Baverstock considered that snails must have emerged from burrows, and this explanation seems to be the only one that would explain his observation.

No separate study of burrowing was made during my own work, but the results reported in Section 1.4 may bear upon the question of burrowing.

In each of the three pens, the numbers of living snails were recorded at each visit and the total number that had died was also noted. It was therefore possible to determine how many of those marked in each pen had not been accounted for at a visit, in spite of the thorough search that was made. The number of snails that was not accounted for is shown in Table 1.10 for each pen for visits made during the period for which snails were present in the long grass area at Northfield.

TABLE 1.10

The number of snails not accounted for at each visit to the pens

Date	Pen Number		
	1	2	3
15.12.66	4	1	1
22.12.66	6	8	5
28.12.66	12	3	2
5. 1.67	4	1	1
12. 1.67	5	7	3
19. 1.67	8	7	3
2, 2.67	6	3	2
9. 2.67	7	1	2
16. 2.67	17	2	2

It is obvious that more snails were not accounted for in the unwatered pen 1 than in either of the other pens. This was true in spite of the fact that there was less ground cover in this pen and it should have been easier to see any marked snails there than in the watered pens.

The explanation for this was seen to lie in the fact that snails entered cracks in the soil in pen 1. The soil at Northfield is a red clay-loam which is very susceptible to shrinkage when dry. Cracks form readily. In both watered pens, the soil remained wet enough so that cracking did not occur. In pen 1, cracks generally formed along the line where the wall of the pen entered the ground, and these cracks were often shaded by the walls themselves. Pen 1 contained almost no vegetation that was over two inches in height. It seems possible that by moving down temperature gradients, snails would have been likely to enter these cracks which were at times in excess of one inch deep.

It may be that this observation is distinct from the situation where a snail digs its own hole. I have not observed a snail to do this, except during egg-laying, throughout the course of my studies at Northfield, but it may be that some of the snails do bury themselves rather than simply enter cracks in the ground. It seems likely that if one intended to pursue this question it would be necessary to study the behaviour of the animal on different soil types in different parts of its range.

SECTION 2. THE PERMEABILITY OF DORMANT SNAILS TO WATER

2.0 INTRODUCTION

Active land-snails do not appear able to control the loss of water from their bodies. Thus Helix aspersa loses water by evaporation almost as fast as a free water surface (Machin 1965). During dormancy, however, the rate of loss of water is much reduced. Pomeroy (1966) found that at 25°C dormant Helicella virgata lost approximately 5 per cent of their wet weight per week.

This reduction in permeability shown during dormancy is clearly a property of the living animal, as after death the rate of loss of water increases greatly (Andrewartha 1956; Machin 1966; Pomeroy 1966).

Pomeroy (1966) pointed out that it has often been stated or implied that the shells and epiphragms of terrestrial snails are impermeable to water, and that it is the waterproof nature of these structures which permits snails to live in dry places. However as he further pointed out, the evidence for the above statement is equivocal.

Studies on the shells of terrestrial snails have indicated that these structures are relatively impermeable to water, but the degree of permeability has been debated (Mazek-Fialla 1933, 1934; Gebhardt-Dunkel 1953; Fischer 1950; Warburg 1965; Pomeroy 1966; Machin 1967).

Studies on the epiphragm (Fischer 1950; Machin 1968) have shown that these structures are too permeable to be the only mechanism retarding the loss of water from the aperture of dormant snails.

Machin (1967) has claimed that the presence of an epiphragm reduces the water-loss from the mantle-collar in Helix aspersa, Otala lactea and Sphincterochila boissieri. Pomeroy (1966) did not find a significant difference between the rates of water-loss in still air among Helicella virgata with and without epiphragms.

Warburg (1965) has suggested that although the epiphragm is permeable to water molecules, the space between it and the collar would contain saturated air. He suggested that although water evaporates through the epiphragm continuously, it does so at a lower rate than if the body were exposed to moving air.

Machin (1966) has shown that dormant Helix aspersa are able to regulate evaporative water-loss from the mantle surface, where this is not overlain by shell (the mantle-collar). In this paper Machin has shown also that low permeability seems to be a unique property of the living tissues of the mantle-collar. The tissues of the dorsal body wall, which underlie the shell, are at least forty times as permeable to the passage of water as the tissues of the living mantle-collar. Thus the shell itself must be responsible for the regulation of water-loss from the tissues beneath it, and this is supported by the work of Gebhardt-Dunkel (1953) where she produced evidence to suggest that the rate of water-loss through the shells of freshly killed snails was similar to that through the shells of living animals.

2.1 GENERAL OBSERVATIONS ON THE SHELLS AND EPIPHRAGMS OF Helicella virgata

2.11 Shells

Helicella virgata is possessed of a moderately thick, heavily calcified shell of the form shown in Plate 2.1A. The populations of snails in different areas contain different proportions of two morphs, a banded and a white shelled form. The population at Northfield is approximately 50 per cent banded and 50 per cent unbanded (Baverstock, pers. com.).

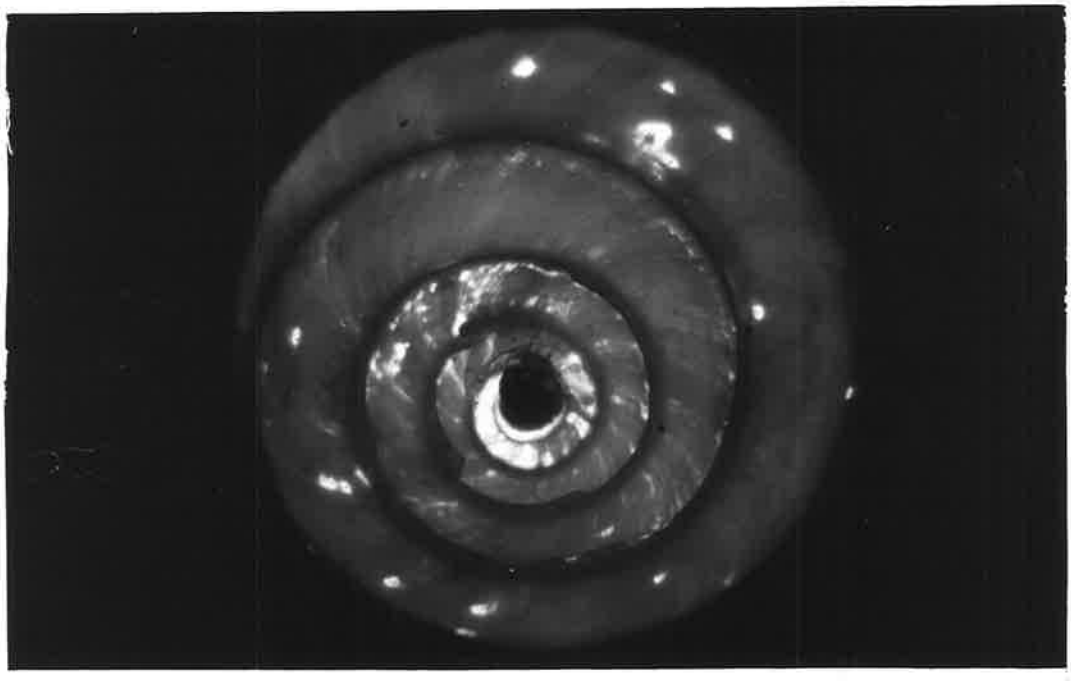
The outer layers of the shells appear chalky, and in most specimens the periostracum appears to be partially rubbed off. Melchinger (1955) found for Helicella ericetorum and Helicella obvia that calcium carbonate made up 55 to 60 per cent of the shell. The calcium carbonate was present as aragonite which is associated with a low magnesium content. The inner layer of the shell differs in colour amongst individuals but is always very smooth, hard and transparent. When held to catch the light this surface is highly reflective and appears glassy. In some animals this layer appears brownish, but in others is quite clear. There is no suggestion that this lining of the shell is perforated when shells are examined under the microscope, but detailed studies of the structure of the shells have not been undertaken.

During summer, when snails were dormant in the field, it was noticed that the shells of many specimens showed small dark spots on otherwise white shells. These spots proved to be clear areas of shell, and the dark appearance was due to the colour of the underlying mantle tissue.

PLATE 2.1

A. Form of the shell of Helicella virgata. Dorsal and ventral aspect (scale in millimeters).

B. Clear areas in the shell. From ventral with part of the coils removed to show the inner surface of the dorsal coils. Mounted with a strong light behind the shell.



This same phenomenon was observed amongst snails dormant in the laboratory, and in some treatments where snails remained dormant for very long periods it was further observed that the shells of many of the snails became generally more fragile and easily broken by handling. These observations suggested that calcium might have been withdrawn from the shells by these animals. The clear areas of shell from a snail taken from the field during summer are shown in Plate 2.1B. The shell has been mounted with a strong light behind it and the clear areas show as bright spots in this photograph.

2.12 Epiphragms

It is usual for Helicella virgata to build some form of mucous structure when the animal retracts into the shell.

In winter, during short periods of inactivity in the field, many snails produced thin uncalcified structures that appeared similar to those described as mucous veils by Howes and Wells (1934a) for Helix pomatia. Often these structures served to support the snail so that the aperture of the shell was raised about one centimetre off the surface of the ground.

In summer, at the onset of periods of dormancy, snails would climb on to vegetation and become attached by means of a thin, uncalcified epiphragm. This structure was often incomplete or broken, such that it did not cover the aperture completely. Even though such epiphragms were usually transparent and uncalcified over most of their area it was a rule that there would be present one heavily calcified spot, lying directly over the pneumostome as illustrated in Plate 2.2A. It seems probable that

PLATE 2.2

A. Ventral aspect of shell showing one complete epiphragm containing little calcium except in the region above the pneumostome.

B. Dorsal view of two snails from which part of the dorsal surface of the first coil has been removed to show the arrangement of multiple epiphragms (scale in 1/16 inch).

in Helicella virgata, like Helix aspersa, this spot is the result of secretion from a concentration of calcium secreting glands which surround the pneumostome (Campion 1961).

During dormancy the tissues of the snail retreat away from this initial epiphragm, and other more heavily calcified epiphragms are produced successively, one behind the other. In the field it was uncommon to find more than five or six of these calcified epiphragms in any one snail. During protracted dormancy in the laboratory, very many heavily calcified epiphragms were produced, with as many as 55 having been counted in a single specimen that had been dormant at 10°C for approximately three years. Two such snails are shown in Plate 2.2B. In these, the dorsal surface of the outer coil of shell has been removed to show the position of the epiphragms.

When dormancy was terminated it was usual for snails to ingest the epiphragm material. However, in those snails containing many epiphragms only a portion of this material was ingested, the remainder was expelled from the aperture and was often ingested by other snails.

2.2 WATER-LOSS FROM THE SHELLS AND APERTURES OF DORMANT SNAILS

The aim of this experiment was to determine the total water-loss from dormant specimens of Helicella virgata and to compare this with the amount lost through the shells. In this way I hoped to estimate the relative amounts of water lost through the shell and aperture of snails

during dormancy.

Fifty snails of a size class 1.0 ± 0.1 cm were collected from Northfield (9.2.67). The method of measuring the snails is discussed in Appendix 2 (Section 4.2). The snails were randomly assigned to two groups of fifteen animals after discarding any damaged individuals.

Snails in one group were placed individually into numbered and weighed nylon bags and weighed on the day of collection. The snails were then placed over silica gel in a desiccator maintained at 20°C in a constant temperature cabinet. After fourteen days the snails were weighed again, and thereafter at approximately weekly intervals until the ninety-seventh day after collection. The silica gel was renewed at each weighing.

Following the last weighing, the snails were removed from the bags and broken open to make sure that no deaths had occurred that were not observed clearly during the determination.

Snails in the second group were stored over silica gel at 20°C until the fifteenth day after collection. They were then killed in a cyanide bottle and the bodies removed from the shells. The shells were thoroughly washed with distilled water and dried in a desiccator over silica gel at 25°C.

When dry, each shell was numbered and glued, aperture down, to a glass coverslip with "Araldite" epoxy resin. A small hole was then made into the top spiral of each shell with a number 19 hypodermic syringe needle, and distilled water introduced from a syringe through this hole. The holes were then sealed with a little spot of histological wax and

covered with a layer of "Araldite" to protect this seal.

All shells were weighed individually on day twenty and then placed over silica gel in a desiccator at 20°C. They were reweighed at approximately weekly intervals and the silica gel changed at each weighing.

The mean loss of weight of the whole animals for the period day 0 to day 97 is shown in Figure 2.01. It is clear that the rate of loss of weight changed markedly during the period of measurement.

Pomeroy (1966) showed that the loss of weight of dormant Helicella virgata could be accounted for entirely by loss of water, and his result is confirmed in Section 3 of this thesis for snails dormant at 20 and 25°C. Thus the loss in weight shown in Figure 2.01 can be considered as loss of water.

From Figure 2.01 it is obvious that the rate of loss of water from these snails was very different after day 20 from the initial rate. Clearly, the initial rate cannot be considered to reflect the true rate of water-loss during dormancy, and probably arose from snails becoming active during this time.

For the purpose of comparing the rate of water-loss from whole snails with that of the shells, the changes in weight from day 20 to day 56 have been considered. The weighings of the shells began on day 20. Figure 2.02 shows the mean loss of water for whole snails and shells for the period day 20 to day 56. It is clear that there was little change in the rate of water-loss from whole animals during this period but after day 49 some increase in permeability occurred in the shells, suggesting

FIGURE 2.01. Cumulative mean loss of water (mg. per animal) from dormant snails at 20°C over silica gel.

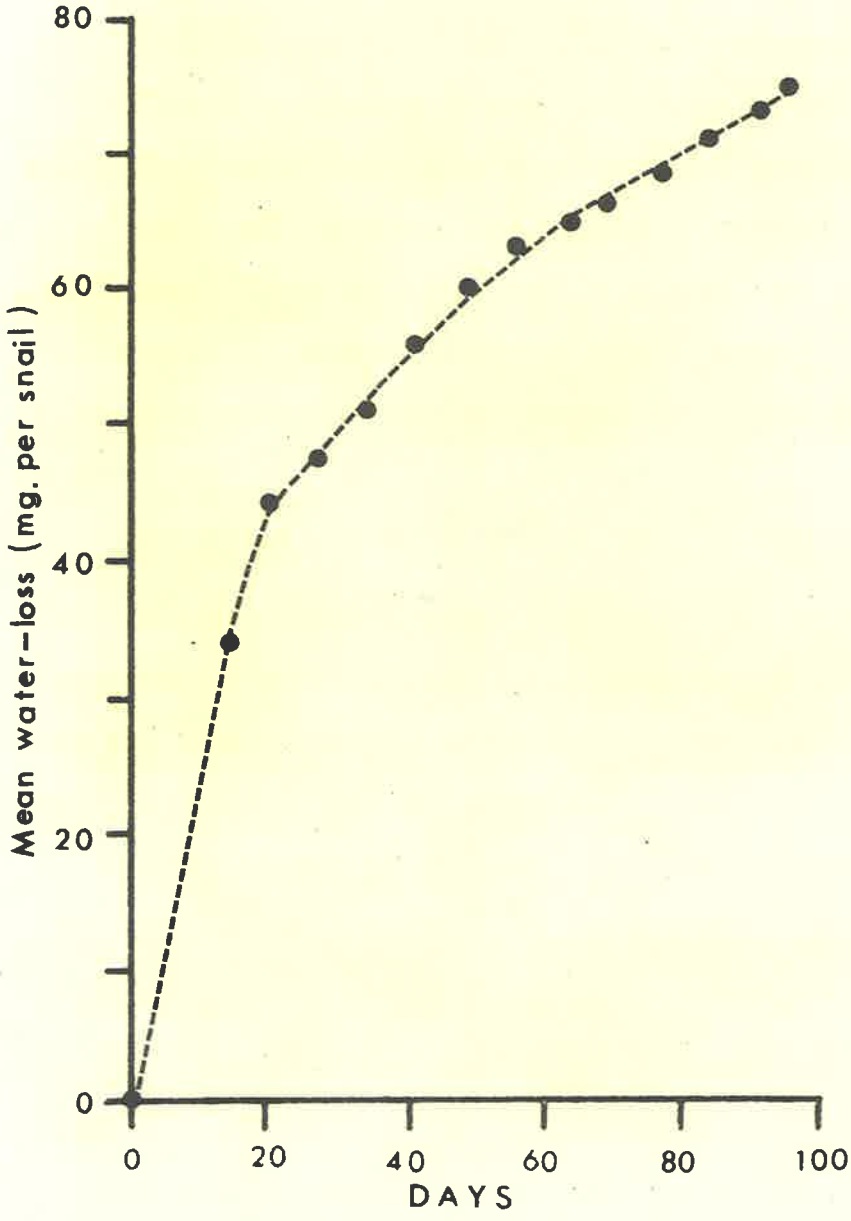
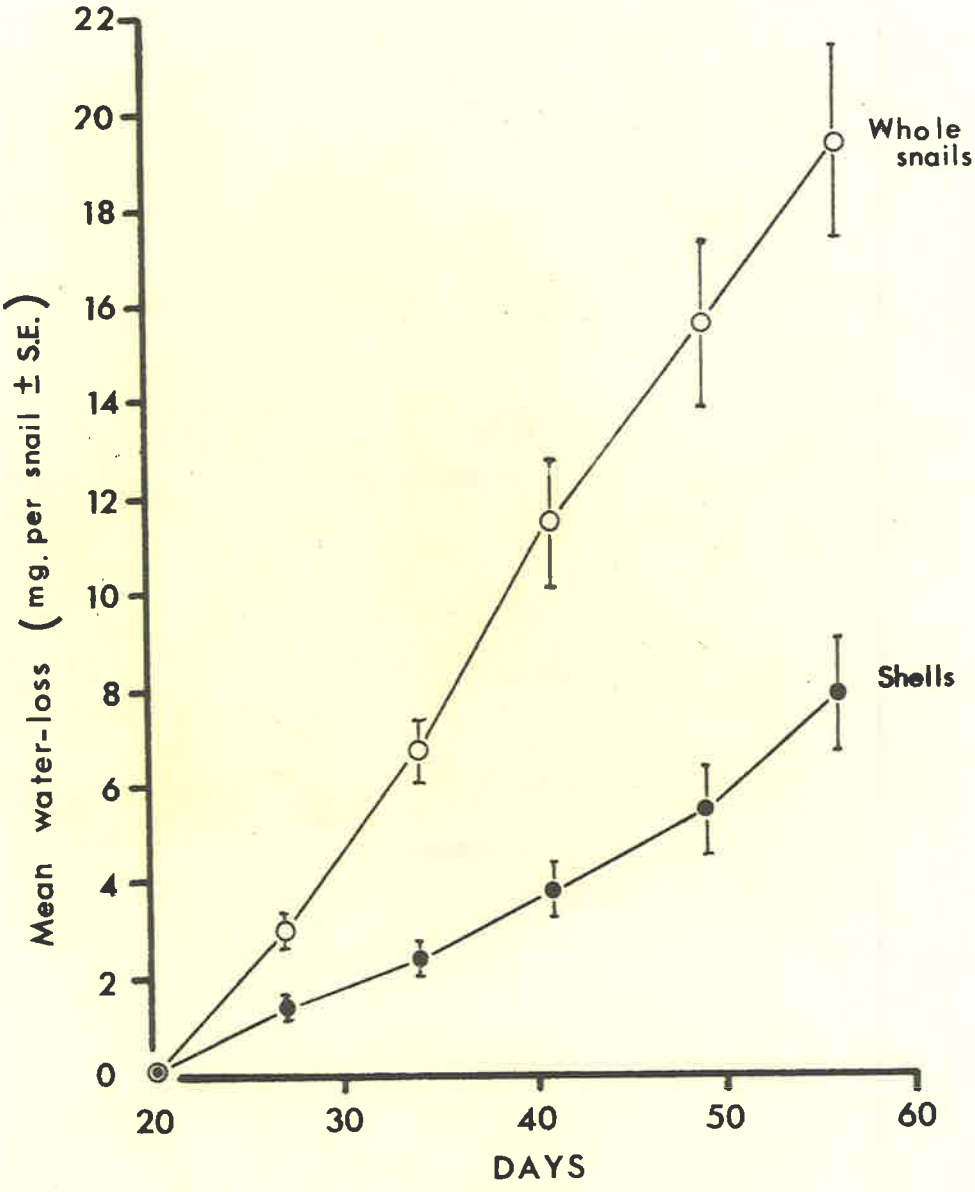


FIGURE 2.02. Cumulative mean loss of water from whole snails and shells with plugged apertures at 20°C over silica gel.



that the results from the shells should be discarded after day 49.

From Figure 2.02 it may be seen that for the period from day 20 to day 49, the mean rate of water-loss from whole snails was 15.76 mg., and that from the shells 5.56 mg. Thus the loss of water via the aperture was approximately

$$\frac{100\ (15.76 - 5.56)}{15.76} \quad \text{or} \quad 65\%$$

of the total loss from the whole animal.

Ives (1967 and pers. com.) found that the water-loss through the aperture of retracted Helicella virgata was approximately 90 per cent of the total loss from the whole animals. Her animals were of similar size, and calculations based on her results show that the rate of loss of water through the shells of her animals was very similar to that found in this study, however much greater amounts of water were lost from the apertures of her snails. She has stated that she is now aware that the snails used in her study were not truly dormant and that she does not regard her results as being in conflict with the above.

It is of interest to compare this finding, that approximately 65% of the total water-loss was via the aperture, with the published results of Machin's (1967) study on Helix aspersa, Otala lactea and Sphincterochila boissieri. Machin calculated the loss of water, in still air at 22°C over fused calcium chloride, through the shells and apertures of these snails. From his published figures it is possible to calculate

approximately the percentage of the total water-loss that occurred through the aperture (assuming that this was covered by an epiphragm). The results of these calculations are H. aspersa 92.6%, O. lactea 79.8%, S. boissieri 52.9%. This suggested that Helicella virgata falls somewhere between O. lactea and S. boissieri with respect to the percentage loss of water through the shell.

The area of the evaporative surfaces

A gastropod of given volume will clearly present a smaller evaporative surface if the shell is flattened, for in this way more of the area of each coil of shell is overlain by another coil. Also if the mantle-collar is more permeable to water than is the shell the adaptive significance of a reduction of the area of the aperture in relation to the total surface area of the animal can be appreciated.

To determine the surface area of Helicella virgata fifty snails of different sizes were collected from Northfield. These animals were inspected for damage in the laboratory and the surface area determined on undamaged specimens in the following way.

(i) Shell area. This was determined by coating the shells with a silicone rubber compound ("Silastomer" 9161 with N9162 catalyst - Midland Silicones Ltd.). The snails were measured to the nearest 0.5 mm in the manner described in Appendix 2 (Section 4.2) and supported above a block of plasticene on a fine wire. The coating compound was brushed on thinly with a fine paintbrush, hardening in approximately 30 minutes.

The coating was then stripped off the shells giving thin moulds of the shells with the texture of soft rubber. This material when hardened is totally elastic (Green pers. com.).

The shell moulds were cut so that they lay flat. The outlines of each mould were then traced with a planimeter calibrated in square centimetres. No snails smaller than 9.0 mm were used as they were too easily damaged by handling.

(ii) Area of aperture. This was determined on the same snails used for the determination of shell area, however no snails smaller than 13.0 mm were used as the shell edges were too fine and easily damaged by handling. The snails were mounted on a block of plasticene with the aperture upwards. They were supported firmly so that they could not be shaken, and the outline of the aperture was traced directly by running a needle-pointed planimeter around the inside of the aperture.

The results of these determinations of area are presented in Table 2.1 below.

TABLE 2.1

The area of shells and apertures of snails of different sizes

Diameter of shell (cm)	Area of aperture (sq. cm)	Area of shell (sq. cm)	Aperture area x 100 / Aperture area + shell area (%)
0.90	-	1.86	-
0.90	-	1.86	-
1.00	-	1.99	-
1.05	-	2.31	-
1.10	-	1.99	-
1.20	-	2.70	-
1.30	0.256	3.78	6.34
1.30	0.256	3.33	7.15
1.30	0.256	3.59	6.70
1.30	0.256	3.27	7.25
1.35	0.256	3.85	6.23
1.35	0.320	3.91	7.56
1.40	0.320	4.23	7.03
1.40	0.320	3.82	7.72
1.40	0.320	4.23	7.03
1.50	0.385	5.00	7.15
1.50	0.449	5.32	6.75
1.55	0.449	5.64	7.37
1.60	0.449	6.20	6.70
1.60	0.449	6.13	6.80
1.65	0.449	6.15	6.78

The mean aperture area as a percentage of the total area is 6.97 ± 0.11 (Std. Error)

The mean aperture area as a percentage of the shell area is 7.57 ± 0.14 (Std. Error)

It is of interest to compare Machin's (1967) figures for the relative area of the aperture in the three species of snails that he studied. His figures are H. aspersa 12.1%, O. lactea 7.8%, and S. boissieri 4.3%.

Again, as with the relative loss of water through the aperture, Helicella virgata appears to lie between O. lactea and S. boissieri with respect to the percentage of the total evaporative area that is comprised of the aperture (6.97 ± 0.11).

The relationship between the diameter of the shells and the logarithm of their areas is shown in Figure 2.03 together with the equation for the fitted regression line. From the equation it may be seen that for snails of 1.0 cm shell diameter the area of the shell is approximately 2.04 square centimetres. Thus, in the previous section, this result indicates that the permeability of the shells to water was approximately

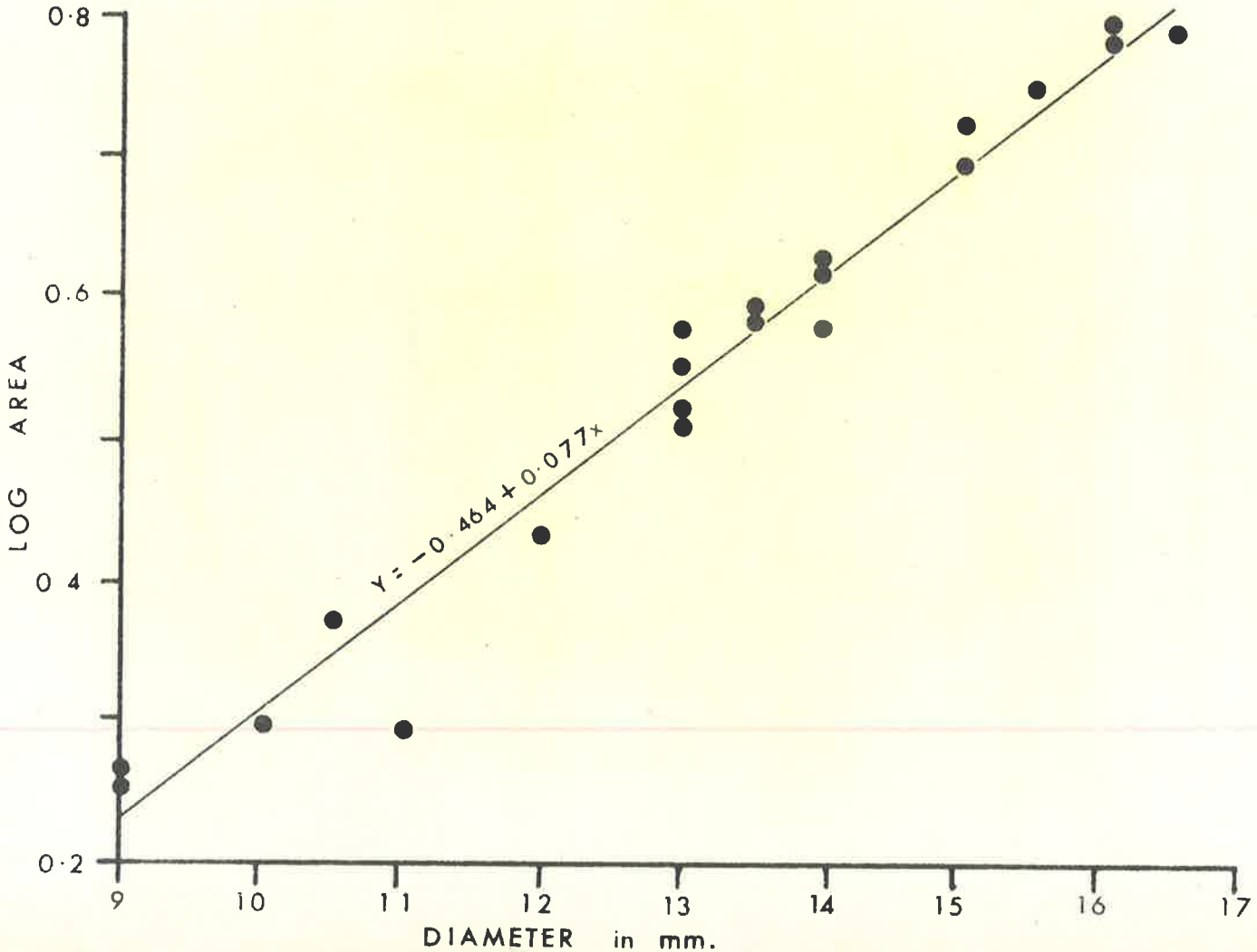
$$\frac{5.56}{29 \times 2.04} \quad \text{or} \quad 0.094 \text{ mg/cm}^2\text{/day}$$

in a saturation deficit of approximately 17 mm of mercury.

Similarly the approximate rate of loss per unit area through the aperture may be calculated. The area of aperture for a snail of 1.0 cm diameter is approximately 0.154 sq. cms so the rate of loss per unit area is given by

$$\frac{10.20}{29 \times 0.154} \quad \text{or} \quad 2.28 \text{ mg/cm}^2\text{/day}$$

FIGURE 2.03. The relationship between the overall diameter of the shells in mm. and the logarithm of their surface areas in sq. cms.



Thus the rate of loss of water per unit area of aperture was approximately 200 times as great as that from the shell in the snails at 20°C over silica gel.

2.3 THE PERMEABILITY AND SITE OF WATERPROOFING OF THE SHELLS

In appearance the shells of Helicella virgata are very different on the inner and outer surfaces, thus, while Machin (1967) claimed that for the three species of snails in his study water-loss depended strictly upon thickness and total area, I thought it possible that particular layers of the shell might be more waterproof than others, and that the shells might display some assymetry with respect to the passage of water through these layers.

2.31 Permeability of shell fragments with unsealed margins

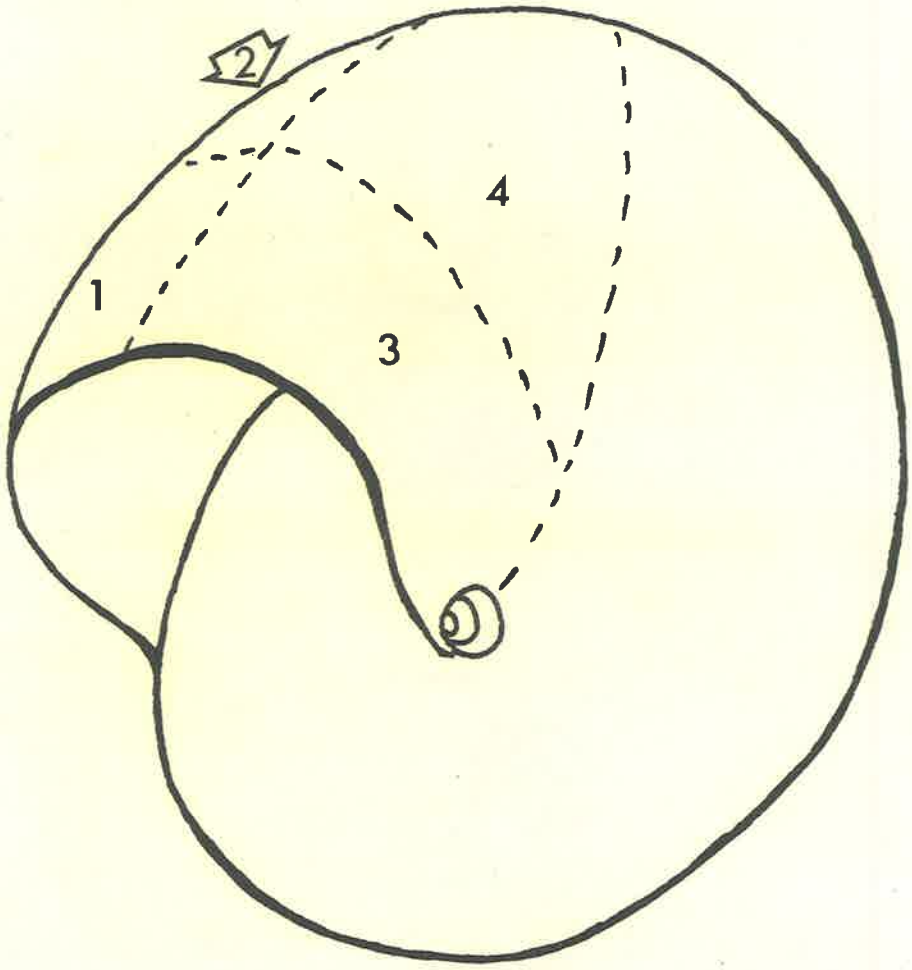
Eleven snails were collected from Northfield in March 1967. These were killed in the manner previously described, the bodies removed and the shells washed and dried.

Each shell was then broken so that part of the dorsal surface of the first coil could be removed as shown in Figure 2.04. The section removed was then broken into four fragments, numbered 1 to 4 in Figure 2.04.

The thickness of each fragment was measured with a micrometer gauge. The measurements were made to the nearest 0.0005 inch.

All fragments were glued to short lengths of glass tubing with

FIGURE 2.04. Sketch of the ventral aspect of a shell to show the sections of shell removed for division into fragments.



epoxy resin. Two fragments from each shell were glued with the inner surface of the shell towards the tube (referred to as upright fragments), and two with the outer surface towards the tube (referred to as inverted fragments), in the manner shown in Figure 2.05 (1).

The diameter of the bore of the glass tube was measured with a micrometer eyepiece, under a binocular microscope.

Small rubber stoppers were cut from a large rubber bung. These were pierced with a 30 gauge hypodermic syringe needle and inserted into the lower end of each glass tube when the epoxy resin had hardened. The needle served to provide exit for the air in the tubes. If the stoppers were pushed in without the needle in place it was thought that the increase in air pressure within the tube might weaken the shell fragment and cause leakage during the experiment.

When all the tubes had been stoppered they were taken into a 30°C constant temperature room and distilled water at 30°C was introduced through the stopper to within 1.0 cm from the shell fragment by means of a hypodermic syringe and a 30 gauge needle. After each tube had been filled both needles were withdrawn and a layer of histological wax was painted over the epoxy resin seal around the shell fragment and also over the stopper at the lower end of the tube.

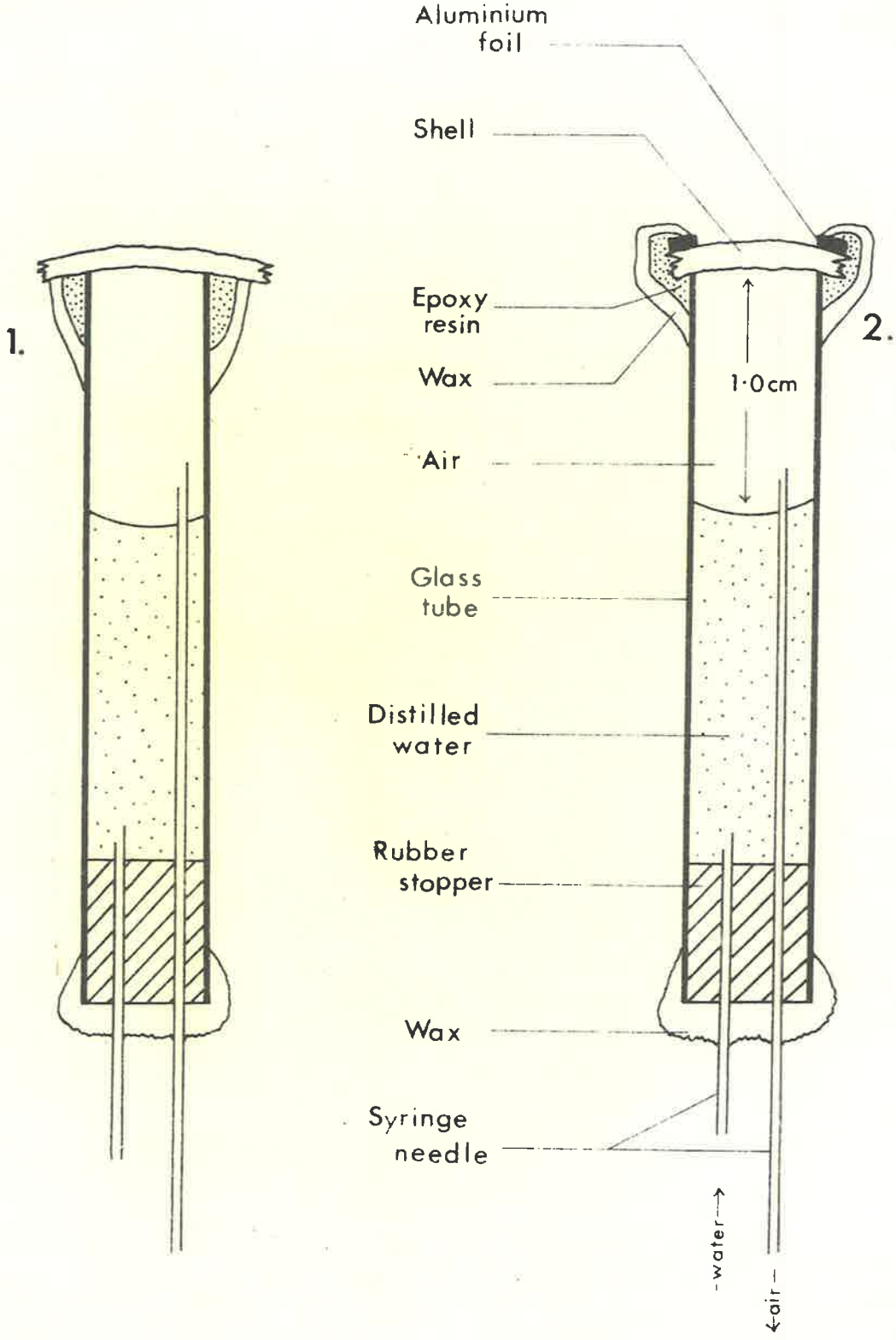
The tubes were mounted, fragment uppermost, in a block of wood with holes drilled in it. Each hole was numbered so that a particular tube could be identified.

All tubes were weighed to the nearest 0.025 mg on a Mettler H.16

FIGURE 2.05. The two methods for mounting shell fragments on tubes (not to scale).

1. Fragments mounted so that the margins were unsealed.

2. Fragments mounted with the margins sealed.


1.
2.
Aluminium foil
Shell
Epoxy resin
Wax
Air
1·0cm
Glass tube
Distilled water
Rubber stopper
Wax
Syringe needle
water
air

balance, then placed over silica gel in a desiccator and maintained at 30°C in a constant temperature cabinet for fourteen days before being reweighed. The rate of loss of water for each fragment was calculated in mg/cm²/day, and the results of this experiment are shown in Table 2.2.

TABLE 2.2

The thickness and rate of loss of water through upright and inverted shell fragments (sat. deficit 30 mm Hg)

Shell No.	Upright fragments		Inverted fragments	
	Thickness (0.001")	Rate of water-loss (mg/cm²/day)	Thickness (0.001")	Rate of water-loss (mg/cm²/day)
1	5.0	0.27	5.0	0.45
1	5.0	-	4.5	-
2	7.5	0.09	7.5	0.36
2	7.5	0.09	7.5	0.31
3	4.0	0.09	3.5	0.62
3	5.0	0.18	4.5	0.36
4	13.5	0.22	12.0	0.49
4	12.5	0.22	11.0	0.45
5	2.0	0.31	2.0	0.49
5	2.5	-	2.0	-
6	6.0	0.40	6.0	0.89
6	6.0	0.58	6.0	0.85
7	4.5	0.62	4.5	0.76
7	5.0	-	5.0	1.03
8	3.0	-	2.5	0.89
8	3.0	-	3.0	0.80
9	2.5	-	2.5	0.71
9	3.5	0.36	3.5	1.16
10	4.0	0.40	4.5	0.89
10	5.0	-	5.0	0.80
11	8.0	0.18	8.0	0.62
11	7.5	0.09	8.0	0.71

For the comparison of water-loss due to differences in orientation only the data from pairs of fragments were considered. The difference between the rate of water-loss from the inverted and the upright fragment was obtained for each pair. These differences are shown in Table 2.3 and represent the extra rate of water-loss from the inverted fragment.

TABLE 2.3

The extra rate of water-loss shown by the inverted fragment of each pair ($mg/cm^2/day$)

Shell No.	Extra rate of water-loss
1	0.18
2	0.27
2	0.22
3	0.53
3	0.18
4	0.27
4	0.23
5	0.18
6	0.49
6	0.27
7	0.14
9	0.80
10	0.49
11	0.44
11	0.62

The appropriate method of analysis for such data would be to compare the mean of the differences between pairs of observations with zero by means of a t-test. However, in this instance no statistical analysis is required to show that there was a difference in the rate of water-loss between pairs of fragments. Without exception the rate was greater from the inverted fragments.

Because of this result only data from shells in the upright position have been used to investigate the relationship between the thickness of the shell and the amount of water lost in fourteen days (Figure 2.06). It is clear from the scattered nature of the points that there was little obvious relationship between these quantities.

2.32 Permeability of shell fragments with sealed margins

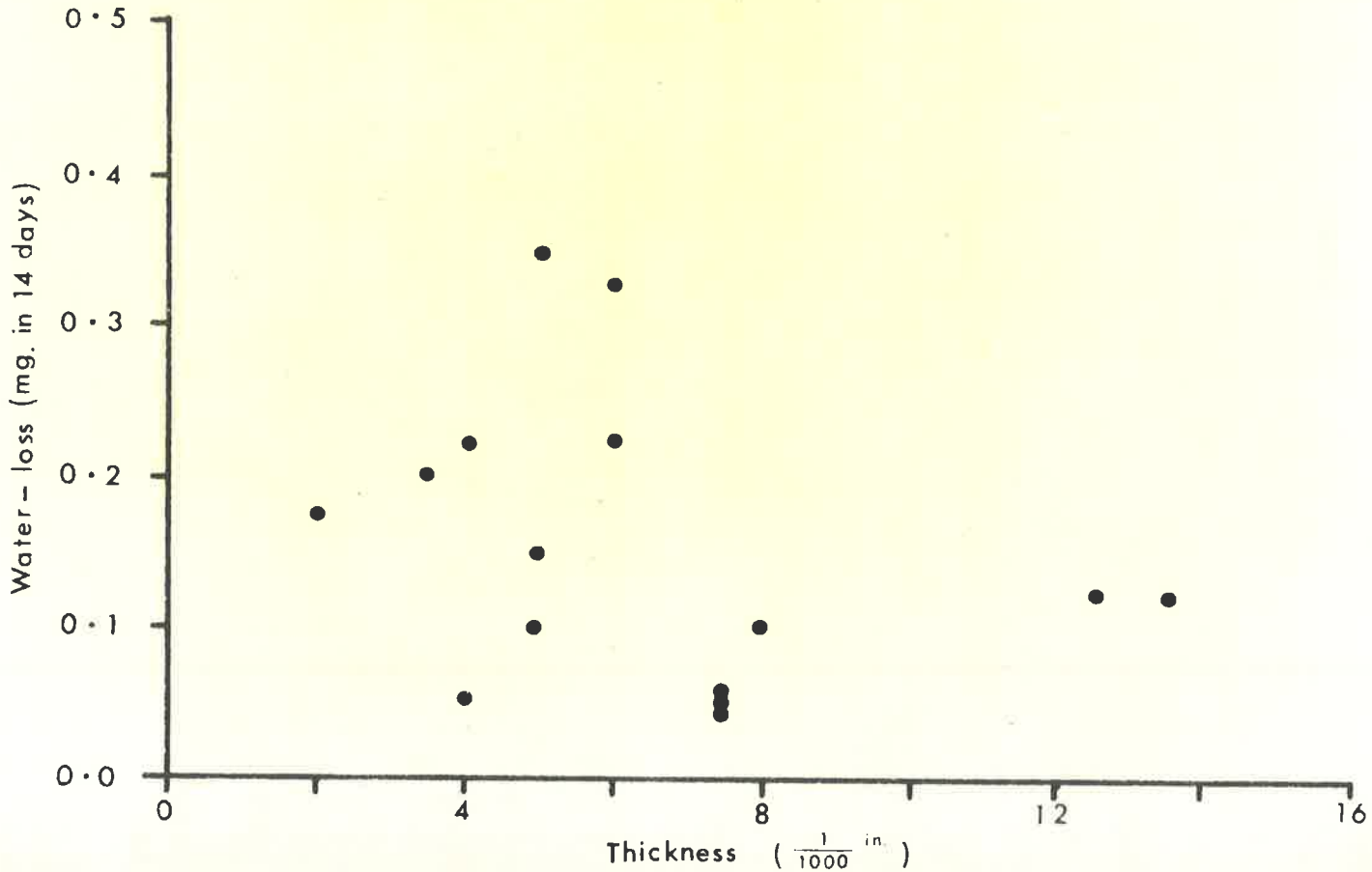
The observation that water was lost at a greater rate through inverted shells with unsealed margins could be explained in two ways.

Firstly, such a result would be expected if there was an assymetrical passage of water through the shell. Secondly, if the inner layer only of the shell was waterproof then such a result could be observed if water was leaking into the more porous outer layer of the shell and thence laterally through the unsealed broken margins.

The second alternative appeared to be the easier to test, and the following experiment was performed to that end.

Ten large snails were collected and the shell fragments obtained as before. Only ten pairs of fragments were used in this experiment owing

FIGURE 2.06. The relationship between thickness of shell and the amount of water lost through it in 14 days (30°C over silica gel).



to the amount of time that was necessary to prepare each one. The shells were mounted on the tubes as previously described, however on the side of the shell opposite the tube a small mask was mounted as shown in Figure 2.05 (2) to prevent side leaks and provide uniform evaporative area for all shell fragments. This mask was made from aluminium foil, and had a hole of the same diameter as the bore of the glass tube. The holes in the masks were aligned with the holes in the tubes, and the masks were glued to the surface of the shell fragments with epoxy resin. When the epoxy resin had hardened the tubes were filled and waxed in the manner previously described. All the tubes were weighed and stored over silica gel at 30°C for fourteen days. They were then reweighed and the rates of water-loss calculated as before.

The mean rates of water-loss in the two groups were compared by means of a t-test.

TABLE 2.4

Comparison of the daily rates of water-loss from upright and inverted shell fragments with sealed margins

Treatment	n	Mean rate of water-loss ($mg/cm^2/day$)	Variance	Var. Ratio	P
Upright	10	0.38	0.01	1.11	
Inverted	10	0.40	0.009		$0.7 > P > 0.6$

The probability value of $0.7 > P > 0.6$ did not reject the hypothesis that there was no significant difference in the mean rate of water-loss in the two groups of fragments. It seemed unlikely then that there was any real assymetry in the passage of water through the shells. The differences noted in the previous experiment were thus likely to have arisen as a result of leakage from the inverted fragments. This suggested that the inner layer of the shell might have been contributing more heavily to the waterproof nature of the shells than did the outer layer.

2.33 The site and nature of the impermeable regions of the shell

The suggestion above is in agreement with the results of Ives (1967 and pers. com.). She was unable to demonstrate that greater losses of water occurred from snails with holed shells than in those from a control group having holes drilled in the shells which were then sealed with paraffin wax. In all her determinations the snails had repaired the holes during the experimental period. It seemed that the initial repair was sufficient to prevent large losses in water through the hole, although the hole remained obvious and was only thinly repaired.

Wilbur (1964) has reviewed the subject of shell formation and regeneration, and stated that the first eventfollowing damage to the shell is for fluid to collect at the site of the hole. From the fluid is formed a thin organic membrane which gradually becomes calcified. In Helix, as in Helicella, the initial covering of the defect takes place within a few hours although complete repair requires much longer.

The following experiment was performed to test the hypothesis that the inner layer of the shell was the principal site of waterproofing.

From snails collected at Northfield twenty-eight shell fragments were mounted on tubes, fourteen upright and fourteen inverted, in the manner described in Section 2.32.

All tubes were weighed and placed over silica gel at 30°C for 29 days. On day 29 all tubes were reweighed and those that were obviously leaking were discarded from the experiment.

The remaining tubes of both types were randomized into two groups, such that for both upright and inverted fragments there was a set of control and experimental tubes. All tubes were replaced over silica gel at 30°C and reweighed on day 36.

On day 36, the exposed surface of each fragment on the experimental tubes was washed with chloroform. This washing was done by stroking the fragment with a fine camel-hair brush soaked in chloroform. All tubes were replaced over silica gel at 30°C until day 43 when they were reweighed.

On day 43, the exposed surface of each fragment on all the experimental tubes was scratched. This scratching was done with a small piece of fine emery paper glued to the end of a wooden matchstick. So that uniform scratching could be carried out a 10 gm piece of plasticene was wrapped around the matchstick, the emery paper was placed on the exposed surface of the fragment and the matchstick rotated five times.

Care was taken so that very little downward pressure was exerted on the matchstick. When all the experimental tubes had been treated in this way they were replaced together with the controls over silica gel at 30°C until day 56, when they were all weighed.

The rate of water-loss was calculated for each fragment in each weekly period. The results are presented in Table 2.5 as means of the daily rate of water-loss from each set of tubes for each week.

TABLE 2.5

The mean daily water-loss of the shell fragments over silica gel at 30°C after various treatments

Tubes	n	Mean rate of water-loss ($mg/cm^2/day \pm$ Std. Error)		
		(Week 1) Day 29 - 36	(Week 2) Day 36 - 43	(Week 3) Day 43 - 56
Upright Controls	5	0.36 ± 0.05	0.32 ± 0.06	0.36 ± 0.06
Inverted Controls	6	0.42 ± 0.04	0.40 ± 0.03	0.42 ± 0.04
Upright Experiment	5	0.39 ± 0.03	0.38 ± 0.04	0.41 ± 0.03
Inverted Experiment	7	0.44 ± 0.03	0.43 ± 0.04	1.00 ± 0.05

For the purpose of analysis, the mean change in rate of water-loss between weeks one and two, two and three and one and three was compared with zero for each group of fragments by means of a t-test. The null hypothesis was that no significant changes in rate took place between any weeks (i.e. following any treatment) during the period of the experiment. The probability values obtained are shown in Table 2.6.

TABLE 2.6

The probability that there was no difference in the change of rate of water-loss between weeks amongst tubes in a group

Tubes		Probability that $\Delta R = 0$		
		Between Weeks 1 and 2	Between Weeks 2 and 3	Between Weeks 1 and 3
Upright	Control	$0.7 > P > 0.6$ N.S.	$0.6 > P > 0.5$ N.S.	$P > 0.9$ N.S.
	Expt.	$0.7 > P > 0.6$ N.S.	$0.6 > P > 0.5$ N.S.	$0.7 > P > 0.6$ N.S.
Inverted	Control	$P = 0.9$ N.S.	$0.9 > P > 0.8$ N.S.	$P > 0.9$ N.S.
	Expt.	$0.9 > P > 0.8$ N.S.	$0.05 > P > 0.02$ *	$0.02 > P > 0.01$ **

From Table 2.6 it is obvious that the null hypothesis must be discarded for two values of Δ R. Thus,

(1) scratching the inverted fragments caused a significant increase in the rate of loss of water through these fragments, but this treatment did not alter the rate of water-loss from upright fragments.

(2) Washing fragments with chloroform did not cause a significant change in the rate of water-loss in either groups of tubes.

(3) From the control tubes it is obvious that there was no significant change in permeability in either upright or inverted fragments during the period of the experiment.

The results reported in Figure 2.06, which showed the rate of water-loss to be independent of the thickness of the shell, are consistent with the conclusion reached in this section that the impermeability of the shell to water is caused chiefly by a thin inner layer.

Although, as Wilbur (1964) pointed out, studies of the shell have contributed perhaps the largest fraction of the literature on Mollusca, none of these studies appear to have considered in detail the structural elements that might be responsible for the waterproof nature of some shells.

It is known that the organic matrix of the shell is secreted in soluble form from the tissues of the mantle and is deposited as a layer on the inner surface of the shell. Studies using the electron microscope

have shown the matrix to be a continuous sheet or a fenestrated sheet with the pattern of the holes a characteristic of the taxonomic group (Gregoire et al., 1955; Gregoire, 1957, 1960). X-ray diffraction studies have indicated that the matrix has an α or β -type keratin structure depending upon the species from which it comes (Wilbur 1964). Present also in the organic matrix is polysaccaride material, probably acid mucopolysaccaride (Durning 1957).

Studies of regenerating shells have shown that during repair the organic matrix is laid down first. The membrane becomes gradually calcified, and the shell thickened on the side external to the mantle. It is difficult to imagine how these processes could occur without a considerable amount of water being also passed through the inner layer of the shell. These processes are extremely complex, and as yet relatively little understood.

In formed shell it is possible that a waterproof layer is secreted on to or into the organic matrix. Campion (1961) has shown the presence of lipid secreting glands in the mantle of Helix aspersa. These glands were not abundant, and could only be demonstrated in frozen-dried tissues. It seemed possible that such glands, if present in Helicella virgata, might enable a lipid monolayer, or a lipoprotein to be secreted on or in the inner surface of the shell. This suggestion was not supported by the results of the previous experiment, where washing the inner surface of the shell fragments with chloroform did not appear to alter the rate

of water-loss through these fragments, however if such a lipid were present and bound in pores, or protected from the solvent in some other way, this result could still have been obtained. In the absence of any real knowledge of the detailed structure of the shell it would be unwise to reject the hypothesis at this stage.

In Section 2.11 it has been suggested that calcium may have been withdrawn from the shells of dormant snails. Clearly if this is to happen, the calcium ions must be transported through the inner layer of the shell.

Ives (1967) suggested that as the thin organic membrane is laid down prior to those parts of the shell external to it, there must be pores through which calcium carbonate, protein and polysaccarides can pass in soluble form. Such pores could be used for the resorption of calcium carbonate from the shell during dormancy. She further suggested that the principle of economy of hypotheses indicated that if such pores existed they would be permanent, yet scattered, and altogether making up a very small percentage of the total surface area of the otherwise impermeable inner layer of the shell.

Her model, in which the shell acts simply as a physical barrier to water, comprising a waterproof layer of closely packed, surface orientated, hydrophobic molecules, perforated at intervals by pores which pass to the more permeable laminae of calcium carbonate crystals and protein-polysaccaride matrix, probably cannot be improved upon at this stage of our knowledge.

Clearly this question of the structural nature of the waterproofing is of considerable complexity. It promises a new and potentially exciting field for investigation.

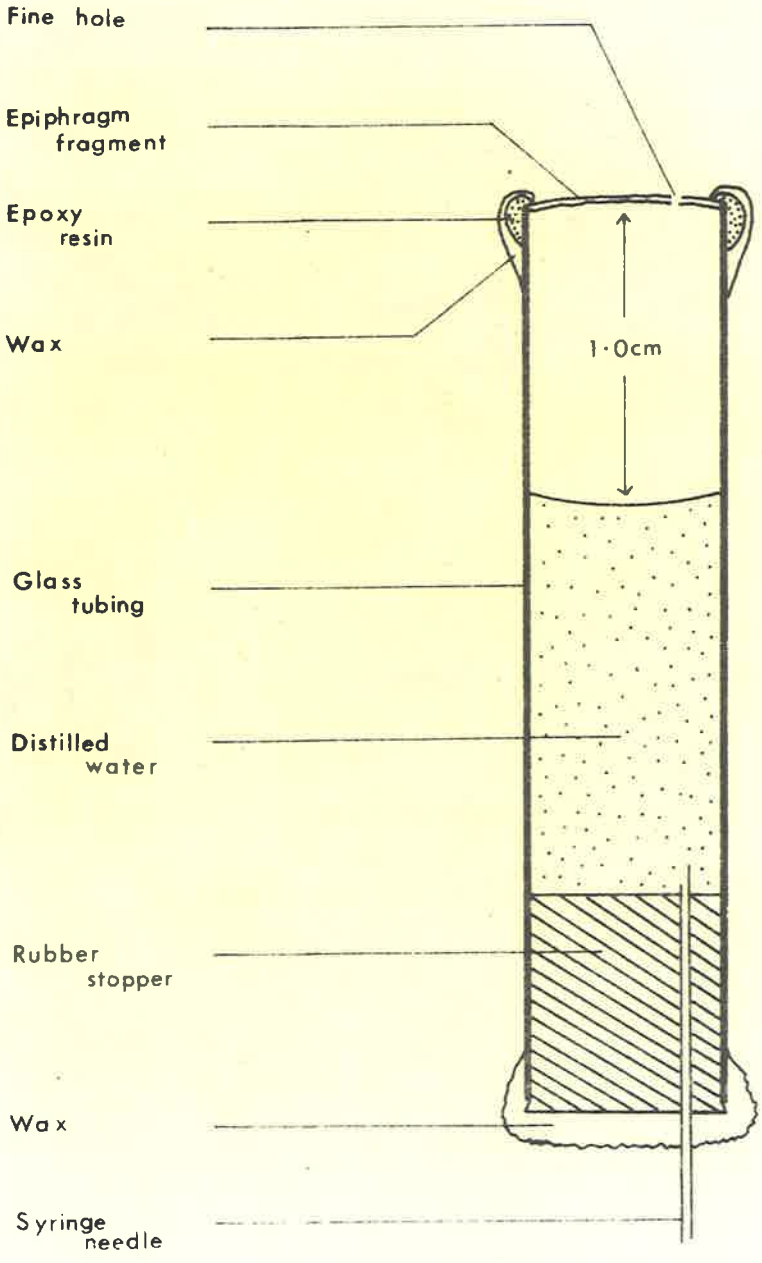
2.4 THE PERMEABILITY OF THE EPIPHRAGMS TO WATER

With a sharp scalpel and watchmakers' forceps, epiphragms were removed from snails that had been dormant for approximately four months on a bench in the laboratory. The thickness of these epiphragms was measured with a micrometer gauge to which an extra vernier scale had been fitted so that readings could be made to 0.0001 of an inch.

The epiphragms were glued to glass tubes of measured diameter with epoxy resin. In all preparations the outer surface of the epiphragm, as it occurred in the shell, was mounted with this surface facing outward from the glass tube. The method of mounting isolated epiphragms is shown in Figure 2.07.

When the epoxy resin had hardened, a very small hole was made through the epiphragms using only the tip of a 30 gauge hypodermic syringe needle mounted in a micromanipulator. This was done to ensure that the pressure on either side of the epiphragm would be equal, as damage might otherwise have resulted to the epiphragm when the tube was filled with water and sealed. The method of stoppering, filling and waxing the tubes was similar to that described in Section 2.31 for the shell fragments. Six control tubes, with aperture open, were also set up in this same way.

FIGURE 2.07. Method of mounting epiphragms for the determination of water loss (not to scale).



All tubes were weighed after filling and then placed over silica gel in a prewarmed desiccator at 30°C. The tubes were weighed again four and a half hours later.

The results of this experiment are presented in Table 2.7.

TABLE 2.7

Loss of water from unsealed tubes and tubes sealed with epiphragms of different thickness over silica gel at 30°C

Treatment	Thickness of Epiphragm (0.001 in)	Loss of water in 4.5 hrs (mg)	Rate of water-loss (mg/cm²/hr)
Unsealed tubes	-	3.7	25.7
	-	3.6	25.0
	-	3.8	26.4
	-	3.7	25.7
	-	3.7	25.7
	-	3.6	25.0
Sealed tubes	0.1	2.5	17.4
	0.1	3.0	20.8
	0.1	2.5	17.4
	0.2	2.6	18.0
	0.5	2.8	19.4
	0.6	2.5	17.4
	0.8	2.5	17.4
	1.0	2.5	17.4
	1.2	2.0	13.9
	1.2	2.2	15.3
	1.5	2.3	13.9
	1.5	2.0	16.0
	2.0	1.4	9.7
	2.0	1.2	8.3
	2.1	1.4	9.7
	2.2	1.7	11.8
	2.3	1.2	8.3

The results from Table 2.7 for the sealed tubes have been used to plot Figure 2.08, in which the relationship between thickness of the epiphragm and the rate of loss of water is shown. These results indicate that the permeability of the epiphragms to water was a function of their thickness. Thicker epiphragms retarded the passage of water molecules more than did thinner ones. Further, inspection of the figures in Table 2.7 indicates that even the presence of an extremely thin epiphragm caused a reduction in the rate of loss of water from those tubes compared with the unsealed control tubes. These results are in general agreement with those obtained by Machin (1968) for the epiphragms of Helix aspersa and Helix pomatia.

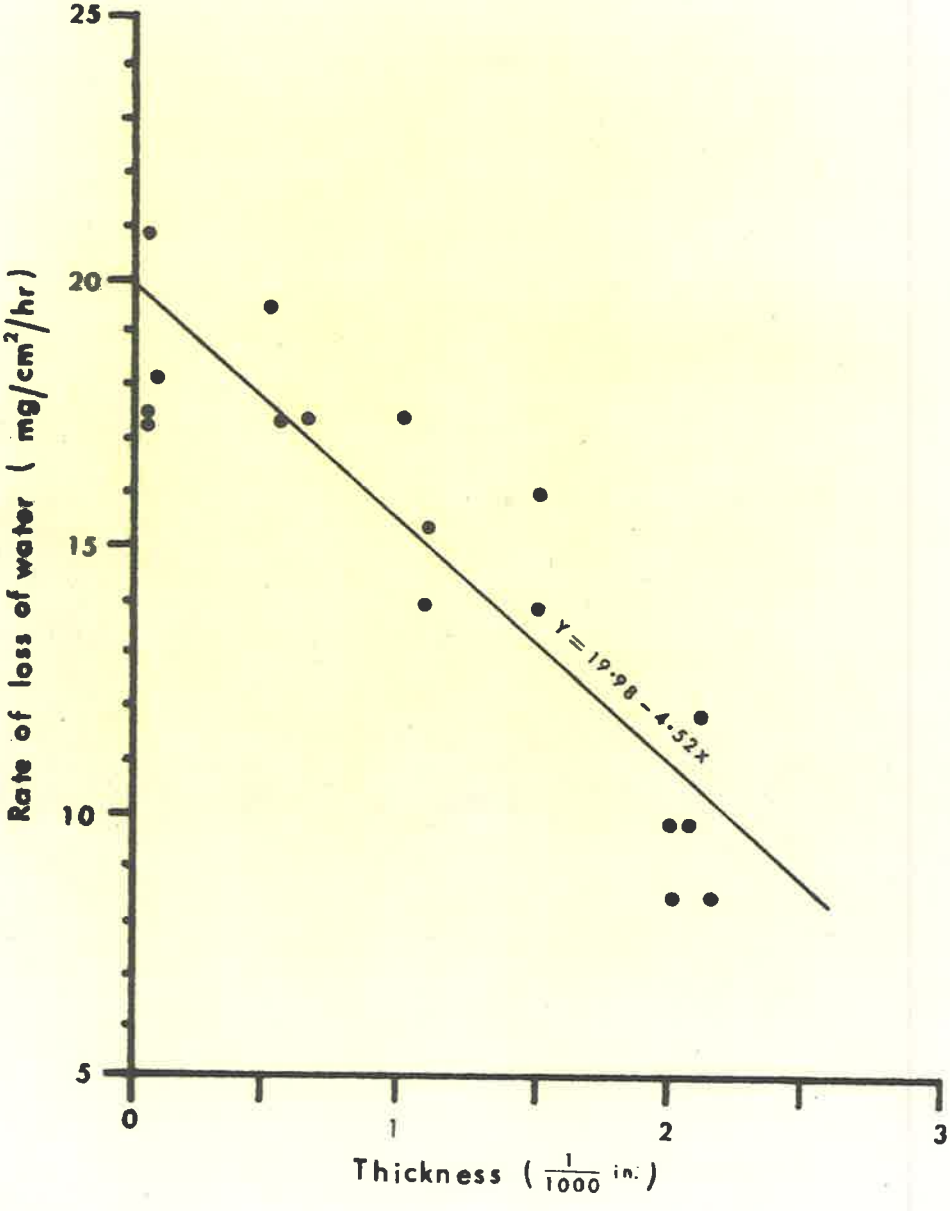
From the results in Table 2.5 it may be seen that the rate of water-loss through upright shell fragments was approximately 0.36 mg/cm^2/day. The results in Table 2.7 indicate that the mean rate of water-loss from the tubes covered by epiphragms was approximately 14.8 mg/cm^2/hr. Thus the epiphragm was seen to be approximately

$$\frac{24 \times 14.8}{0.36} \quad \text{or} \quad 987 \text{ times}$$

as permeable to the passage of water as the shell under similar experimental conditions (over silica gel at 30°C).

It is concluded from this experiment that while the epiphragms were approximately 1,000 times as permeable to water as the shells, they might reduce the amount of water lost from the aperture of dormant snails

FIGURE 2.08. The relationship between thickness of epiphragm and the rate of water-loss through it (30°C over silica gel).



under conditions of extreme saturation deficit like those employed in this experiment.

The epiphragms of Helicella virgata are variable with respect to the amount of calcareous inclusions that they contain. Very thin epiphragms may appear almost uncalcified, except for the spot above the pneumostome, thicker epiphragms appear to contain much inorganic material. Campion (1961) has suggested that in Helix aspersa at least, the organic fraction of the epiphragm is composed of mucopolysaccaride and protein. Mucoprotein is absent.

Machin (1968) did not find any assymetry in the passage of water through the epiphragms of Helix aspersa or Helix pomatia, indicating that the structure and properties of the epiphragm were uniform throughout its thickness. He suggested also that the calcium carbonate fraction of the epiphragm was unimportant as far as the permeability to water was concerned. The permeability of a given epiphragm simply varied inversely with its thickness.

Meenakshi (1964) considered that the "mucous seal" around the operculum of dormant Pila virens was "differentially susceptible to water". She considered that water could pass into the shell through this mucus but that none could pass out. It seemed worthwhile to investigate the epiphragms of Helicella virgata with respect to possible assymetrical passage of water molecules through the material.

Epiphragms were removed from snails that had been dormant for 4 months in the laboratory, and their thickness measured. The epiphragms

were placed into numbered tubes with care being taken to preserve the initial orientation of the material. Only epiphragms between 1.0 and 2.0 thousandths of an inch thick were used. The tubes were randomly divided into two groups. Ten epiphragms were glued upright, and ten inverted, to glass tubes in the manner described previously and the loss of water from these tubes was determined after four and a half hours over silica gel at 30°C. The results are shown in Table 2.8.

TABLE 2.8

The rate of loss of water through upright and inverted epiphragms over silica gel at 30°C

Rate of loss of water ($mg/cm^2/hr$)	
Upright	Inverted
10.4	12.3
10.8	15.7
18.4	11.1
15.3	10.6
14.6	13.9
12.7	15.2
15.9	18.2
16.5	17.0
16.3	16.5
13.7	14.7

The mean rate of water-loss in the two groups of tubes was compared using a t-test and the results of the comparison are shown in Table 2.9.

TABLE 2.9

Comparison of the mean rates of water-loss amongst tubes sealed with upright and inverted epiphragms

Treatment	n	Mean rate of water-loss ($mg/cm^2/hr$)	Variance	Var. ratio	P
Upright	10	4.5	6.6	1.03	
Inverted	10	4.5	6.4		$P > 0.9$

Clearly there was no significant difference between the mean rates of water-loss in the two groups. There is no evidence to suggest that any assymetry exists with respect to the passage of water through the epiphragms. These results agree with those of Machin (1968) for Helix aspersa and Helix pomatia.

2.5 FACTORS RESPONSIBLE FOR THE CONTROL OF WATER-LOSS FROM THE APERTURE

That the living tissues of the mantle-collar may retard the loss of water from the aperture is clear from the work of Machin (1966), Pomeroy (1966) and Ives (1967), but the means by which this is achieved is not well understood.

Machin (1966) concluded that in Helix aspersa there must be an active or passive barrier to water, and since tissue hydration was never observed in living snails, he concluded that this barrier must be a superficial one, but that it was unlikely to lie in the mucus covering the epidermis of the mantle-collar. He considered that such a barrier would be more likely to lie in a particular layer, 2μ thick, on the surface of the epidermis itself.

Pomeroy (1966) calculated for Helicella virgata the amount of energy that would have been required to keep water within the body of the snail on the assumption that an active transport process, or water pump, was operating. His results indicated that for such a process to operate more energy would have been required than was contained within the food reserves, and he favoured the hypothesis that the waterproof barrier was a passive, or structural one, possibly involving lipid or lipo-protein. Pomeroy, like Machin, stated that logically one would expect to find such a membrane located on the external surface of the epidermal cells of the mantle-collar and not in the mucus which overlies the cells.

Campion (1961) has demonstrated the presence of lipid secreting cells

in the mantle collar of Helix aspersa, and while these were sparsely distributed, it seems probable that they could secrete sufficient material, if only as a monolayer, to cover the surface of the epidermis in the region of the mantle-collar. If such a monolayer were produced it would tend to be protected by the overlying mucus.

Such a model raises an interesting question. If indeed such a monolayer did exist on the surface of the epidermis, what might one expect to happen to this when the snail produces another epiphragm and retracts further into its shell? It seems possible that such activity would disrupt any barrier, and might result in a brief period during which the rate of loss of water might increase.

Pomeroy (1966 p. 178) stated that few such "jumps" in the rate of water-loss occurred in snails during dormancy, and those that did occur were a feature of particular snails. All of his figures are presented in the form of "transformed rates of water-loss", derived from a formula in which the rate of water-loss was measured relative to the surface area of the snail, calculated as a function of it weight. As Ives (1967) pointed out, the application of this transformation depends on the assumption that "the water is lost in a similar manner through the shell and through the aperture". Her observations, and those in this thesis suggest that this assumption is not justified. It is likely that at least some of Pomeroy's conclusions when drawn from these figures may be in error, particularly as his method of calculating rates of water-loss would tend to mask any small changes in these rates.

Pomeroy (1966) was unable to show for Helicella virgata that the mean rate of water-loss differed significantly in snails with an epiphragm from those without one. From this he concluded that there was no evidence to suggest that epiphragms were in any way concerned with reducing the rates of water-loss from snails.

On the other hand, Machin (1967) found evidence in Helix aspersa, Otala lactea and Sphincterochila boissieri which suggested that the presence of an epiphragm reduced the amount of water lost from the aperture in all three species. Calculations based on his published figures show that for H. aspersa the rate of loss of water in snails with epiphragms was approximately 59 percent of that from snails without them, and for O. lactea and S. boissieri the figures were 64 and 69 per cent respectively.

In view of Machin's results it seemed wise to question Pomeroy's conclusion that the epiphragms of Helicella virgata were not in any way concerned with reducing water-loss from these snails, and the following experiment was performed to investigate this problem.

Fifty snails were collected in November from Northfield at the beginning of the summer of 1967. These snails were all of the size class 1.0 ± 0.1 cm with respect to the diameter of the shell. The animals were kept in an open cage in the laboratory for two months, and during this time all snails secreted calcareous epiphragms.

In January 1968, forty live snails were removed from the cage and divided into two groups of twenty animals. From one group the epiphragms

were removed with the aid of a sharp scalpel and fine forceps. The other group of animals was left with inact epiphragms. From the snails in which the epiphragms were removed it was observed that most snails had secreted two or three separate epiphragms, and it was concluded that those in the control group were probably possessed of similar numbers.

The removal of the epiphragms caused many of the snails to retract into the shells, and some muscular activity was noted in all the animals from this group.

All snails from both groups were then placed into small numbered bags, made from fine nylon mosquito netting, through which it was possible to observe the aperture of the snails. The snails were then placed over silica gel in a desiccator maintained at 25°C in a constant temperature cabinet. After 4 days the snails were again inspected and those which had formed epiphragms were discarded in the experimental group. All remaining snails were then weighed in their bags to the nearest 0.25 mg on a Mettler H.16 balance, and were returned to the desiccator for a further 14 days before being weighed again.

The rate of water-loss from these snails is shown in Table 2.10. Only eleven of the twenty snails from which epiphragms were removed had not secreted another during the period of the experiment, and the results from the nine snails that did secrete an epiphragm have been discarded.

TABLE 2.10

Rate of water-loss (mg/day) in snails with and without epiphragms at 25°C over silica gel

Rate of water-loss (mg/day)	
With Epiphragms	Without Epiphragms
0.52	0.65
0.70	1.30
0.65	0.95
0.97	2.00
1.01	1.60
0.95	0.75
1.01	0.95
0.63	2.10
0.84	1.75
1.21	2.04
1.00	1.52
0.75	-
0.59	-
1.05	-
0.76	-
0.63	-
0.57	-
1.02	-
0.63	-
1.00	-

The mean rates of water-loss in the two groups of snails were compared by means of a modified t-test (Bailey 1959 p. 51) as the variances

were not homogeneous. The results of this comparison are shown in Table 2.11.

TABLE 2.11

Results of a t-test comparing the mean rate of water-loss from snails with and without epiphragms

Treatment	n	Mean rate of water-loss (mg/day)	Variance	Var. ratio
Without Epiphragms	11	1.42	0.28	7.0
With Epiphragms	20	0.82	0.04	$[F^{10}_{20} = 2.51\ (5\%)]$

Calculated degrees of freedom = 11

d = 3.66 $\qquad\qquad$ $0.01 > P > 0.001$

The null hypothesis that there was no difference in the rates of water-loss between the two groups of snails was rejected at long odds. When the means are compared it may be seen that the mean rate of water-loss in snails with epiphragms is approximately 58 per cent of the mean loss in those with no epiphragm, and this figure seems to be in agreement with

Machin's results for the three species that he studied.

This result indicates that the epiphragms of Helicella virgata were acting to retard the loss of water from the aperture of the snails in this experiment.

The reasons for the difference in this result and Pomeroy's are not clear. However, I consider that Pomeroy's method of handling snails during weighing might have influenced the results that he obtained. He kept his snails in cotton bags that were opaque and removed the snail from the bag at each weighing. It is possible that in this way he might have unwittingly destroyed any epiphragms that the snails had produced in the interval between weighings. Further, his result was based on a set of experiments that were not primarily designed to test the hypothesis that the presence of an epiphragm conferred some degree of impermeability on the snails.

In Section 2.12 attention has been drawn to the fact that very many epiphragms may be produced by individual snails during protracted dormancy. The effect of multiple epiphragms on the reduction of water-loss would be difficult to assess, but as Machin (1967) pointed out it is probable that a greater degree of impermeability is achieved by such animals.

In Section 2.2, from the results shown in Figure 2.01, it was stated that there was little change in the rate of water-loss from whole snails at 20°C after the twentieth day of dormancy. In fact, the rate of loss between days 20 to 60 appears to have been somewhat greater than

that after day 60. It could be thought that such a result may have reflected the production of another epiphragm by many of the snails in the experiment.

One further point should be considered in connection with the function of the epiphragm. Measurements made in still air may underestimate the importance of an epiphragm in reducing water-loss from snails in nature.

Consider the situation where the aperture is sealed only with a thin, rather permeable epiphragm. Studies in still air could indicate that this sort of structure conferred no measurable degree of impermeability upon such an animal, and the conclusion might rightly be that the rate limiting barrier was the mantle-surface. In nature, however, even such a thin structure would almost certainly function to prevent the movement of air currents over the surface of the mantle. It is conceivable that had measurements been made in moving air the conclusion that the epiphragm conferred no extra impermeability on the snail might not have been drawn.

It does not seem far-fetched to suggest that this prevention of circulation of air over the mantle-tissues may be a prime function of the epiphragm to snails in the field, particularly in a species like Helicella virgata where relatively few individuals within a population become attached to objects that enable them to seal the aperture tightly against some impermeable material.

SECTION 3. THE PHYSIOLOGY OF DORMANCY

3.0 INTRODUCTION

Little is known about the physiological processes that occur within dormant snails. However in view of the importance of dormant phases in the life-cycle of many molluscs, exposed at times to environments that prevent normal activity, consideration of the processes involved in such animals is of considerable interest.

That dormancy may be a profound phenomenon is shown by the great length of time that some species may remain alive. Some specimens of Helicella virgata have survived for three years during the present study, but reports in the literature suggest that much greater periods of dormancy may be possible for other species. Baker (1935) reported that Oxystyla capax had remained alive for 23 years. Comfort (1957) doubted Baker's observation but accepted the report by Stearns (1877) of six years for Buliminus pallidor.

Such records may have prompted certain authors (among them Pelseneer, 1935; Comfort, 1957; Hunter, 1964; Owen, 1965) to use the term diapause to describe this phenomenon. The choice of this term is perhaps unfortunate, as it implies parallels with the insect condition of diapause which probably do not exist.

There is some justification for rejecting the terms hibernation and aestivation in gastropod studies. If these terms are to be retained as useful concepts they must be strictly defined and these definitions observed.

3.1 THE USAGE AND MEANINGS OF THE TERMS HIBERNATION AND AESTIVATION

There exists within the literature what I believe to be a largely unrecognised and potentially misleading situation arising from the terminology used to describe the kinds of "resting phases" shown by gastropods.

In studies of terrestrial pulmonates the terms "hibernation" and "aestivation" have been traditionally used to denote quiescence during winter and summer respectively. However the situation has been complicated by the fact that some workers have considered these conditions to be rather different, and have implied that different physiological phenomena occur within hibernating and aestivating snails of the same species. It is not my intention to review the literature in which these terms have appeared, but simply to discuss particular papers which I feel illustrate the possible confusion that has arisen from the use of these terms.

Howes and Wells (1934a) stated that active life in Helix pomatia was interrupted at intervals by resting phases "of two types between which it is important to distinguish". Hibernation occurred during the winter months and the mouth of the shell in such snails was said to be "typically closed by a dense calcareous plate, the epiphragm, whose (sic) secretion necessitates elaborate metabolic preparation". Aestivation, on the other hand, they described as a "phase of comparatively short duration, occurring occasionally during the summer, and believed to be a response to unfavourable

conditions". The mouth of the shell in such animals "is closed merely by thin films of dried mucus, running either transversely across the shell mouth or from the rim of the shell to some hard object ...". Such thin mucus films were described as "mucous veils" by Howes and Wells. These authors also distinguished between snails that were retracted and possessed no mucous veils and those that were aestivating. They termed snails in the former condition to be "withdrawn" but did not comment further.

Howes and Wells noted that "the view has been expressed that hibernation and aestivation may be passive results of environmental influences leading to dehydration of the animal". But on the basis of Kühn's (1914) and Künkel's (1916) observations (that they were unable to prevent hibernation in autumn by warmth, moisture and food), and that of Fischer (1931) who was unable to induce hibernation by injections of calcium salts or by cold, Howes and Wells stated that "there can be no doubt of the importance of internal factors in determining the onset of hibernation - the onset of hibernation seems to be a necessary outcome of metabolic preparations made during the summer".

It seems possible that Fischer's (1931) observation contributes little to the question of the role of dryness in causing hibernation. There is perhaps little reason to suppose that injections of calcium salts constitute "dryness" in the sense that these might influence a snail to enter hibernation.

Kühn's and Künkel's observations have not been repeated to my knowledge. As it stands their work constitutes the only evidence that some form of obligatory dormancy occurs in snails, a suggestion which may have prompted certain authors to use the term "diapause" to describe such dormancy. This work should be re-examined critically before these statements are accepted.

Howes and Wells (1934a) made what they termed a casual observation which suggested to them that "aestivation also might be determined in some measure by conditions internal to the organism". They noted that in a large stock of snails there were usually a certain number of aestivating animals, while others in the same container were active. They inferred that "either the snail population included individuals of different activity, some being inclined for reasons of their own to aestivate, or that there was a tendency for phases of aestivation to alternate with phases of activity during the life of any one individual, even under approximately constant external conditions ....".

These authors concluded that "evidently desiccating conditions will promote and often cause aestivation. But we (the authors) believe that the normal hydration cycles of the animals may also play a part".

Meyer and Thibaudet (1937) stated that they had confirmed the observations of Fischer (1931) on Helix pomatia, finding that animals would become inactive at any time of the year if kept without food in a dry place. These authors were unable to show seasonal differences in the response of Helix pomatia to such conditions, however they made the

distinction between hibernation and aestivation in a study of the changes in weight of snails kept without food under dry conditions. Snails kept over fused calcium chloride at 0 to 4°C were said to be in hibernation while those kept at 20 to 30°C over fused calcium chloride were considered to be in aestivation. Meyer and Thibaudet remarked that hibernating snails produced opaque calcareous epiphragms, often as many as 6 or 7, one behind the other. The animals at 20 to 30°C formed epiphragms that were generally thin and transparent.

It is obvious from this paper that Meyer and Thibaudet used the terms in a different sense from Howes and Wells who considered aestivation to be a short term phenomenon. Meyer and Thibaudet stated that aestivating animals lived for approximately the same lengths of time as hibernating animals, but produced no data to support this statement, neither does their paper indicate how such an experiment might have been performed.

These authors state further that after 8 months of hibernation snails were aroused to the active state by food, humidity and warmth. Aestivating animals were aroused after 5 months of dormancy, but the authors do not indicate how this was done or why these particular times were chosen.

Meyer and Thibaudet further suggested that during hibernation the loss of weight from individual snails was due principally to loss of water, however in "les animeux inanities 4 mois a 20° a 30°" (aestivating) the loss in weight was due partly to loss of water and partly to a decrease in tissue weight due to metabolism. Loss of water was greater in the aestivating snails, although the data presented in support of this claim

are somewhat less than convincing.

Without actually stating it, these authors convey the impression that this difference in the amount of water lost is due to some difference in permeability between aestivating and hibernating animals. It seems however, that while the humidity may have been similar at 0 - 4°C and at 20 - 30°C, due to the presence of calcium chloride, the saturation deficit at the higher storage temperature would have been very considerably greater. Thus it is not necessary to postulate a difference in the water retaining properties of the hibernating snails relative to those aestivating in order to explain the different amounts of water lost by snails in these treatments. There is little justification it seems for the remark by Little (1968) that it is "interesting to see that Meyer and Thibaudet showed that Helix pomatia died after similar periods of aestivation and of hibernation, although water loss was much greater during aestivation". Such a remark leaves one wondering whether Little was attempting to comment on what he believed to be a difference between two different physiological states or a simple temperature effect.

Hunter (1964) distinguished between hibernation and aestivation with reference to Helix spp. In his review he noted that hibernation is characterised by snails remaining dormant for long periods, usually over winter. Such snails possess hardened calcareous epiphragms. He suggested that the stimulus causing snails to hibernate is "unknown, but internal, requiring a longer period (than aestivation) of preparatory metabolic changes which may involve blood calcium". He stated that

aestivating snails were characterised by the fact that they became inactive for short periods at any time of the year and usually possessed thin transparent films of dried mucus across the opening of the shell. He suggested that snails might be stimulated to aestivate when their water content was low or possibly as a consequence of starvation. It may be seen that Hunter has used the terms in very much the same way as did Howes and Wells, implying differences in the physiological processes that occur in these two conditions.

Burton (1965) stated that "for much of its life, Helix pomatia is inactive, either hibernating with the shell closed by a calcareous epiphragm, or estivating, with the shell usually closed with one or more membranes of mucus". His description of these states is the same as Howes and Wells and Hunter but he refrains from suggesting that there are physiological differences between the two states.

The situation is further complicated by the use of the term aestivation to describe the kind of dormancy shown by fresh-water and semi-aquatic gastropods (Little, 1968; Meenakshi, 1954b, 1956a, 1958, 1964). These animals generally become dormant during summer, usually in response to the drying up of the pools and streams in which they live. Such animals may exhibit profound dormancy spanning considerable lengths of time, and generally resume activity only when water returns to the habitat. There are no reports to suggest that any form of short term inactivity occurs in these forms during periods when water is abundant.

Meenakshi (1956b) has shown for Pila virens that during this dormancy profound metabolic changes occur within the snails; blood calcium is involved in buffering the products of anaerobic metabolism; to this extent the form of dormancy found in freshwater snails resembles less the phenomenon of aestivation, as described by Howes and Wells and Hunter, than the condition described by those authors as hibernation.

Meenakshi described the dormancy in Pila virens as aestivation in all but one of her papers, however this paper (Meenakshi, 1956b) is titled "Physiology of hibernation of the apple-snail Pila virens (Lamarck)". In it she describes the condition of the snails as aestivation except for a single sentence in which they are described as hibernating. It is obvious that the same condition is being discussed throughout the paper so that one must infer that Meenakshi is either not aware of, or does not regard as important, the distinction made by such authors as Howes and Wells, Hunter, and Meyer and Thibaudet.

Little (1968) appeared to distinguish between hibernation and aestivation, but he did not make the distinction clear.

Machin, on the other hand, in a series of papers dealing with Helix aspersa has apparently rejected these terms (Machin, 1964, 1965, 1966, 1968). He referred only to "inactive" snails.

In his study of Helicella virgata, Pomeroy (1966, 1968) described any snail that was withdrawn into its shell as "dormant". In his 1968 paper he used the term aestivation to indicate any lengthy period of dormancy (several days or more) that occurred during summer, and he stated

that hibernation did not occur in H. virgata in South Australia.

Pomeroy's and my own observations on Helicella virgata have shown that, like Helix, individuals would retract into the shell (sometimes producing a "mucous veil" and sometimes nothing) on odd occasions during what appeared to be favourable conditions for activity. At such times many snails in the same area were active, crawling and apparently feeding. Such a condition was, it seemed, exactly analogous to the observations made on Helix pomatia by Howes and Wells (1934a) and quoted above. I considered that to describe this condition as aestivation in Helicella virgata would be misleading; it always occurred during winter, and often after rain. Further, the protracted period of dormancy in this species occurs over the hot, dry summer months in South Australia. At these times snails climb off the ground on to taller vegetation where they may remain for the entire summer of some three to four months. During this time the snails produce thickened calcareous epiphragms. It was tempting to follow Pomeroy and describe this condition as aestivation, however again it appeared unwise to do so as many of the features of what Howes and Wells described as hibernation were shown by Helicella during this period. To term such dormancy hibernation seemed absurd.

I consider that there is a need to distinguish between the two kinds of resting phases shown by gastropods. For this reason I have described the condition shown by H. virgata during summer as "dormancy", and I have retained the term "inactivity" to describe that condition shown by this snail during winter in South Australia. I consider that "inactive"

snails are merely interrupting briefly the activities that are normal to the season when they feed and reproduce (which is summer in the northern hemisphere and winter in the southern). "Dormant" snails, however, are avoiding through dormancy the rigours of a long unfavourable season (winter in the northern hemisphere and summer in the southern). There should be no semantic difficulty in recognising aestivation as dormancy during the summer, and hibernation as dormancy during the winter once the condition that I have called "inactivity" is distinguished from "dormancy". To clarify this point I would consider that what Howes and Wells (1934a) considered to be aestivation is better described as inactivity, while their condition of hibernation is in fact dormancy during winter.

The terms hibernation and aestivation, as defined above, cannot strictly be applied to studies in the laboratory because they imply seasonal differences. It seems illogical to extend the definitions of these terms to take account of the temperature conditions. Thus while both groups of snails in the experiments of Meyer and Thibaudet were clearly dormant, it does not seem sensible to term one group hibernating and the other aestivating. I would prefer to describe the former group as dormant between $0^{\circ}C$ and $4^{\circ}C$ and the latter as dormant between $20^{\circ}C$ and $30^{\circ}C$. In this way any possible confusion which may result from the use of the terms hibernation and aestivation is avoided. It may be that these terms should now be discarded from the field of molluscan studies.

3.2 PREVIOUS WORK ON DORMANT Helicella virgata

Pomeroy (1966) showed that in general, for temperatures between 25 and 40°C, the length of life in dormant H. virgata was related to the humidity at which they were kept, and inversely related to the temperature. At low humidities (less than 6% R.H.) and at temperatures 25°C to 30°C snails survived in excess of 200 days.

The length of life under such conditions varied depending upon the time of the year at which the snails were collected. In general, snails collected during the early winter did not survive as long under warm, dry conditions as did those collected in late Spring when they were ready to climb.

Pomeroy considered the causes of death amongst dormant Helicella, and concluded that it was unlikely that snails in his experiments died from desiccation. He considered also the possibility that the snails were dying as a result of their having accumulated metabolic products within the tissues, and found no evidence to support this. He therefore suggested that the most likely cause of death amongst dormant H. virgata was starvation resulting from the expenditure of the animal's food reserves.

Pomeroy also investigated the change in dry weight of samples of snails kept dormant at different temperatures and humidities. He found that although the length of life varied greatly in the different treatments, the mean dry weights of the samples did not decrease significantly from the dry weight of an initial sample taken at the time of collection.

He even suggested that the dry weight may have increased slightly, but was not able to offer an explanation for this observation. He therefore suggested that the changes in weight that occurred in dormant H. virgata could be accounted for entirely by the amount of water lost from the animal.

3.3 THE LENGTH OF LIFE AND CHANGES IN WATER AND DRY MATTER CONTENT OF DORMANT SNAILS

The observation made by Pomeroy (1966), that no decrease in dry weight occurred in snails during dormancy, seemed surprising. I decided to perform a similar experiment in order to confirm his observation.

3.31 Experiment 3.1

To investigate the changes in dry weight of samples of snails kept dormant at 20°C relative to the weight of an initial sample taken at the time of collection

Two hundred snails were collected from Buckland Park (2.10.65). These snails were inspected for damage in the laboratory and 160 were then distributed randomly between four treatments each of 40 snails. The experiment was conducted in the following way.

On day 0 (2.10.65), the snails from the first group were killed by brief immersion in hot water. They were then dried to constant weight in a vacuum drying apparatus, which consisted of a chamber connected to an electrically operated vacuum pump. The snails were then weighed

individually.

The remaining treatments were similarly killed, dried and weighed after 7, 35 and 100 days at 20°C in a dessicator containing silica gel. Table 3.1 shows the analysis of variance of these data.

TABLE 3.1

Analysis of variance of effect of time in dormancy on dry weight

Source of Variance	D.F.	S.S.	M.S.	V.R.
Between treatments	3	1337.1	445.7	2.55
Within treatments	156	27247.7	174.66	
Total	159	28584.8		

This gives $P > 0.05$, so that the null hypothesis that the dry weights of snails was unaffected by the time in dormancy is not rejected by these data and the results agree with those of Pomeroy (1966).

It was therefore apparent that dry weights of snails could not be used to measure the decrease in stored food reserves of dormant snails. Some direct measure of the changes in energy content of the tissues would have to be used.

A preliminary experiment to observe the decrease in energy content of the snail tissues was designed using the bomb calorimeter to measure the total amount of energy liberated by known weight of snail tissue on complete combustion to carbon dioxide and water. In this experiment it was impossible to work with individual snails as the amount of tissue, and hence heat of combustion was too small to be measured accurately on the type of bomb calorimeter used.

3.32 Experiment 3.2

A preliminary experiment to observe changes in the energy and water content of dormant snails

Approximately 1,200 snails were collected from Buckland Park, about three weeks after climbing had begun, in December 1965. These snails were randomized amongst 20 small cages, of the sort described by Pomeroy (1966) as "probit boxes", so that each cage contained 50 snails. One cage was chosen at random for the initial sample and the remainder were randomized amongst three desiccators containing silica gel. The desiccators were maintained at 20°C in a constant temperature cabinet.

On day 0 (7.12.65), the initial sample of 50 snails was weighed alive, the snails were then killed by brief immersion in hot water and the body tissues withdrawn complete from the shells. The shells and bodies were then dried to constant weight in the vacuum drying unit and weighed separately. The bodies were burnt in a "Gallenkamp Ballistic Bomb Calorimeter" (which had previously been calibrated with Benzoic Acid)

and the heat content of the tissues was expressed as calories per milligram dry-body weight. At approximately monthly intervals a further sample of 50 animals was withdrawn from the desiccators and treated in the manner described above. At each determination the silica gel was replaced in all the desiccators.

The results of this experiment are presented in Table 3.2.

3.321 Changes in dry weight

To determine whether changes in dry weight had occurred during dormancy the data was subjected to regression analysis. The regression equations were calculated for the relationship between time dormant and mean dry weight of snails in each sample, and for the relationship between time dormant and mean dry tissue weight.

In both, the coefficient of the calculated regression line was compared with zero. The null hypothesis being that neither of the regression coefficients differed significantly from zero.

The plots, together with the equation for the line and the probability of the regression coefficient being equal to zero are shown in Figures 3.01A and 3.01B for the relationship time dormant against mean dry weight and mean dry weight of tissues respectively. It may be seen from these data that the probability values are large for both quantities. Therefore the null hypothesis was accepted, indicating that no significant changes had occurred in either the mean dry weight, or the mean weight of dry body matter, in the samples during the period of dormancy.

FIGURE 3.01. A. (above) The relationship between time in dormancy at 20°C over silica gel (days) and the mean dry weight of snails in each sample (mg.)

The probability that the slope of the regression line does not differ from zero is $0.4 > P > 0.3$.

FIGURE 3.01. B. (below) The relationship between time in dormancy at 20°C over silica gel (days) and the mean dry tissue weight (mg.).

The probability that the slope of the regression line does not differ from zero is $0.2 > P > 0.1$.

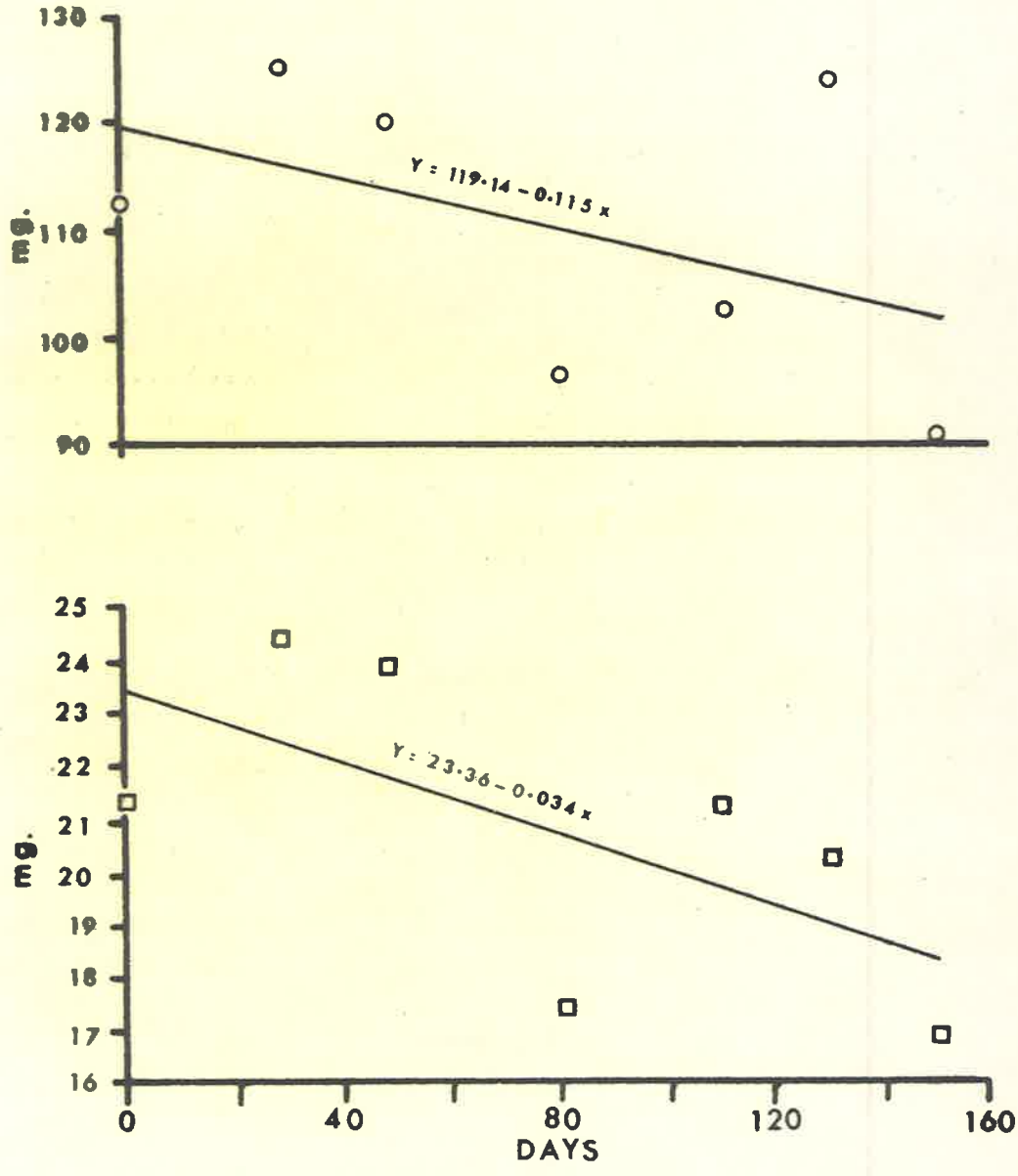

130
120
110
100
90
mg.
Y = 119.14 - 0.115 x
25
24
23
22
21
20
19
18
17
16
mg.
Y = 23.36 - 0.034 x
0
40
80
120
160
DAYS

TABLE 3.2

Results of Experiment 3.2 with snails from Buckland Park

Days Dormant	Sample size	Live wgt. (mg.)	Total dry wgt. (mg.)	Dry wgt. bodies (mg.)	Wgt. water in bodies (mg.)	Total energy (cal./mg.)	Dry body / shell
0	50	18411.60	5580.85	1064.35	12830.75	4.73	0.235
28	50	16425.00	6273.50	1220.50	10151.50	4.59	0.241
48	50	14421.00	6010.65	1192.15	8410.35	4.51	0.246
80	50	10477.20	4815.95	868.45	5661.25	4.33	0.220
110	49	10646.90	5029.45	950.95	5617.45	4.19	0.231
130	50	9339.15	6227.70	1008.40	4711.40	4.03	0.239
150	50	7920.05	4519.70	839.70	3400.35	3.85	0.228
170	more than 50% of snails in all desiccators had died						

3.322 Change in water content

The live weight of the samples decreased during dormancy to less than half the weight of the initial sample. As no decrease in the total dry weight (shells and tissues) of the samples could be demonstrated the results agree with those of Pomeroy (1966) and this decrease in weight was caused by water loss. Figure 3.02 shows the water content, expressed as a percentage of the total wet tissue weight, of the sample at each determination. The plot suggests that the amount of water present in the tissues decreased steadily from an initial 92% of the tissue weight to approximately 80% before death.

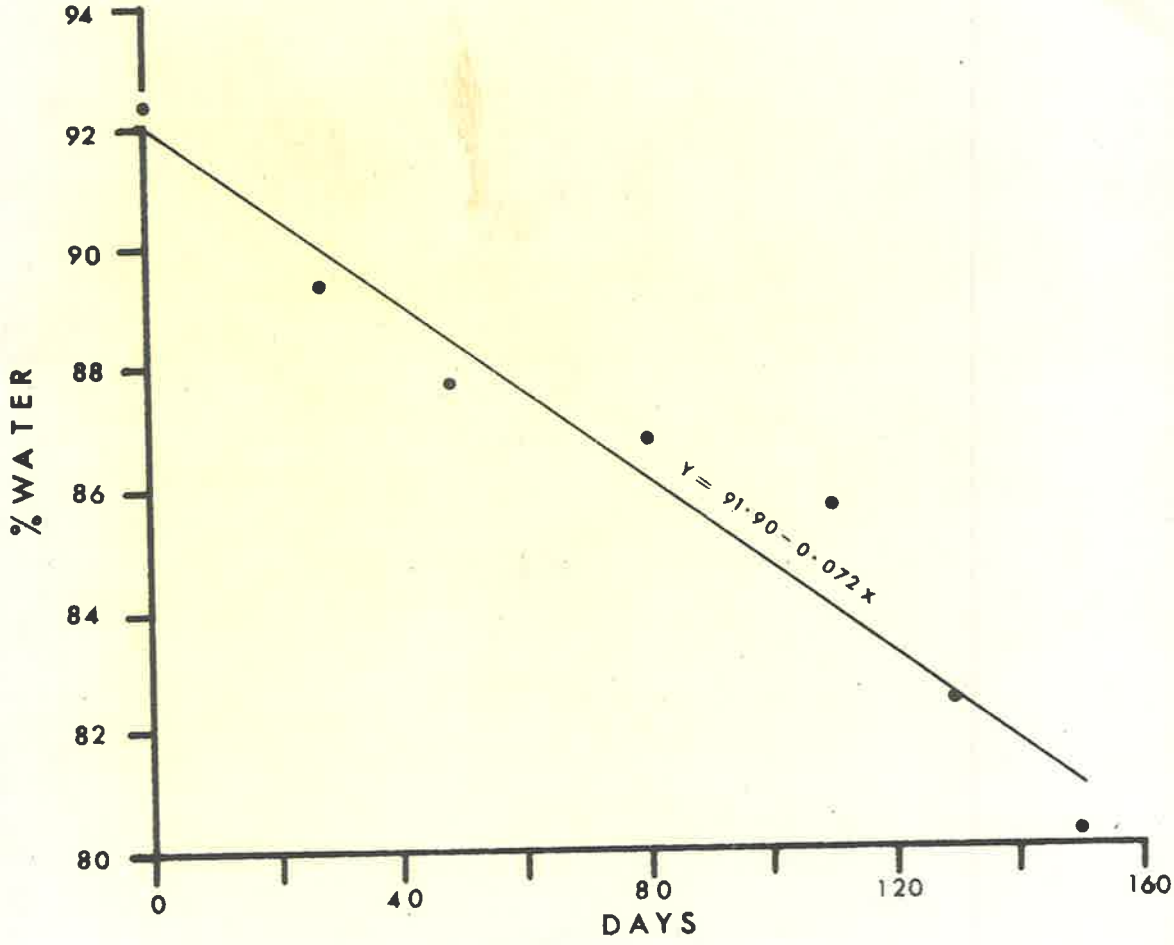
3.323 Change in energy

There was a decrease in the energy content of the body tissues in dormant snails (Table 3.2). Because of the lack of any significant change in the mean dry weight of tissue in the samples during dormancy, the difference in the quantity of energy contained per unit dry weight of tissue at any two observations, was thought to constitute a valid estimate of the change in energy content between those two observations. In this way the energy expenditure per unit time of "an average snail" from the population within the desiccators could be estimated directly (assuming complete randomization).

3.324 Time at which snails died

After the determination at 150 days, and before day 170, most of the remaining snails in the desiccators had died. It seemed unreasonable

FIGURE 3.02. The relationship between the time in dormancy at 20°C over silica gel (days) and the water content of the samples (expressed as a percentage of the wet tissue weight).



to suppose that individual snails had died simultaneously and independently as a result of their being exactly alike with respect to rate of metabolism or water-loss. The more likely explanation seemed to be that some snails in each desiccator had died and that this had caused the death of the remaining animals.

Pomeroy (1966) pointed out that snails collected at the end of summer lived for only a short time when exposed to high temperature, but those collected at the beginning of summer were able to survive for longer periods at the same temperature. He found that at 35°C and approximately 5% Relative Humidity, neither the crowding of living snails, nor the introduction of freshly killed animals caused a significant difference in the length of life of animals from that of a control group. Pomeroy did not record the date of collection of snails used in this experiment but it must be presumed from the length of life of the animals (mean of 55 days) that they were not collected at the end of summer when either food-reserves or water-contents were low.

The effect of dead snails may be different on snails collected towards the end of dormancy. Pomeroy's experiment does not indicate the effect of dead animals on snails with depleted reserves of food and water. It is probable that by the time snails were beginning to die the dead animals introduced at the beginning of his experiment were quite dry and unlikely to influence the living specimens.

Pomeroy also found that the length of life of 50 individuals placed in a desiccator together with 400 others did not differ significantly from

that of 50 control snails. This was evidence contrary to the suggestion that dying snails kill others, so I decided to conduct a similar experiment to attempt to resolve this possible discrepancy.

3.33 Experiment 3.3

To investigate the effect of crowding on the length of life of dormant snails

"Crowding" may not simply be a function of the number of snails within a desiccator. For example the micro-environment within a cage containing 50 snails may be similar irrespective of the number of other cages present in the dessicator, hence the spatial arrangment of snails may in part decide whether similar results are observed in treatments containing very different numbers of individuals.

Fifty snails were collected from Northfield (1.11.66) at the end of Spring before climbing had begun. These snails were of one size class (1.5 ± 0.1 cm) with respect to the diameter of the shells. These snails were then randomized into two treatments of 25 snails each. Snails in the first treatment ("individually stored") were placed in small numbered bags made from nylon mesh. They were weighed individually and each snail was placed into a 2" x 1" glass specimen tube containing silica gel. Each tube was fitted with a plastic top.

Snails in the second treatment ("crowded") were also placed into individual nylon bags, weighed and then placed together into a screw-top bottle containing silica gel. The volume of air contained in the bottle

was approximately 25 times as great as that of an individual tube. Both the tubes and the bottle were kept in a desiccator at 25°C in a constant temperature cabinet.

At weekly intervals all snails were individually weighed in their bags, the time of death being shown by a sudden and large decrease in the weight of an individual (Pomeroy 1966). At each weighing the silica gel was replaced in all tubes and in the bottle. All snails were replaced in the treatments after each weighing. The results are presented in Figure 3.03, where the numbers of snails dying in a particular week are shown for both individually stored and crowded animals.

For the purposes of analysis the length of life of an individual snail was arbitrarily considered to be given by the time from the beginning of the experiment to the end of the last week in which it was known to be alive. In practise this would tend to underestimate the length of life for most snails.

The mean length of life for snails in both treatments was compared by means of a t-test. The null hypothesis being that there was no significant difference between the mean length of life of crowded snails with respect to that for snails kept individually. The results of this comparison are shown in Table 3.3.

FIGURE 3.03. The time at which snails died at 25°C over silica gel.

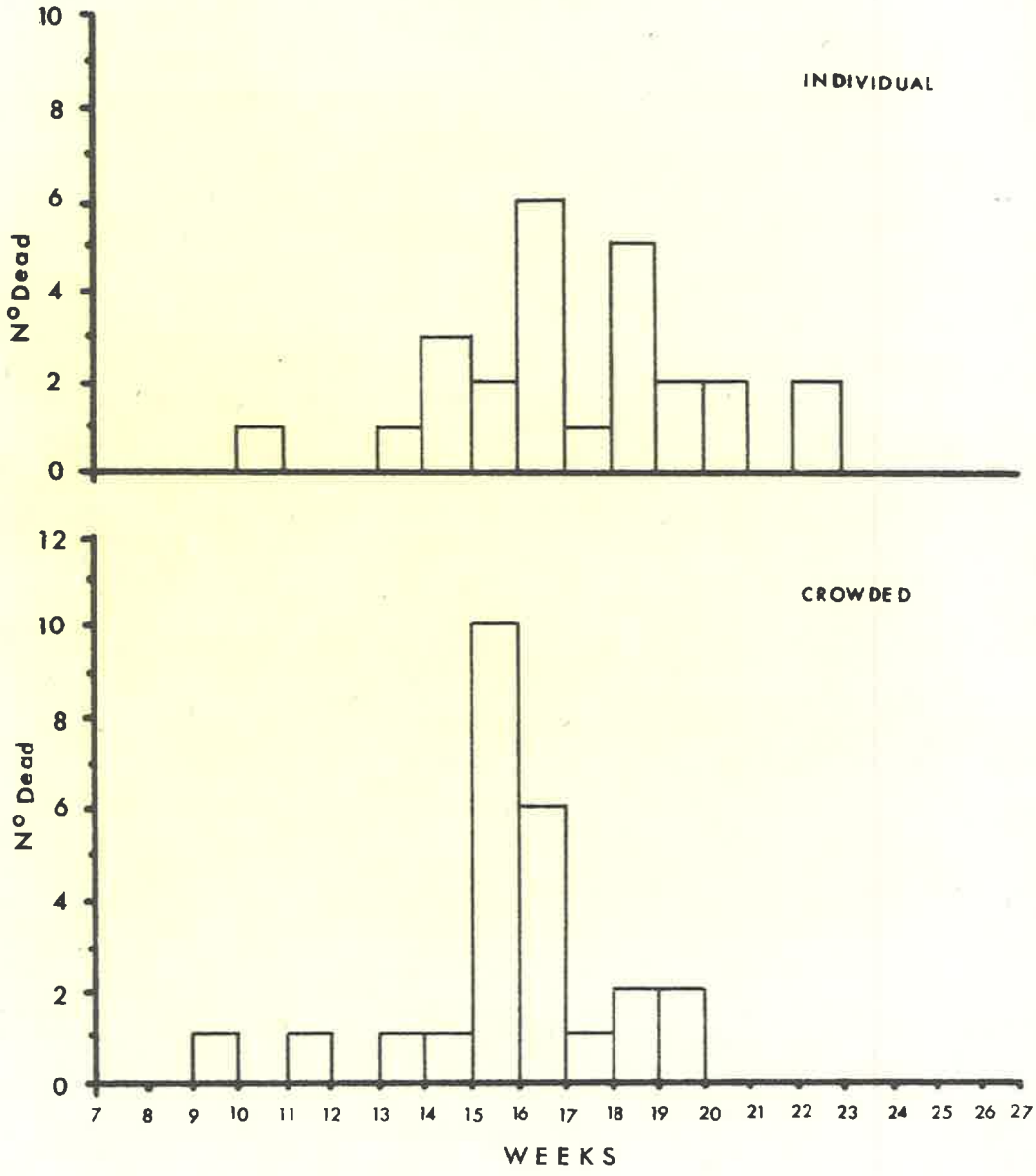


TABLE 3.3

Results of the t-test comparing the means of the length of life in crowded and individual snails

Treatment	Mean (wks)	Variance	Var. ratio	Probability
Individuals	16.80	7.75	1.63	
Crowded	15.36	4.75		$0.6 > P > 0.5$

Neither the means nor the variances are significantly different (at the 5% level) in the two groups. The null hypothesis is not rejected by this data and I concluded that crowding had not affected the length of life of snails relative to individual animals that were maintained singly. This result supports Pomeroy's (1966) finding and suggests also that the snails in Experiment 3.2 from Buckland Park were not dying as a result of their having been crowded.

3.34 Experiment 3.4

An experiment to observe the length of life and changes in energy and water content of dormant snails kept at different temperatures

The length of life of dormant snails differs at different temperatures (Pomeroy 1966). If one assumes that the cause of death is the same at all temperatures within certain physiological limits, then the changes in energy and water content, and the value of these quantities at death, should indicate the relative importance of starvation and desiccation as causes of death in dormant snails.

A similar experiment to that described for snails from Buckland Park (Experiment 3.2) was performed. Approximately 1,500 snails were collected from Northfield (1.11.66) on the same date as those for Experiment 3.3. The snails were all of one size class (1.5 ± 0.1 cm) with respect to the diameter of the shells.

In the laboratory the snails were randomized into 48 cages (each containing 25 snails). One cage was withdrawn for the initial sample and the remainder randomized between six desiccators. Two desiccators were then placed at random in each of three constant temperature cabinets at 20, 25 and 30°C.

At approximately fortnightly intervals a sample of 25 snails was withdrawn from a desiccator at each temperature for the determinations and the procedure described above for Experiment 3.2 was followed. Determinations were continued until more than 50 per cent of the snails

contained in a sample box at a particular temperature had died.

The results appear in Tables 3.4, 3.5 and 3.6 for temperatures of 20, 25 and 30°C.

Regression analysis has been employed to investigate whether any significant changes occurred in mean dry weight or the mean weight of the dry body matter during dormancy. The plots, together with the equation for the line and the probability of the regression coefficient being equal to zero, are shown in Figures 3.05 and 3.06 for the relationships time dormant against mean dry weight and time dormant against mean dry tissue weight respectively.

TABLE 3.4

Results of Experiment 3.4 Northfield snails dormant at 20°C

Days Dormant	Sample size	Live wgt. (mg.)	Total dry wgt. (mg.)	Dry wgt. bodies (mg.)	Wgt. water in bodies (mg.)	Total energy (cal./mg.)	Dry body / shell
0	25	14205.90	3972.95	1204.35	10232.95	4.87	0.435
14	25	13058.90	3770.70	1191.55	9288.20	4.83	0.462
28	25	12219.40	3935.75	1234.50	8283.65	4.75	0.457
48	25	10975.95	3846.50	1197.40	7129.45	4.58	0.452
66	25	10699.90	3937.00	1193.45	6762.90	4.47	0.435
95	25	9428.85	3601.75	1091.80	5827.10	4.34	0.435
107	25	9670.70	3812.65	1177.80	5858.05	4.31	0.447
120	25	9709.60	4003.55	1267.00	5706.05	4.24	0.463
130	25	8334.30	3684.90	1089.90	4649.40	4.14	0.420
140	24	7727.85	3521.75	1009.80	4206.10	4.06	0.402
150	25	8401.65	3847.70	1120.75	4553.95	3.94	0.411
160	22	7871.50	3643.50	1095.60	4228.00	3.74	0.430
170	more than 50% of snails in both desiccators had died						

TABLE 3.5

Results of Experiment 3.4 Northfield snails dormant at 25°C

Days Dormant	Sample size	Live wgt. (mg.)	Total dry wgt. (mg.)	Dry wgt. bodies (mg.)	Wgt. water in bodies (mg.)	Total energy (cal./mg.)	Dry body / shell
0	25	14205.90	3972.95	1204.35	10232.95	4.87	0.435
14	25	12669.15	3794.25	1179.25	8874.90	4.85	0.451
28	25	10686.75	3896.55	1175.50	6790.20	4.57	0.432
48	25	9837.92	3853.20	1192.15	5984.72	4.51	0.448
66	25	9085.10	3666.40	1130.85	5418.70	4.42	0.446
79	25	9044.65	3669.45	1150.95	5375.20	4.18	0.457
95	25	8458.70	3847.95	1197.85	4610.75	4.02	0.452
107	25	7593.00	3668.20	1135.70	3924.80	3.87	0.449
120	more than 50% of snails in both desiccators had died						

TABLE 3.6

Results of Experiment 3.4 Northfield snails dormant at 30°C

Days Dormant	Sample size	Live wgt. (mg.)	Total dry wgt. (mg.)	Dry wgt. bodies (mg.)	Wgt. water in bodies (mg.)	Total energy (cal./mg.)	Dry body / shell
0	25	14205.90	3972.95	1204.35	10232.95	4.87	0.435
14	25	11485.50	3810.80	1206.00	7674.70	4.74	0.463
28	25	9694.45	3897.90	1187.25	5796.55	4.56	0.438
48	25	8801.10	3768.50	1171.30	5032.60	4.36	0.451
66	25	8251.60	3843.40	1102.05	4408.20	4.13	0.402
79	25	7921.70	3742.55	1088.25	4179.15	3.90	0.410
86	24	6349.05	3550.10	1010.70	2798.95	3.76	0.398
98	more than 50% of snails in both desiccatorshad died						

Discussion of Experiment 3.4

3.341 Length of life

The length of life of "an average snail" was an estimate based on the time taken for more than 50 per cent of the snails in each treatment to die. At all three temperatures very few snails had died before the last determination shown in Tables 3.4, 3.5 and 3.6. However, 50 per cent or more snails had died quite suddenly in all treatments before the next determination for that treatment became due. On the basis of the result of Experiment 3.3 it is suggested that this sudden mortality was probably not the result of the snails having been crowded in the desiccators, and the length of life of "an average snail" was thus taken to be approximately 98 days at 30°C, 120 days at 25°C and 170 days at 20°C.

Pomeroy (1966) found that at the same temperatures, and at humidities of less than 6 per cent, H. virgata lived for approximately 236, 250 and 260 days at 30, 25 and 20°C respectively. His experiments were very much more precise and were based on records for individual animals.

Baverstock (pers. com.) found that, for H. virgata collected at Northfield, the length of life at temperatures slightly above 30°C was only about half of that observed for snails at 30°C in Experiment 3.4 and very considerably less than the figures given by Pomeroy. It seems likely that these differences are due to differences in the snails. These observations are likely to be equally valid, but great care should be exercised in comparing the results of different workers.

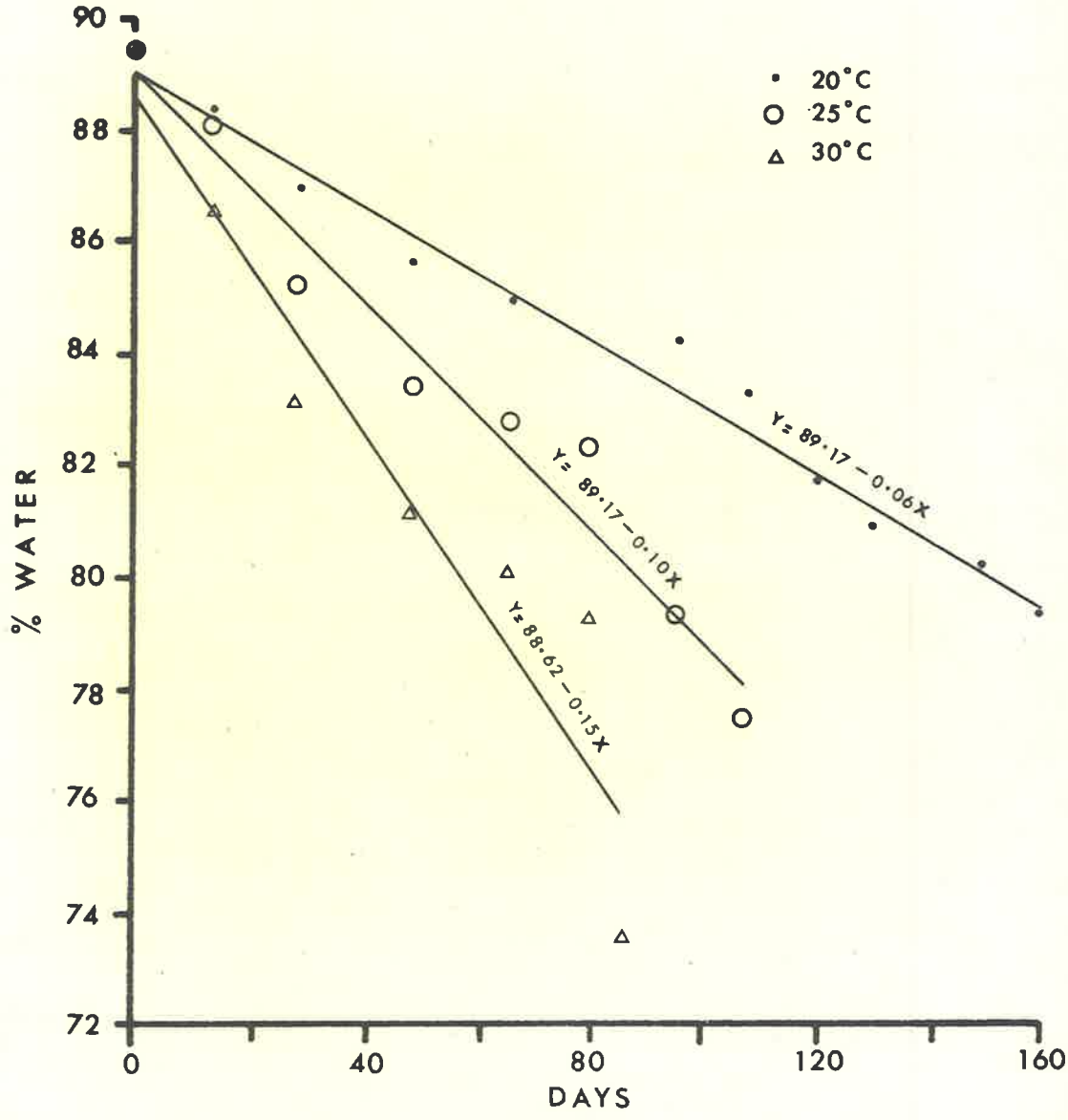
3.342 Changes in water content

Figure 3.04 shows the amount of water (expressed as a percentage of the wet weight of tissues) that was present in the samples, at each determination, for each temperature. At the last determinations, water made up 79.4 per cent of the tissue weight in snails dormant at 20°C, but only 77.5 per cent and 73.5 per cent of the tissue weight in those dormant at 25° and 30°C respectively, thus it is apparent that snails at higher temperature lost water at a faster rate, and also more water, than snails at lower temperature.

The amount of water lost before death from the Buckland Park snails (Experiment 3.2) is considerably greater at 20°C than the amount lost by Northfield snails at the same temperature. It is unwise to attempt to compare these losses because the collections of animals were made in different years and from areas approximately 30 miles apart. Further, the collection at Buckland Park was made some weeks after the onset of natural dormancy, during rain. It seems likely from consideration of the amount of water present initially in these snails that not only were the tissues fully hydrated at the time of collection, but free water may also have been present, perhaps in the mantle cavity (Blinn 1964).

Pomeroy (1966) showed that at death the average loss in weight of specimens of Helicella virgata, kept at different temperatures, varied. This loss in weight was due to loss of water from the animals. His experiments showed that the greatest loss occurred in snails kept at

FIGURE 3.04. The relationship between time in dormancy over silica gel (days) and the water content of the samples (expressed as a percentage of the wet tissue weight).



30°C, where approximately 23 per cent of the initial weight was lost before death. At 35°C and 40°C snails in his experiments lost an average of 10 per cent and 1 per cent respectively of the initial weight, and at 25°C approximately 20 per cent.

In the Experiment 3.4 similar differences to those noted by Pomeroy were found to exist in the mean amounts of weight lost per snail to the last determination before death. The losses are of greater magnitude than those reported by Pomeroy, however, greatest loss occurred in the snails kept at 30°C (53% of initial weight) and least loss (37% of initial weight) in those snails kept at 20°C.

3.343 Changes in dry weight

As has been pointed out, Experiment 3.1 failed to show that any significant change occurred in the total amount of dry weight of the snails during dormancy. This result agreed with Pomeroy's finding. Further, Experiment 3.2 with snails from Buckland Park also suggested that during dormancy at 20°C no significant change (5% level) occurred in either the mean dry weight or the mean weight of the dried body tissues.

Experiment 3.4, with Northfield snails indicated that, when the regression coefficients of the calculated regression equations, for the relationship between time dormant and the mean dry weight of snails in each sample, were compared with zero, only that for the snails at 30°C showed a departure from zero at the 5 per cent level of significance. The slope of the regression did not differ significantly (5% level) from zero at

20°C or 25°C (Figure 3.05).

A similar result was obtained for the relationship between time dormant and mean dry tissue weight (Figure.3.06). Only amongst snails dormant at 30°C was there a significant change in the mean dry weight of the body tissue during dormancy.

Those results from snails dormant at 20°C lend support to Pomeroy's suggestion that no change in dry weight takes place during dormancy. The results from Experiment 3.1 and 3.2 are also from snails kept at 20°C and they too support Pomeroy's finding, however the results from 30°C do not.

These results are based on samples of snails of a particular size class and particular care was taken to ensure that the snails were completely randomized between the three treatments. Further, if small changes in these quantities were occurring, regression analysis of the form used should have provided a sensitive test of the significance of these changes. For these reasons I consider that at temperatures above 25°C (although this may vary with the acclimatized state of the snails) small, but significant losses in dry weight do occur. This result is in general agreement with that of Meyer and Thibaudet (1937). These authors reported that in specimens of Helix pomatia, dormant at 0 to 4°C, the loss in weight could be accounted for entirely by loss of water from the animals. However in those specimens dormant at 20 to 30°C some losses in dry weight did occur.

From Table 3.2 and Tables 3.4, 3.5 and 3.6 it is clear that the ratio of the weight of the dried body tissue to that of the shell was different in those snails from Northfield and Buckland Park. The Northfield

FIGURE 3.05. The relationship between the time in dormancy over silica gel and the mean dry weight (mg) of individual snails in the samples at different temperatures

A. (above) Snails dormant at 30°C

The regression equation is $Y = 157.03 - 0.093x$

The probability that the coefficient of regression does not differ from zero is $0.05 > P > 0.02$

B. (centre) Snails dormant at 25°C

The regression equation is $Y = 156.38 - 0.083x$

The probability that the coefficient of regression does not differ from zero is $0.2 > P > 0.1$

C. (below) Snails dormant at 20°C

The regression equation is $Y = 154.60 - 0.006x$

The probability that the coefficient of regression does not differ from zero is $0.9 > P > 0.8$

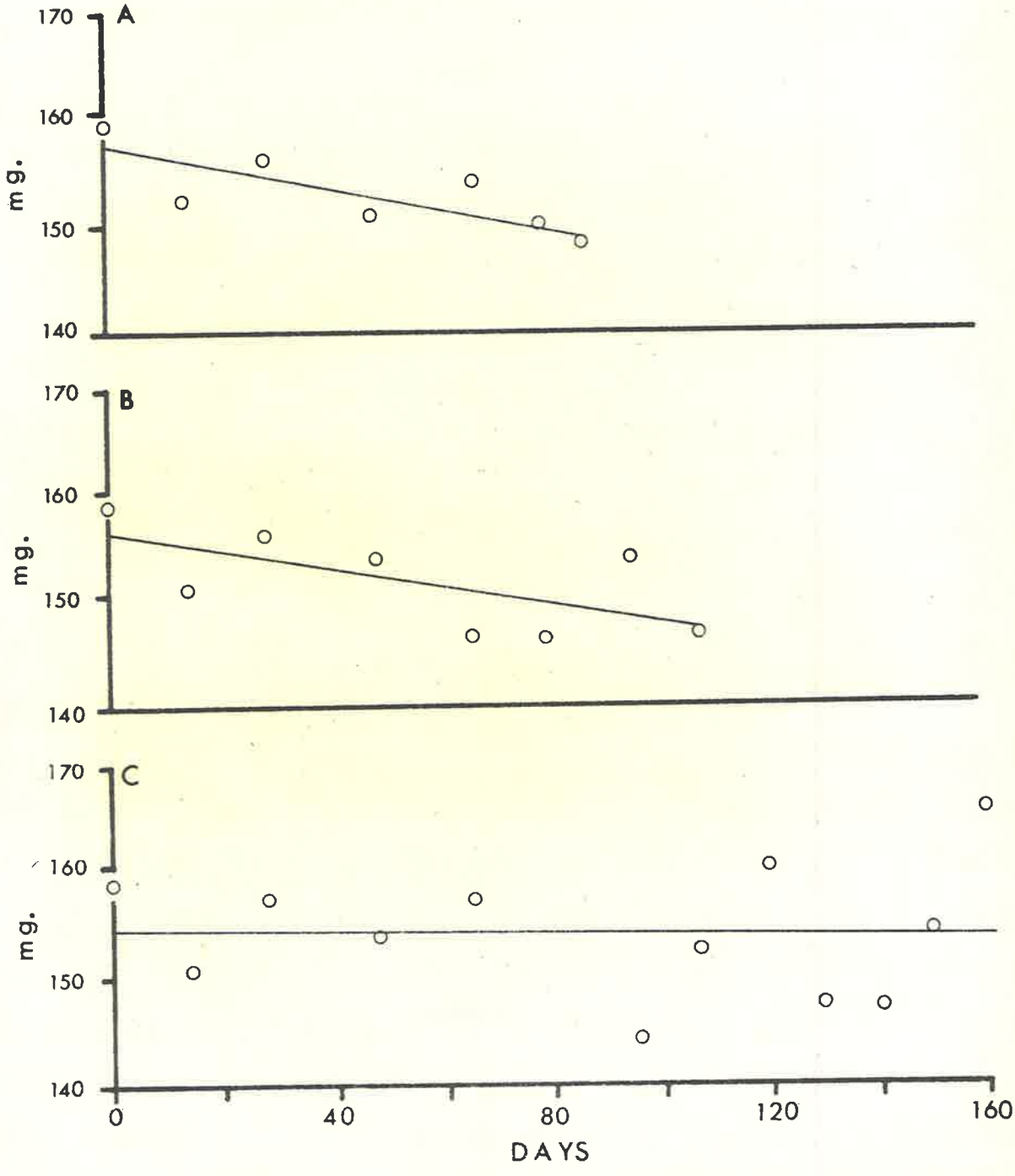

A
B
C
170
160
150
140
mg.
0
40
80
120
160
DAYS

FIGURE 3.06. The relationship between the time in dormancy over silica gel (days) and the mean dry tissue weight (mg) of snails in the samples at different temperatures

A. (above) Snails dormant at 30°C

The regression equation is $Y = 49.08 - 0.072x$

The probability that the coefficient of regression does not differ from zero is $P < 0.001$

B. (centre) Snails dormant at 25°C

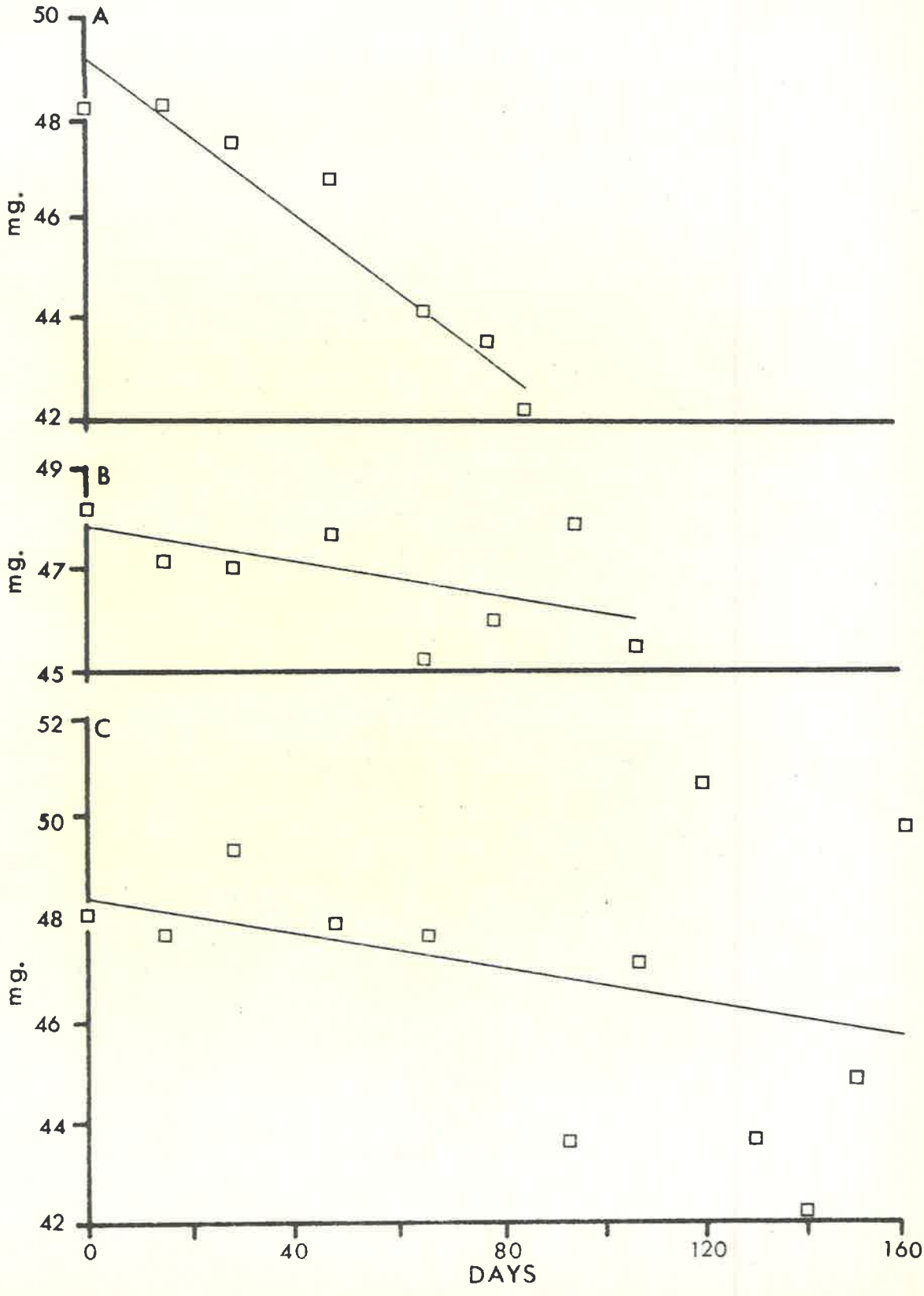
The regression equation is $Y = 47.70 - 0.016x$

The probability that the coefficient of regression does not differ from zero is $0.2 > P > 0.1$

C. (below) Snails dormant at 20°C

The regression equation is $Y = 48.38 - 0.017x$

The probability that the coefficient of regression does not differ from zero is $0.3 > P > 0.2$

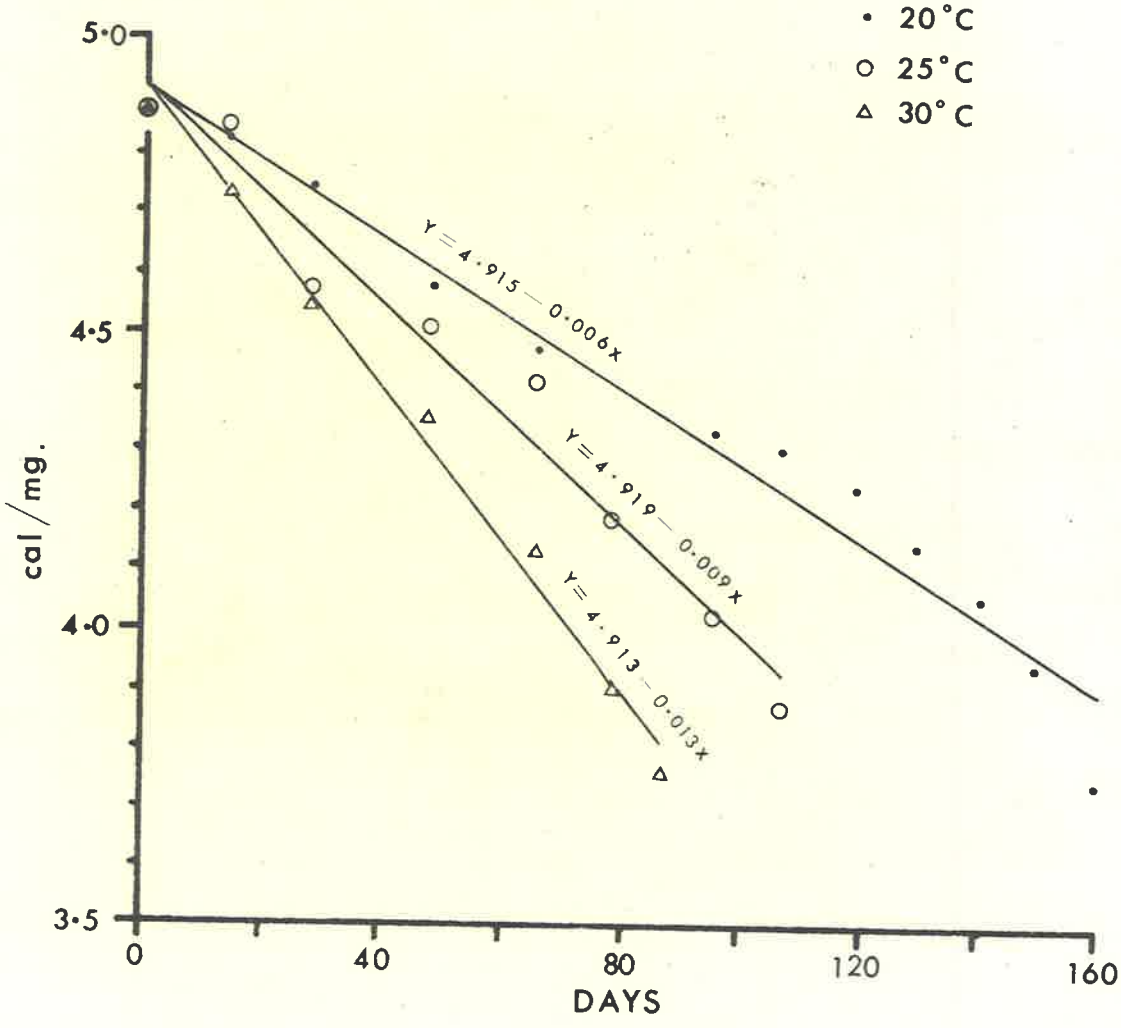

A
50
48
46
44
42
mg.
B
49
47
45
mg.
C
52
50
48
46
44
42
mg.
0
40
80
120
160
DAYS

snails possessed a value of about twice that from Buckland Park snails. Butler (pers. com.) suggested that the shells of the snails from Buckland Park seemed to be heavier and more massive than those from Northfield, and my own observations have confirmed that this was so. The difference may be, in part, correlated with the amount of calcium present in the environments in the two areas. The soil at Northfield is a clay loam and that at Buckland Park coastal sand dune containing much calcareous matter in the form of shellgrit. There is, however, little reason to believe that a shortage of calcium is limiting the growth of shells in animals at Northfield.

3.344 Changes in energy content

The decrease in the total amount of energy contained per milligram of dry body tissue is shown for all samples in Figure 3.07. It is apparent that at higher temperature the energy content of the tissue decreased more rapidly than at lower temperature. However, at the last determination, just before many snails died in each treatment, the energy content of the samples was very similar regardless of the temperature at which the snails had been dormant (Tables 3.4, 3.5 and 3.6). Furthermore, the energy content of the last sample of Buckland Park snails (Table 3.2) is in close agreement with that found in the snails from Northfield.

FIGURE 3.07. The relationship between the time in dormancy over silica gel (days) and the energy content of the samples (calories per mg. dry tissue).



3.4 CAUSES OF DEATH IN DORMANT SNAILS

To my knowledge few studies have provided information on the causes of death in dormant snails. It seems to have been generally assumed that such animals die as a result of becoming desiccated, and most of the studies on dormancy have been concerned with elucidating the mechanisms by which the animals reduce water-loss from their bodies.

Machin (1967) calculated approximately the length of "inactivity" possible for Helix aspersa, Otala lactea and Sphincterochila boissieri at typical room temperature, on the assumptions that the initial water content of these animals was 86 per cent of the wet body weight, and that they could tolerate water loss to the extent of 35% of the wet body weight. He stated that the general agreement between the survival times observed by various workers and the times calculated by himself, lent support to his assumption that the water reserves of the three species of snails were basically similar and that the length of survival depended on the rate of water-loss.

This extremely general result cannot be taken as evidence to show that the snails did not die from some other cause.

Pomeroy (1966) showed that for Helicella virgata the amount of water lost at death was very different at different temperatures. Further, more water was lost at 30°C than was lost at 25, 35 or 40°C. Because of the widely different amounts of water lost it seemed unreasonable to suppose that death was due to desiccation.

In Experiment 3.4 there were also differences in the amounts of water lost from dormant specimens of Helicella virgata at the last determination before death, and these results agree with Pomeroy's.

Pomeroy also considered the possibility that death was due to an accumulation of metabolic products in the tissues of Helicella. He found no evidence to support this suggestion and concluded that the snails were probably dying from starvation as a result of the exhaustion of food reserves.

The results of the changes in total energy of snails at different temperatures, in Experiment 3.4, support Pomeroy's suggestion. The energy levels of these snails fell from approximately 4.9 calories to 3.8 calories per milligram of dry body tissue from the time of collection until the last determination before many snails died. Clearly, although the rate of change of energy was different at different temperatures, the total energy expenditure was remarkably similar at all temperatures.

These observations support the hypothesis that the snails were dying in the laboratory as a result of starvation, the residual energy of approximately 3.8 calories per milligram being derived from the combustion of structural material within the body that is not available for metabolism.

It should be stressed that the causes of death amongst snails in the field may be different, and neither the work of Pomeroy (1966) nor the results of this experiment should necessarily be applied to snails in nature.

3.5 SOURCE OF ENERGY DURING DORMANCY

Glycogen and fat are quantitatively the most important of the insoluble energy-stores in animals. During starvation or dormancy, when replenishment of these energy-stores is not possible, a decline in the energy reserves will occur at a rate determined by the energy requirement of the particular organism. It is convenient to consider together the fate of the energy-stores under conditions of starvation and dormancy, but it should be borne in mind that, while there is at present little evidence to show this, the metabolism during starvation may differ from the metabolism during dormancy in the same organism.

Previous studies on starving snails have indicated that there may be fundamental differences in the type of food materials stored amongst different species. Von Brand et al. (1948) showed that well-fed specimens of Australorbis glabratus possessed relatively high respiratory quotients which decreased as the snails were starved. They suggested that, while glycogen was probably the main source of energy in well-fed snails, the glycogen reserve did not seem to last for very long. Quite quickly, values of respiratory quotient were obtained which were typical of those for the metabolism of fat and protein, but did not preclude the possibility that the values obtained were due to the retention of carbon dioxide by the animals. In a later study, von Brand et al. (1957) showed that A. glabratus used an appreciable amount of polysaccarides and lipids during 30 days of starvation. They did not present quantitative data,

but suggested on the basis of oxygen consumption that protein may have been the principal source of energy during protracted periods of starvation.

Emerson (1967) has shown for Planorbis corneus that during starvation most of the energy used was derived from the metabolism of polysaccarides. Death appeared to be due to a depletion of carbhohydrate reserves, since 95 per cent of the original polysaccarides had been metabolised. At death there were still substantial amounts of lipid present, although some had been metabolised together with some protein.

Emerson and Duerr (1967) showed that the marine prosobranch Littorina planaxis used lipids as the main source of energy during a 70 day period of starvation. In their study neither glycogen nor protein nitrogen decreased significantly.

Meenakshi (1956a) investigated the amounts of both glycogen and fat in Pila virens. In animals that were well-fed glycogen accounted for a maximum of 3.2 per cent of the wet weight of the animals, and fat for a similar figure (3.4 per cent in females and 2.7 per cent in males). Meenakshi showed that in six months the dormant snails metabolised approximately four times as much glycogen as fat, and she concluded that glycogen was the major source of energy during dormancy.

Meenakshi's findings for Pila virens are in contrast with those of George and Desai (1954) for Pila globosa. They stated that in P. globosa, fat provided the energy source during aestivation. Meenakshi

(1956a) stated that this discrepancy was probably due to the fact that George and Desai studied only the fat content of the digestive gland,not that of the whole animal. They did not study the glycogen content in the remainder of the animal either.

Meenakshi (1956a) found that the decrease in glycogen during a twelve month period of aestivation differed between male and female Pila virens. The rate of decrease of glycogen was similar for both sexes for the first six months of dormancy, but after the sixth month the decrease in glycogen in female snails became less rapid. After twelve months female snails had lost approximately 9.3 milligrams of glycogen per gram of wet weight and males had lost 15.3. In a previous study (Meenakshi, 1954b) she showed that the galactogen content of the "uterus" began to decline slowly after six months' dormancy. It seems likely that the decrease in glycogen utilization is a result of the mobilization of galactogen in these animals.

Von Brand (1931) followed the changes that occurred in the amounts of polysaccarides and fat in Helix pomatia during an entire year. He found that in the period just before hibernation polysaccarides were deposited to a maximum of approximately four per cent of the wet weight of the snails. The amount of polysaccaride declined steadily during dormancy, the glycogen content decreasing from about 2.5 per cent to 1.5 per cent of the fresh body weight. Thiele (1959) confirmed these results for Helix pomatia.

Von Brand found very little fat in Helix pomatia. His results show that fat made up approximately 0.6 per cent of the wet weight of the animals, and there was little or no change in the amount of fat present in the animals throughout the year. Thiele (1959) also found that the fat content of Helix pomatia was low. He found rather more fat (1.6% of the wet weight) than did von Brand, and suggested that von Brand may not have extracted all the fat in snails during his study. Thiele noted that there seemed to be a trend shown in his results, such that the fat content at the end of hibernation in March appeared to be slightly less than it had been prior to the onset of hibernation in November, but the differences were not significant and in the main he agreed with von Brand that stored fat was not a significant source of energy for Helix pomatia during dormancy or at any other time.

May (1934b, 1934c) examined the changes in polysaccaride content in various tissues throughout the year. He found that in addition to glycogen, Helix pomatia contained galactogen and that this was confined to the albumen gland. During hibernation May (1934b) found that there was a steady decrease in the amount of glycogen present in the tissues, but that the amount of galactogen in the albumen gland remained constant. May (1934c) then allowed snails to emerge from dormancy, but prevented them from feeding. The glycogen reserves were used up within the first 10 days, but during this time no decrease in galactogen took place. Only after all the glycogen had been metabolized did the galactogen levels begin to decrease, and the snails survived on galactogen for a

period of about 20 days after the glycogen stores had been exhausted. Martin (1961) has commented on the possible advantage that such a system might confer upon these animals, with respect to reproductive success.

Baecker (1932) and Filhol (1938) found that the albumen gland in Helix was relatively smaller in winter, and Yung (1911) reported a progressive decline in the amount of secretory material present in the albumen gland during hibernation. Goddard et al. (1961), in their review of the literature on changes in the albumen gland, have pointed out that the above observations tend to contradict May's findings, that galactogen is not mobilized until the glycogen stores have been exhausted.

In the present study I considered it likely that similarities might exist in the energy stores of both Helix and Helicella.

3.51 Experiment 3.5

To determine the amount of fat present in Helicella virgata prior to, and the changes in fat content during, dormancy

In order to discover whether significant amounts of fat were present in specimens of H. virgata prior to dormancy, and whether changes occurred in the fat content during dormancy, the following experiment was performed.

From the Northfield collection of 1.11.66, 50 snails of the size class 1.5 $\pm$ 0.1 cm were randomly distributed between 10 cages such that there were five snails to a cage. One sample of five snails was withdrawn

at random for the initial determination and the remainder were placed in a desiccator containing silica gel. The desiccator was maintained at 25°C in a constant temperature cabinet.

On day 0, the initial sample of snails was killed by brief immersion in hot water. The bodies were withdrawn from the shells and placed individually on small weighed discs of filter paper. These discs were cut from Whatman No. 42 filter paper with a cork borer and greatly simplified the handling of tissues. The individual bodies were placed into numbered glass tubes and dried in the vacuum unit to constant weight. They were reweighed after drying, placed in extraction thimbles and the fat extracted by ethyl ether in Soxhlet apparatus. After extraction for 24 hours, the bodies were returned to the vacuum unit and the ether was extracted. The amount of fat extracted was expressed as a percentage of the initial dry weight of the tissues for each individual.

This procedure was repeated at approximately monthly intervals until dead snails were found in the cages. At each determination the silica gel was renewed in the desiccator. The results of this experiment are presented in Table 3.7.

TABLE 3.7

Fat content (% of initial dry weight of bodies) of snails dormant at 25°C over silica gel

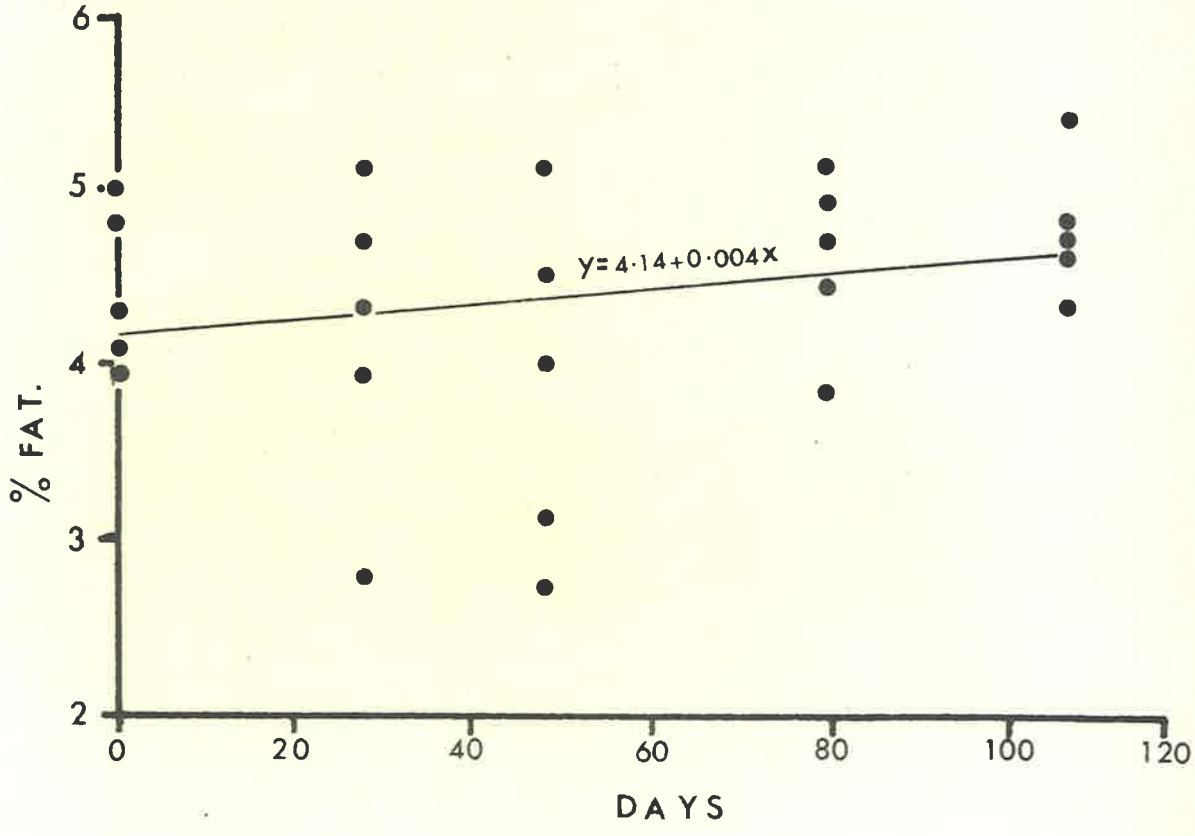
Days dormant				
0	28	48	79	107
4.8	5.1	5.1	4.9	4.6
4.3	4.3	2.7	3.8	4.7
5.0	2.8	4.5	5.1	5.4
4.1	3.9	4.0	4.7	4.3
3.9	4.7	3.1	4.4	4.7

These results are plotted, together with the calculated regression line, in Figure 3.08. The coefficient of the regression line has been compared with zero on the null hypothesis that the observed slope did not differ significantly from zero. The probability value obtained is shown in Figure 3.08.

Both von Brand (1931) and Thiele (1959) presented data for the amount of fat contained in Helix pomatia as percentages of the wet body weight. In the analysis above, fat content has been expressed as a percentage of the dry weight of the tissues, since from Experiment 3.4

FIGURE 3.08. The relationship between the time in dormancy at 25°C over silica gel (days) and the fat content (expressed as a percentage of the initial dry weight of the tissues of individual animals).

The probability that the slope of the regression line does not differ from zero is $0.3 > P > 0.2$.



(Figure 3.04), it is clear that the water content of the tissues decreased markedly during dormancy. The result of this analysis indicated that the percentage fat content did not change significantly with time in dormancy, but it is possible that the value of wgt fat/ wgt dry tissue might remain constant if both the weight of fat and the dry weight of the body tissues changed in the same direction. The results of Experiment 3.4 (Figure 3.06B) suggested that no significant change occurred in the weight of the dried tissues of snails dormant at 25°C, but, in spite of this result, the analysis above has been repeated using the actual weights of fat obtained from the snails. These are shown in Table 3.8.

TABLE 3.8

Fat content (mg.) of snails dormant at 25°C over silica gel

Days dormant				
0	28	48	79	107
1.40	2.50	1.05	0.80	1.60
2.35	1.10	0.85	0.85	1.20
2.40	0.85	0.95	1.35	1.45
2.65	1.95	1.00	2.00	1.45
1.05	3.75	0.90	1.20	1.40

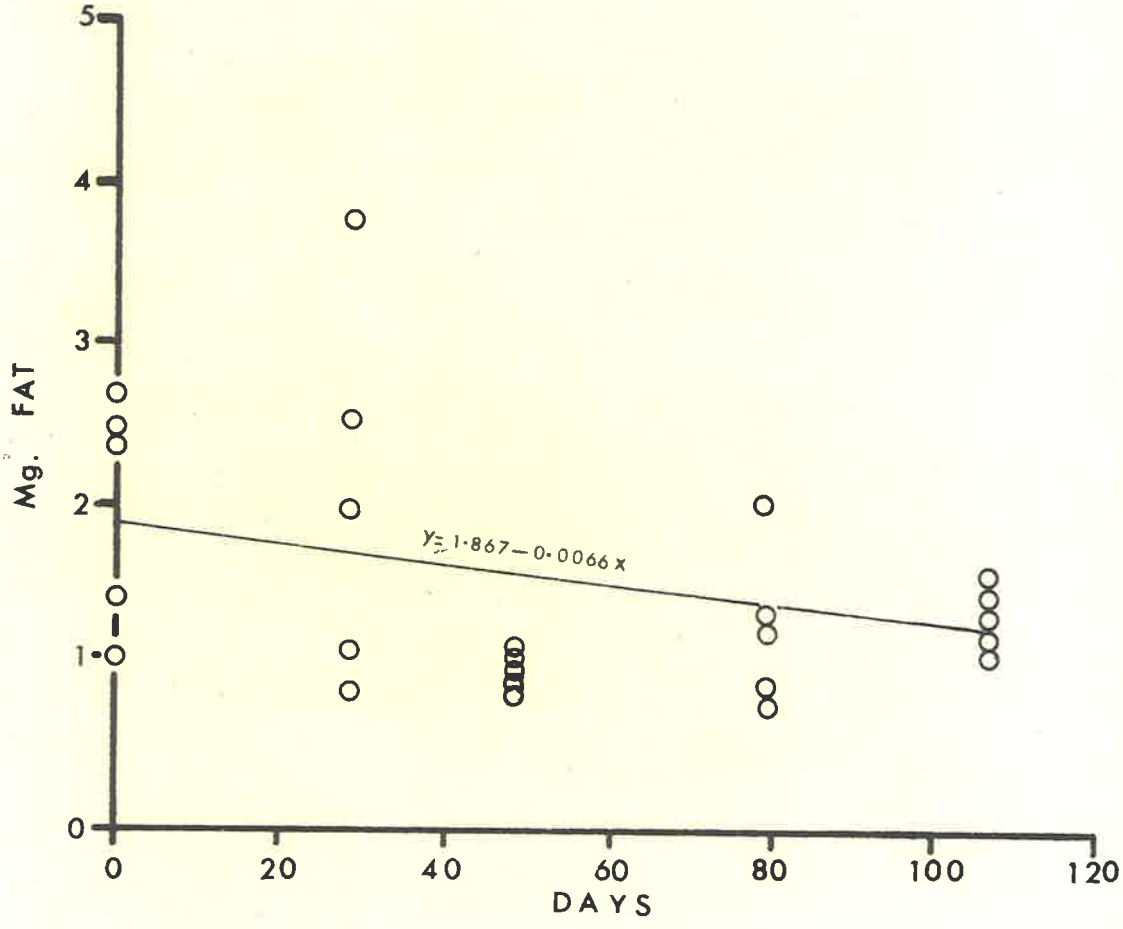
These results are plotted, together with the calculated regression line in Figure 3.09. Again, the coefficient of regression has been compared with zero on the null hypothesis that the observed slope did not differ significantly from zero. The probability value of $0.2 > P > 0.1$ did not reject the null hypothesis.

The results of this experiment indicate that there was little fat in specimens of Helicella virgata prior to the onset of dormancy. Further, the amount of fat present did not change significantly during dormancy. From the regression equation in Figure 3.08, fat content to the value of 4.14 per cent of the dry body was indicated as an initial figure for snails that had not been dormant. Further, consideration of the results of Experiment 3.4 indicates that the dry matter in these snails was likely to have been about 11 per cent of the wet weight of tissue. From these it may be seen that approximately 0.45 per cent of the initial wet weight of the animal was fat. This finding is in general agreement with the amount of fat found by von Brand (1931) for Helix pomatia (0.6 per cent of the wet weight), and indicates that for Helicella virgata, like Helix, fat is of little significance as a source of energy during dormancy.

It seems likely that the energy utilized by dormant H. virgata, like Helix, was derived principally from polysaccaride reserves, although these have not been studied specifically in Helicella. This suggestion is likely in view of the relatively close taxonomic position of these animals.

FIGURE 3.09. The relationship between the time in dormancy at 25°C over silica gel (days) and the weight of fat present in individual snails (mg).

The probability that the slope of the regression line does not differ from zero is $0.2 > P > 0.1$.



3.6 THE TYPE OF METABOLISM EMPLOYED DURING DORMANCY

Few studies have been undertaken to investigate the kinds of metabolic processes that occur within dormant snails.

Meenakshi (1956b, 1958) has shown that in Pila virens, during dormancy, respiration is anaerobic. She was unable to detect any utilization of oxygen by dormant snails, but showed that the polysaccaride content of these animals fell steadily and that lactic acid was accumulated in the tissues. This lactic acid was neutralised by calcium from the blood. The pH of the blood remained constant at about 7.3, and the calcium lactate formed was deposited principally in the tissues of the foot. Following the return of dormant animals to the active state, lactic acid was not excreted but appeared to be completely oxidised. During this time the snails displayed the repayment of an oxygen debt.

Meenakshi also showed that there were marked differences in the metabolic processes of active animals placed in anoxic conditions compared with those in dormancy. The rate of utilization of glycogen was very much greater in anoxic animals. Further, mobilization of calcium did not occur, with the result that lactic acid was rapidly accumulated and the pH of the blood fell from 7.3 to 4.4. Such snails did not survive for more than two days under these conditions.

Meenakshi (1956b) found that in Pila virens "the factors governing aestivation can be traced to biochemical changes in the cerebral ganglia of the animal". Injection of the sterol fraction of the cerebral ganglion

from a dormant specimen into an active snail produced a lowering of the oxygen consumption and an increase in the amounts of calcium and magnesium in the blood of the injected animal.

No comparable studies have been made on Helicid snails, although the available evidence suggests that polysaccarides form the major source of energy during dormancy. There is little evidence to indicate whether such snails obtain their energy aerobically or anaerobically. Little (1968) quoted Fischer (1931) as having suggested that the respiration of "aestivating" Helix pomatia was largely aerobic. Von Brand (1946) remarked that it had not been investigated whether dormant snails ever find themselves in conditions where oxygen tensions are low, however he considered it possible that many burrowing species might find themselves in such conditions. P. virens appears to be such a species, and it is possible that many fresh-water forms might encounter such conditions during dormancy. Helicid snails, however, are unlikely to encounter anaerobic conditions. Helix aspersa tends to be cryptic during its dormancy, but there is little reason to suppose that low oxygen tensions occur in these places.

Helicella virgata generally spends its dormant life on vegetation above the surface of the ground. It is thus never likely to encounter anaerobic conditions in nature. However, as Martin (1966) has pointed out, owing to other climatic conditions, anaerobiosis may be necessary at high temperatures. "The term estivation does not necessarily imply anaerobiosis, but the problem of water conservation is so difficult for

some estivators that anaerobiosis is superimposed."

Pomeroy (1966) apparently considered that Helicella virgata was such an animal. He stated that "the retention of lactic acid, carbon dioxide and excretory material presumably accounts for the fact that the dry weight of aestivating H. virgata does not decrease with time - and may even increase". He did not verify this conclusion experimentally.

For the purpose of investigating the metabolism of Helicella, it seemed profitable to attempt to construct a model for the dormant snail. In this way predications based upon the model could be tested experimentally to see whether they could be verified.

The following observations and assumptions were considered in the setting up of the model.

During dormancy, "pitting" occurred in the shells of most snails (Section 2.11). Many snails produced a number of heavily calcified epiphragms. It seemed possible that calcium was being withdrawn from the shell by the dormant animal, and on the basis of Meenakshi's work, this suggested that calcium might be involved in the metabolic processes during dormancy.

There was no apparent decrease in the dry weight of body tissues in snails kept dormant at temperatures of less than 25°C. Above this temperature losses in dry weight did occur but were of relatively small magnitude.

Water was lost at a low rate from dormant snails. In the light of Martin's (1966) comment and Pomeroy's (1966) suggestion, together with

the observation on the "pitting" of shells, it seemed reasonable to postulate that anaerobic glycolysis was involved in metabolism during dormancy. Further, it seemed possible that if gas exchange was occurring in the "lung" of the snail, then evaporation of water from the pneumostome would have followed. It was postulated that this would be unlikely from the amounts of water lost from dormant snails.

No excretion occurs during dormancy, and generally no faeces are produced when the animal is aroused to the active state and prevented from feeding.

Snails ingest the epiphragm material as they emerge from the shell after dormancy.

The Model

The model suggested for dormant Helicella virgata was that energy was derived anaerobically from polysaccaride reserves. Lactic acid was produced and calcium carbonate was withdrawn from the shell to neutralise this acid, the calcium lactate thus produced being deposited in the tissues. The epiphragm might also function as a store for the calcium lactate produced, and on arousal from dormancy the ingestion of the epiphragm material would serve to return to the snail all the calcium and lactate. This latter would then be completely oxidised and during this oxidation the snail would show an oxygen debt.

3.61 Oxygen consumption of dormant snails

If the model was an accurate representation of the dormant snail, then I would have expected little or no oxygen consumption in these animals. I planned a series of experiments using manometers to determine whether dormant snails utilized oxygen, however all of these failed initially. The snails emerged from dormancy within the manometer cups, many ingested their epiphragms and some crawled. It was apparent that any measurements of oxygen consumption that were made could not be considered to apply to dormant snails.

The snails were maintained in a desiccator over silica gel at 25°C. I planned to determine the oxygen consumption at 25°C as this temperature was not exceeded in the laboratory where the manometer bath was kept. The air temperatures fluctuated between approximately 17°C and 22°C. One possible stimulus that I thought might operate to arouse snails was the change in temperature that the animals would have experienced when they were removed from the desiccator and put into the manometer cups. Dainton (1954a) has shown that Agriolimax reticulatus may become active in response to a decrease in temperature of 0.1°C per hour, and on most occasions when I was transferring snails to the manometer cups the temperature of the snails would have dropped from 25°C to approximately 20°C and thenrisen again to 25°C when the animal was immersed in the water bath. It was not possible to prevent this change in temperature.

I then attempted to cause snails to enter dormancy within the

manometer cups at 25°C. The area around the water bath was warmed with several electric radiators and the manometer cups together with the snails were transferred from dessicators to the manometers as rapidly as possible. The snails aroused from dormancy as before.

I considered that the humidity within the manometer cups might operate to arouse the snails, however when silica gel was placed in the cups together with the snails these still aroused from dormancy when placed on the manometer bath.

Wells (1944) suggested that Helix pomatia might become active in response to bumping or knocking. I observed that the vibration set up in the manometers, from the pump used to circulate water in the water bath, was quite noticeable when I placed my finger on the top of the body of any manometer. This vibration was of quite high frequency and it seemed possible that it might stimulate the arousal of dormant snails.

In order to test this hypothesis the water bath was warmed to 25°C and the pump left switched off when the manometers containing the snails were introduced to the bath. On two occasions when this was done only 3 of the 15 snails were obviously aroused, and on another occasion only 4 of the 15. In all previous experiments either all 15 snails had aroused or most of them. It seemed likely that the vibration from the pump was at least partly responsible for the observed activity. No readings of oxygen consumption were possible however when the pump was not operating, as the temperature gradient across the bath made it impossible

to read the changes in volume within the manometers with any degree of accuracy. Further, some snails aroused from dormancy even with the pump motor off. I could not therefore suggest that those snails that were not obviously aroused were still dormant.

I attempted to mount another pump above the manometer bath in such a way that it was isolated from the rack carrying the manometers. This resulted in a reduction in the vibration transmitted to the snails, however some vibration was still apparent and snails awoke in the manometer cups.

I abandoned the use of the manometer bath and set the manometers up in a constant temperature room. The manometers were supported on a wooden frame at 25 $\pm$ 1^{o}C.

The snails used in this experiment had been dormant for six months under room conditions of temperature and humidity. They were placed, together with the manometers and cups, in the constant temperature room one day prior to the setting up of the experiment.

Eight manometers were used, one thermobarometer and seven experimental. Into the experimental cups I placed a layer of non-deliquescent soda lime ("Carbasorb" - BDH) to absorb any carbon dioxide. The soda lime was spread evenly on the bottom of each manometer cup and covered with a piece of filter paper. Into each cup I placed one snail with as little bumping or mechanical stimulation as possible.

Ten minutes after the introduction of the snails to the cups the

taps on the manometers were closed. The manometers were read after 1.5 hours and then at intervals of approximately 24 hours for the following eight days. No snail emerged from its shell during this time.

After the last reading the snails were removed from the manometers and weighed individually. They were then aroused by wetting, cooling and vibration. One snail did not arouse. The snails were then killed in hot water, the shells and bodies separated and the shells dried to constant weight in the vacuum drying apparatus. The snail that did not arouse was found to be dead. It had died during the experiment.

The weight of each shell was subtracted from the total weight of the animal to give the weight of the wet body.

The results of the consumption of oxygen are shown for individual snails in Figure 3.10. The broken line is the result from the snail that died during the experiment. The remaining animals generally showed a greater rate of oxygen consumption over the initial 26.5 hours than during the rest of the experimental period, suggesting that the initial oxygen consumption was high due to handling. For the calculation of the rate of oxygen consumption during dormancy, the figures obtained in the first 26.5 hours have been ignored.

FIGURE 3.10. The oxygen consumption of individual snails dormant at 25 ± 1°C.

The curves are cumulative. The points connected by the broken line were obtained from a snail that was found to have died during the experiment.

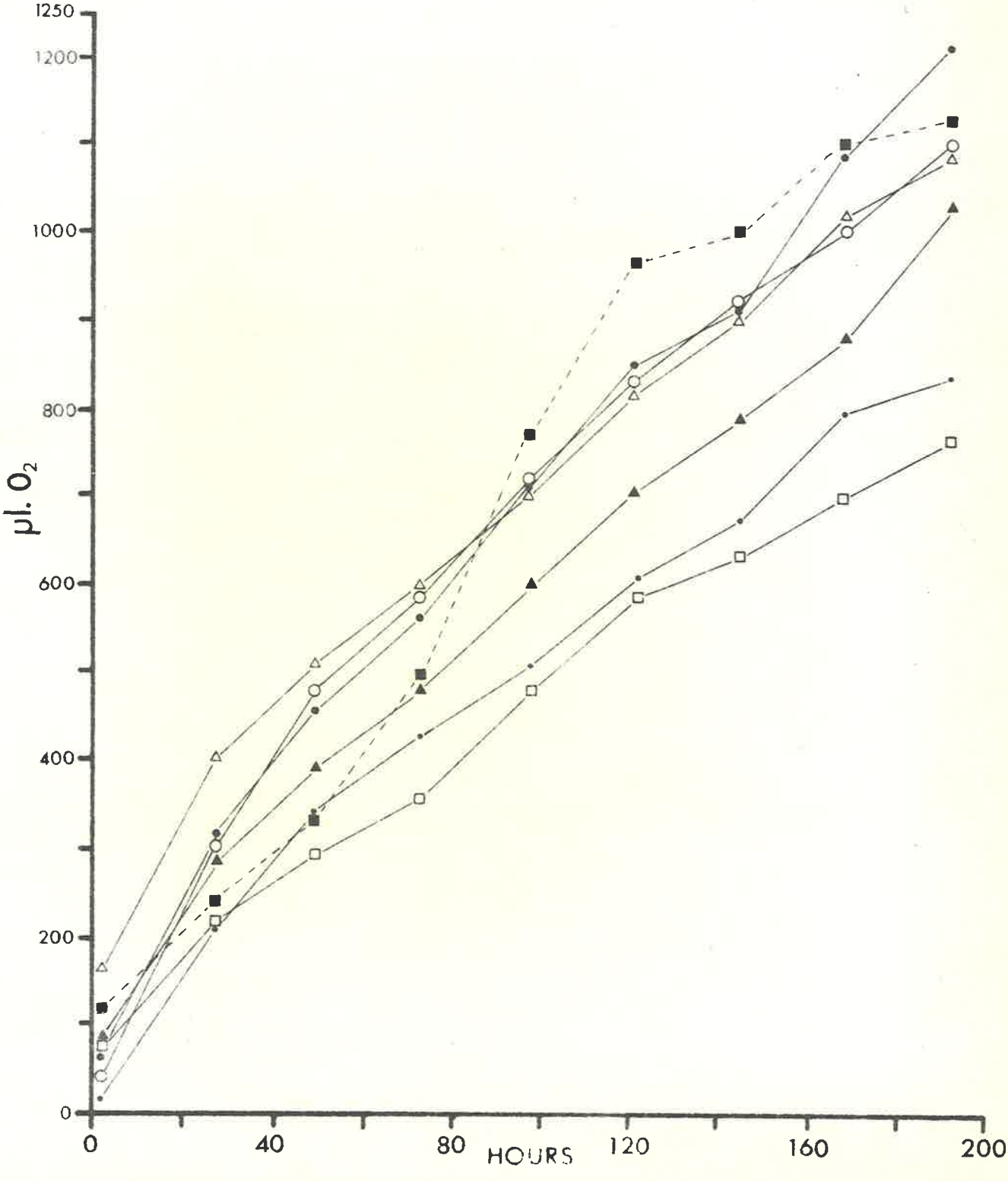

1250
1200
1000
800
600
400
200
0
µl. O2
0
40
80
HOURS
120
160
200

TABLE 3.9

The results of the determination of oxygen consumption of dormant H. virgata at 25 ± 1°C over 166 hours

	Snail No.						
	1	2	3	4	5	6	7
$\mu l.O_2$	1096	1032	1212	764	1128	1092	832
Wet wgt. tissue (mg)	182	146	173	181		242	177
Rate of O_2 consumption (ml/gm/hr)	0.026	0.031	0.032	0.018	-	0.017	0.020

Clearly these snails used oxygen during dormancy. The rate of consumption varied from 17 to 32 $\mu l.O_2$/gm/hr amongst individuals, but was relatively constant over a period of 166 hours for individuals. These results do not support the hypothesis that the metabolism of Helicella virgata is entirely anaerobic during dormancy, rather, they suggest that the metabolism is aerobic and proceeding at a very low rate.

3.62 Transport of calcium

The "pitting" observed in shells of dormant snails suggested that calcium was being withdrawn from the shells. Snails in which "pitting" had occurred generally possessed numbers of heavily calcified epiphragms. It seemed likely that calcium was being transported from the shell through the tissues and being secreted as part of the epiphragm.

In order to test this hypothesis I collected 50 large specimens of Helicella virgata from Northfield (7.8.67). These snails were washed to remove any mud that was adhering to the shells, then dried on towelling paper. They were then placed individually into 2" x 1" glass specimen tubes numbered from one to fifty. The tubes were then randomized into 10 groups of 5 individuals. One group was withdrawn for the initial determinations and the remainder covered with gauze and placed in a desiccator over silica gel. The desiccator was stored at 20°C in a constant temperature cabinet.

The method that I used to determine the amount of calcium present was similar to that used by Corley and Denis (1925) to check their modification for tissue samples. The method involved ashing the tissues instead of autoclaving them with 0.1 N sodium hydroxide.

Snails in the initial sample were killed in boiling water and the bodies (including epiphragm) were removed from the shells. The shell and body of each snail was ashed separately in silica crucibles in a small

furnace. The ash was digested with 2 ml of approximately N HCl. The digest for each shell was washed from the crucible into a volumetric flask and the volume made to 100 ml with distilled deionised water. A 5 ml aliquot was then transferred to a 15 ml centrifuge tube for the precipitation of the calcium. The total digest for each body was used for the precipitation of the calcium.

The calcium was precipitated with 4 per cent ammonium oxalate. The pH of the contents of each tube was adjusted to the neutral point of Methyl Red indicator with concentrated ammonium hydroxide, using approximately N HCl for back titrations if the reaction had passed the neutral point. After standing for approximately two hours the tubes were centrifuged, the precipitate washed with 5 ml of cold distilled water and centrifuged again. The supernatant fluid was discarded and the precipitate dissolved in 1.0 ml of N sulphuric acid. Distilled water was added to make the solution to 4 ml total volume.

The solutions were titrated (in a water bath at 70°C) with 0.01 N potassium permanganate solution. The amount of calcium present in each solution was calculated from the relationship that 1.0 ml of 0.01 N potassium permanganate solution is equivalent to 0.2 mg of calcium.

At approximately monthly intervals a further sample of five snails was withdrawn from the desiccator for the determinations. At each determination the silica gel in the desiccator was renewed.

The experiment was terminated after the fourth determination on the ninetieth day of dormancy. A laboratory accident resulted in the

wetting of the stock of snails in the desiccator. Some of these snails aroused from dormancy and I considered it possible that the results obtained from these animals might not be strictly comparable with those previously obtained.

The results of this experiment are shown in Table 3.10 below. The figures in the body of the table are the weights of calcium in milligrams present in the body and shell of each individual snail.

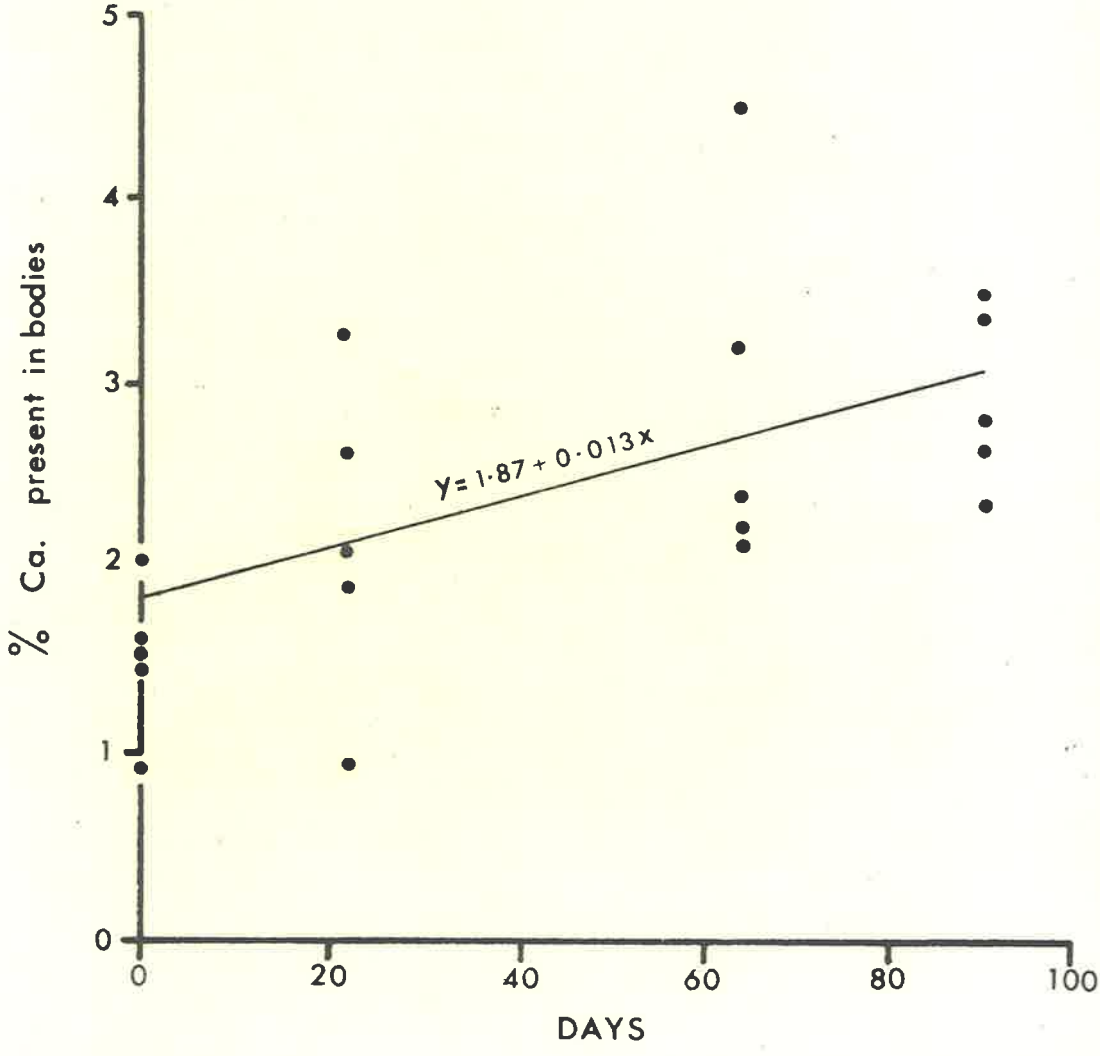
TABLE 3.10

The weight of calcium (mg) in the body and shell of individual snails after varying periods of dormancy at 20°C

Snail No.	Days of dormancy 20°C							
	0		22		64		90	
	Body	Shell	Body	Shell	Body	Shell	Body	Shell
1	0.38	21.73	0.52	19.20	0.70	31.33	0.50	14.80
2	0.36	24.40	0.40	11.73	0.42	13.33	0.72	20.61
3	0.22	10.60	0.60	30.80	0.62	27.86	0.34	12.00
4	0.68	37.86	0.34	23.33	0.34	16.00	0.46	17.25
5	0.36	20.40	0.26	12.13	0.40	16.66	0.62	26.43

FIGURE 3.11. The relationship between time in dormancy at 20°C over silica gel (days) and the amount of calcium present in the body (expressed as a percentage of the total calcium present in an individual).

The probability that the slope of the regression line does not differ from zero is $0.01 > P > 0.001$.



For the purpose of analysis, the amount of calcium in the body of each snail was expressed as a percentage of the total amount of calcium present in the whole animal. If calcium was being transported from the shell to the body this percentage should have increased as the time in dormancy increased.

Figure 3.11 shows the relationship between the time in dormancy and the percentage of calcium present in the bodies for individual snails.

The regression equation has been calculated for this data, and the coefficient of regression compared with zero on the null hypothesis that there was no significant difference between the observed value and zero. The null hypothesis was rejected at long odds ($0.01 > P > 0.001$) and I therefore concluded that during dormancy there was a real increase in the amount of calcium present in the body of individual snails. This increase could only have occurred through the removal of calcium from the shell and its relocation in the tissues of the body and epiphragm.

3.63 The concentration of lactic acid in tissues of dormant and active snails

The transport of calcium from the shell to the body suggested to me that an anaerobic metabolism, like that of *Pila virens* (Meenakshi, 1956b, 1958) might be operating in dormant *Helicella virgata*, this observation was in accordance with the model. In order to test the model, I compared the amount of lactic acid per unit dry weight of tissue, for individual snails that had been dormant for approximately 3 years

over silica gel at 10°C with that of active snails collected in the field during rain.

The epiphragms were removed as completely as possible from the dormant snails, and placed into numbered and weighed 2 ml plastic centrifuge tubes. The snails were then killed in boiling water, the bodies removed from the shells and placed into similar weighed and numbered tubes.

In addition to the individual epiphragms a collection of this material was made from other snails from the same stock in the 10°C desiccator. This material was pooled in one numbered and weighed 2 ml plastic centrifuge tube.

The active snails did not possess epiphragms. They were also killed in hot water, the bodies extracted and placed into numbered, weighed tubes.

All tubes were then placed into the chamber of the vacuum drying apparatus and the tissues were dried to constant weight. The weight of the dry tissues was obtained by subtracting the tare weight of each tube from the weight of the tube and its dry contents.

The individual tissues were ground in glass tissue grinders and hydrolysed. The lactic acid present was determined colourimetrically by the method of Barker and Summerson (1941) as given by O'Brien and Ibbott (1962). The optical density of the samples in 10 mm fused silica cells was read at 570 μ with a Unicam spectrophotometer against standards prepared from zinc lactate. It should be noted that the method of

preparing the standards given by O'Brien and Ibbott (third edition) gives concentration of 10 μg of lactic acid per ml not 1 mg per ml as they appear to suggest.

The results of these determinations are shown below in Table 3.11 for the tissues of the bodies. No lactic acid was found in any of the epiphragm material.

TABLE 3.11

The amount of Lactic Acid present in individual animals

Condition	μg of lactic acid per mg on dry body weight									
Active	0.56	0.66	0.51	0.81	0.67	0.79	0.72	0.67	0.90	-
Dormant	2.19	1.11	2.01	0.71	1.14	0.97	1.13	1.54	0.93	0.78

The mean amount of lactic acid per unit dry weight of body tissue in both groups of snails was compared by means of a t-test. The null hypothesis being that there was no significant difference between the amount of lactic acid per unit dry weight in the dormant snails compared

with that in those that were active. The results of the comparison are shown in Table 3.12.

TABLE 3.12

Results of the t-test comparing the means of the lactic acid contents of dormant and active snails

Treatment	Mean	Variance	Var. Ratio	P
Active	0.69	0.150	1.68	
Dormant	1.25	0.253		$0.02 > P > 0.01$

The null hypothesis is rejected on this data and I concluded that there was significantly more lactic acid present per unit dry weight in dormant snails than in active ones.

The actual amount of lactic acid in dormant snails, however, was only about twice that in active specimens. If the metabolism was principally anaerobic during dormancy, I should have expected relatively greater quantities of organic acid to be present in these animals. Von Brand *et al.* (1950) found that for several species of fresh-water snails, kept under anaerobic conditions, lactic acid never accounted for

more than fifty per cent of the carbohydrate consumed, and in some species as little as one per cent. Helicella virgata might therefore be an animal in which lactic acid is quantitatively a relatively less important product of metabolism than it is in Pila virens, where Meenakshi (1958) showed that in animals dormant for six months the lactate concentration was 450 times as great as that of active animals.

3.64 The composition of the epiphragm with respect to calcium salts

In any organism, the rate of utilization of polysaccaride material is normally greater for a given energy requirement under anaerobic conditions than under aerobic conditions. This phenomenon has been termed the Pasteur effect. It arises from the fact that the oxidation of each 6-carbon unit of glycogen to an organic acid will, under anaerobic conditions, yield only 3 high energy phosphate bonds compared with approximately 40 when complete oxidation occurs during aerobic metabolism (Prosser & Brown 1961). In general then, animals are required to utilize approximately twenty times as much glycogen under anaerobic conditions as they would under aerobic conditions for the same energy requirement. Unless the organic acids produced from glycolysis can be retained in a non-toxic form for later oxidation under aerobic conditions, the process of anaerobic glycolysis is wasteful of the polysaccaride reserves.

The hypothesis that the epiphragm might function as a store for materials excreted by the dormant snails seemed attractive, particularly

as this material was invariably ingested when the snails became active again. Such a system would be efficient if the metabolism of dormant snails was principally anaerobic, providing a site external to the tissues for storing an excretory product which could, nevertheless, be reingested and provide considerable energy when conditions again became favourable for aerobic life.

That no lactic acid could be determined in the epiphragm of dormant snails did not preclude the possibility that a calcium salt of another organic acid might be present.

In order to investigate this hypothesis I decided to investigate the total calcium content of the epiphragm material, together with the amount of calcium present as calcium carbonate. If any excess calcium was found, further studies would be necessary to determine the form in which it was present.

I removed the epiphragms from all the snails that had been dormant at 10°C over silica gel for approximately 3 years. This material was pooled, dried to constant weight in the vacuum pump and ground in a glass tissue grinder.

For the determinations of the total amount of calcium, a weighed sample of dried epiphragm powder was ashed in a silica crucible. The total calcium was determined by the method described in Section 3.62 for that of the shells and bodies.

The carbonate fraction was determined using manometers. One

manometer was run as a thermo-barometer, in another a known weight of ground epiphragm was reacted with 1 ml of approximately 1N hydrochloric acid in the presence of 10 per cent potassium hydroxide to absorb the carbon dioxide. In a third, a known weight of ground epiphragm was reacted with acid with no absorbent for the carbon dioxide.

The manometer bath was run at 25°C; after an equilibration time of 30 minutes the taps on the manometer were closed and the air pressure at that time read from a barometer. The manometers were then tipped to mix the acid with the epiphragm material and read at five minute intervals until three consecutive readings indicated that the reaction had ceased.

Some difficulty was experiencedinitially in obtaining reproducible results. The same weight of epiphragm evolved different amounts of gas when reacted with the acid. This problem was caused by small bubbles of carbon dioxide becoming trapped within the particles of epiphragm material. This material did not dissolve in the acid. Machin (1968) described the epiphragm of Helix aspersa as having hydrofuge surface properties when in contact with water. The epiphragm of Helicella virgata does not dissolve readily in water either, however this difficulty was overcome by warming the weighed epiphragm material, in the manometer cup, with 1 ml of distilled deionized water for five hours at 50°C before the sample was reacted with acid.

From the volume of carbon dioxide evolved at known pressure and temperature, the weight of calcium carbonate was calculated on the

assumption that all the carbon dioxide had been derived from calcium carbonate. From consideration of the atomic weights, the weight of calcium present as carbonate was calculated for this material.

The results of this experiment are shown below in Table 3.13.

TABLE 3.13

Results of the determination of calcium present in epiphragms from snails dormant at 10°C

Replicate	Total Ca (mg) per mg of epiphragm	Carbonate Ca (mg) per mg epiphragm	Wgt of $CaCO_3$ (mg) per mg of epiphragm
1	0.187	0.185	0.448
2	0.184	0.183	0.458
3	0.185	0.185	0.452
4	0.188	0.187	0.456
5	0.184	0.185	0.450

These results do not support the hypothesis that the calcium salt of an organic acid is present in the epiphragm material. They indicate that within the limits of the accuracy of the method, no calcium is present in this material in a form other than calcium carbonate

which makes up approximately 45 per cent of the dry weight of these epiphragms.

I therefore rejected the hypothesis that the epiphragm of Helicella virgata could function as a site of storage for partially oxidised products of metabolism.

3.65 The consumption of oxygen and release of carbon dioxide in snails emerging from dormancy

The epiphragms of dormant snails did not contain any lactic acid, nor (on the basis of the previous experiment) did it seem likely that any other organic acid could be present in this material as a calcium salt. These observations did not preclude the possibility that some other organic acid might have been accumulated, together with lactic acid, in the tissues of the body during dormancy.

If substantial quantities of such acids were present in dormant snails, and were metabolised as the animals resumed active life, then I expected that these animals would show the repayment of an oxygen debt.

In order to investigate the hypothesis that snails displayed an oxygen debt on arousal from dormancy, I performed the following experiment, using manometers to measure the oxygen consumed and the carbon dioxide produced by individual specimens of Helicella virgata, and compared the values obtained with those from active animals.

The dormant snails used in this experiment were collected from Northfield in March 1968. Fifty large snails of the size class

1.5 $\pm$ 0.1 cm with respect to the diameter of the shell were collected and numbered from one to fifty at the time of collection, by painting numbers on the shells. These snails were stored over silica gel in a desiccator at 20°C for approximately 5 months. At the end of this time they possessed thick calcareous epiphragms.

Fifty large active snails of the same size class were collected after rain at Northfield (11.8.68). These were numbered from one to fifty as before and were kept overnight in a cage made from nylon mesh, under fine sprays controlled by a time clock. They remained active and defaecated, but were not permitted to feed.

A circular manometer bath, on which 16 manometers could be mounted simultaneously, was used for the determinations, which were carried out, one per day, for four consecutive days. The determinations were of active, dormant, dormant and active snails on days one to four respectively.

Fourteen snails were withdrawn at random from stock and placed into the manometer cups with as little mechanical stimulation as possible. Into seven of these cups were placed chambers containing folded filter-paper, moistened with 1.0 ml of 10% KOH, to absorb carbon dioxide. These chambers were made from "Clearsite" plastic specimen tubes that had been completely perforated with drill-holes. Their purpose was simply to prevent crawling snails from coming into contact with the KOH.

All cups were placed on the manometers which were then placed in the water bath at 25°C, the taps were closed after 30 minutes equilibration

time. The manometers were read at 30 minute intervals for two and a half hours after the taps were closed.

At the end of two and a half hours, all the manometers were removed from the water-bath. The KOH chambers were removed from the cups, and fresh KOH chambers were introduced into the other seven manometer cups that had not previously contained any. The position of individual snails was not changed. All the cups were replaced on the same manometers as before and the manometers were returned to the water-bath in the same sequence as previously. After 30 minutes the taps were again closed and the manometers read for a further two and a half hours.

Two manometers were run as controls, one empty as a thermobarometer, and the other containing a KOH chamber to check that there was no action of KOH on the plastic from which the chambers were made that might affect the readings of the manometers.

At the end of each determination the snails were removed from the manometer cups and weighed, killed, and the bodies removed from the shells. The shells and bodies were dried in the vacuum drying unit and weighed.

The oxygen consumption of each snail was calculated in ml per gm wet body weight per hour. The results for individual snails are given in Table 3.14 below.

TABLE 3.14

Oxygen consumption (ml/gm wet body/hr) of individual active and dormant snails at 25°C

Hours from start of expt.	Active		Dormant	
	11.8.68	14.8.68	12.8.68	13.8.68
Run 1 (0.5 to 3.0 hrs)	0.185	0.154	0.016	0.028
	0.227	0.033	0.011	0.047
	0.272	0.200	0.034	0.002
	0.209	0.211	0.050	0.048
	0.310	0.115	0.027	0.036
	0.145	0.010	0.020	0.028
	0.431	0.196	0.002	0.027
Run 2 (4.0 to 6.5 hrs)	0.234	0.333	0.000	0.078
	0.261	0.155	0.075	0.141
	0.222	0.209	0.153	0.064
	0.195	0.252	0.224	0.084
	0.256	0.177	0.080	0.061
	0.295	0.208	0.047	0.120

The mean and variance of the oxygen consumption of each group of snails is shown in Table 3.15 for Runs 1 and 2 respectively. These means have been compared using t-tests, where the variances were homogeneous. Where this condition was not met with, the means were compared using the method given by Bailey (1959, p. 51). The probability that the mean oxygen consumption during the first run ($2\frac{1}{2}$ hours) of any determination did not differ from that observed during the second run of the same determination, is also shown in Table 3.15 below.

TABLE 3.15

Mean oxygen consumption (ml/gm wet body/hr) of active and dormant snails at 25°C. [The figures in brackets are the variances associated with each mean]

Hours from start of expt.	Active		Dormant	
	11.8.68	14.8.68	12.8.68	13.8.68
Run 1 (0.5 to 3.0 hrs)	0.254 (0.009)	0.131 (0.006)	0.023 (0.0002)	0.031 (0.0003)
Run 2 (4.0 to 6.5 hrs)	0.238 (0.001)	0.224 (0.003)	0.091 (0.005)	0.085 (0.001)
deg. freedom	7	12	6	12
$P(\bar{x}_1 = \bar{x}_2)$	0.3>P>0.2	0.001>P	0.05>P>0.02	0.01>P>0.001

These results indicate that the consumption of oxygen is markedly less in snails aroused from dormancy than in active animals. Further, consideration of the probability values in Table 3.15, indicates that the metabolism of snails in all groups except the 11.8.68 actives, appeared to change between Runs 1 and 2, more oxygen being consumed during the second run.

It was not possible to determine the amount of carbon dioxide evolved by individual animals. With some individuals the total volume of gas within the manometer increased during a run, and with others a decrease was observed. The results in Table 3.16 are presented in the form of volume increases (ml) shown by individual snails for a two and a half hour period of measurement. A negative figure in the body of the table indicates that a particular snail consumed a greater volume of oxygen than it liberated as carbon dioxide. A positive figure indicates that the reverse situation was observed.

TABLE 3.16

Total volume increase (ml) within the manometers shown by active and dormant snails at 25°C

Hours from start of expt.	Active		Dormant	
	11.8.68	14.8.68	12.8.68	13.8.68
Run 1 (0.5 to 3.0 hr)	-0.084	-0.024	0.124	0.100
	0.008	0.184	0.084	0.464
	0.020	-0.024	0.668	0.188
	-0.016	-0.044	0.684	0.152
	-0.020	0.044	0.060	0.396
	-0.036	-0.028	0.208	0.128
	-0.012	0.012	0.144	0.076
Run 2 (4.0 to 6.5 hr)	-0.020	-0.040	0.096	0.000
	-0.020	0.008	0.080	0.028
	-0.016	0.012	0.072	0.056
	-0.008	-0.024	0.012	0.004
	-0.024	0.004	0.036	0.036
	-0.016	0.064	0.168	0.016
	-0.008	0.012	0.092	0.004

The total increase in volume is presented, together with the total weight of the wet bodies of each group of snails, in Table 3.17 below.

TABLE 3.17

The weight of each group (gm) and the total change in volume (ml) within the manometers for each group of snails

Conditions of snails	Date of determination	Σ wgt (gm)		Σ vol (ml)	
		Run 1	Run 2	Run 1	Run 2
Active	11.8.68	3.23	3.43	-0.128	-0.112
	14.8.68	3.01	3.08	0.120	0.036
Dormant	12.8.68	1.55	1.99	2.728	0.548
	13.8.68	1.77	1.78	1.504	0.144

These results, while they are not amenable to statistical analysis, suggest that again differences in metabolism occurred between the runs. Snails in all groups (other than 11.8.68 actives) showed a greater

production of carbon dioxide during the first run than during the second.

Further, much greater volumes of carbon dioxide were produced by snails that had been dormant than were produced by active animals.

Discussion of Results

Oxygen Consumption

The results of the determinations of the oxygen consumption clearly do not support the hypothesis that an oxygen debt was being repaid by dormant snails on their return to the active state. It is thus unlikely that they contained a pool of organic acid that was metabolised following arousal. Oxygen consumption in active snails from the field was about eight times that of recently aroused animals, when measured from a half to 3 hours after the beginning of the experiment. During the second period of measurement the oxygen consumption of the recently aroused snails rose. Nevertheless, between four and six and a half hours after the start of the experiment, the active snails from the field were still respiring oxygen at approximately two and a half times the rate of those that had been dormant.

It is doubtful if the figures obtained on 14.8.68 can be regarded as having come from truly active animals. These snails had been kept in a cage made from nylon mesh, under a spraying system, since their collection on 10.8.68. They had therefore been stored in this way without food for four days prior to the determination. During this time

some of these snails crawled up the sides of the cage and became inactive in spite of the spray. It seemed logical to describe these snails as active for the purpose of comparing them with those that had been truly dormant for approximately five months. The results suggest, however, that these 14.8.68 snails differed from those that were kept only overnight under the sprays. They consumed a little more than half as much oxygen initially as did the truly active snails, but their oxygen consumption was greater by a factor of some four to five times than that observed for the truly dormant animals. Further, by the second run, the oxygen consumption of this so-called active group had increased to a similar value to that observed for the truly active animals.

It seems likely that this group of so-called active snails contained some animals that were either inactive or had begun to enter dormancy as a result of the conditions under which they were kept. Whatever the effects of this change in condition, they were rapidly overcome, so that, unlike the snails that had been truly dormant, the performance of this group of animals with respect to the consumption of oxygen during run 2, was essentially similar to that of the active snails kept only overnight under the sprays.

Production of Carbon Dioxide

The results from that part of the experiment designed to measure the amount of carbon dioxide liberated by individual snails are difficult to interpret. However, even though the results are based on quite small

samples, there is clear evidence to suggest that in all groups, except those containing truly active snails, more carbon dioxide was evolved during the first run of a determination than during the second.

The results of the changes in volume during runs 1 and 2 are presented for individual animals in Figures 3.12 and 3.13. The determinations were made on 12.8.68 and 13.8.68 respectively, using snails that had been truly dormant. Clearly there was considerable variation amongst individuals within a run, with respect to the volume and rate of liberation of carbon dioxide, although this variation is more obvious for the snails within the first run of each determination.

In Figure 3.12 two snails show a very rapid evolution of gas during run 1. This period of very rapid evolution of gas was for the most part begun and concluded within the interval between successive 30 minute readings of the manometers. A similar result is shown by one snail for run 1 in Figure 3.13, although the change in volume was less for this animal. It should be noted that if any other snail had performed similarly in the first 30 minutes of the experiment, during the equilibration time before the manometer taps were closed, then that result would not have been recorded.

I consider that such large and rapid changes in volume are unlikely to have been the results of metabolic processes occurring during the actual time at which these changes were observed. It seems far more likely that they resulted from the elimination from the tissues of the snails of

FIGURE 3.12. The increase in volume within the manometer cup shown by individual snails aroused on 12.8.68 after 5 months' dormancy.

(plotted in manometer scale units, 1 unit ~ 0.004 ml.)

A. (above) Run 1.
30 mins. to 3 hours after start of experiment.

B. (below) Run 2.
4.0 to 6.5 hours after start of experiment.

In both, the abscissa is shown as minutes after the manometer taps were closed.

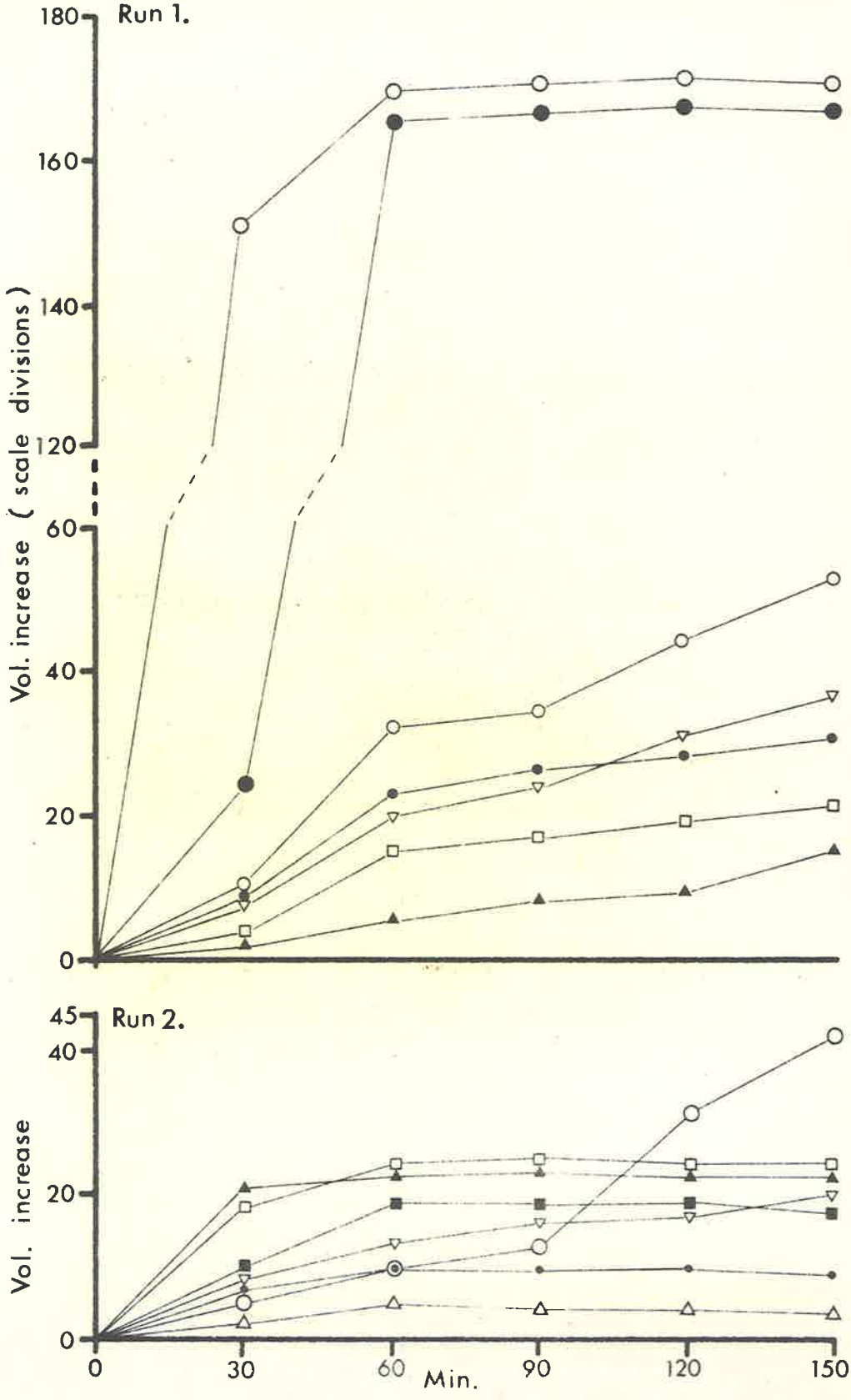

Run 1.
180
160
140
120
60
40
20
0
Vol. increase (scale divisions)
Run 2.
45
40
20
0
Vol. increase
0
30
60
90
120
150
Min.

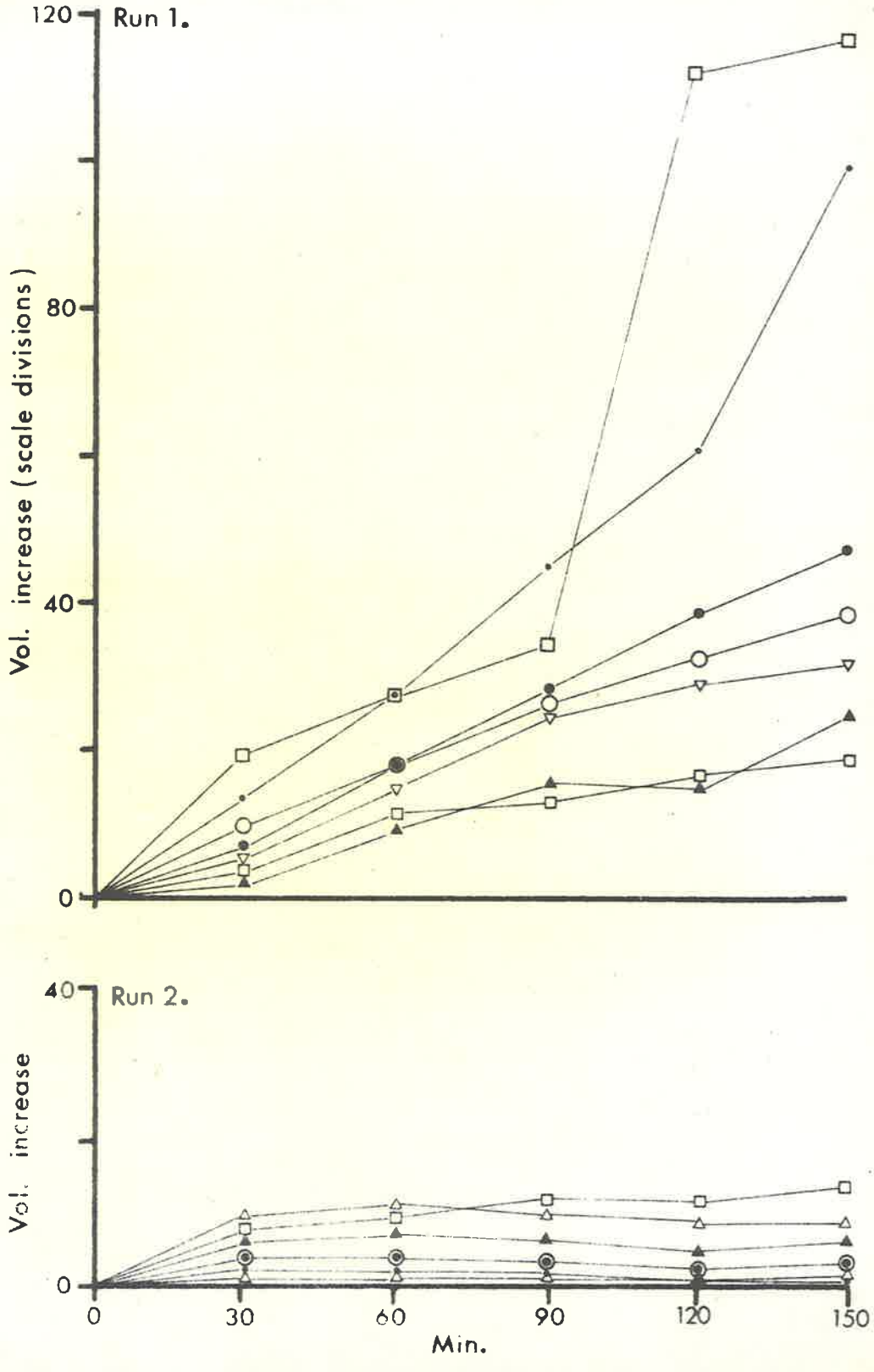
FIGURE 3.13. The increase in volume within the manometer cup shown by individual snails aroused on 13.8.68 after 5 months' dormancy.

(plotted in manometer scale units, 1 unit ~ 0.004 ml.)

A. (above) Run 1.
30 mins. to 3 hours after start of experiment.

B. (below) Run 2.
4.0 to 6.5 hours after start of experiment.

In both, the abscissa is shown as minutes after the manometer taps were closed.


Run 1.
120
80
40
0
Vol. increase (scale divisions)
Run 2.
40
0
Vol. increase
0
30
60
90
120
150
Min.

a pool of carbonate or bicarbonate, accumulated as a result of previous metabolic processes during the period of dormancy.

Further, these rapid changes in volume tended to be associated with the time at which the snail first crawled in the manometer cup. This activity followed the ingestion of the epiphragm, and I would speculate that a further source of the change in volume might have been associated with the action of the gut on the carbonate fraction of the epiphragm.

Clearly retention of carbon dioxide occurs within dormant snails. Meyer and Thibaudet (1937) reported that at 0° to 4°C the loss in weight from "hibernating" Helix pomatia could be accounted for entirely by the loss of water from the animal. Pomeroy (1966) found that the same general result was true for Helicella virgata, and my own experiments confirm this observation for specimens of Helicella virgata kept dormant at temperatures of less than 25°C.

Above this temperature I found that some loss in dry weight did occur in dormant specimens of Helicella virgata, and this result appears to agree with that of Meyer and Thibaudet (1937) for "aestivating" Helix pomatia kept at 20° to 30°C.

The dormant snails, used in the experiment to determine the amount of carbon dioxide that was produced following arousal, were kept at room temperature during their period of dormancy. The temperatures in this room fluctuated between approximately 17° and 22°C.

3.66 Conclusions about the type of metabolism employed during dormancy by Helicella virgata

The experiments reported in Section 3.6 lend little support to the model that was proposed for the dormant snail. This model was largely based on the work of Meenakshi with Pila virens, a snail that is anaerobic during dormancy. It is unlikely that the physiology of dormant Helicella is like that in dormant Pila.

The model was supported by two observations. Firstly that calcium was transported from the shell to the body of dormant snails, and secondly that some increase in the amount of lactic acid in the tissues of dormant Helicella virgata occurred. Both of these observations suggested the presence of an anaerobic metabolism.

Meenakshi (1958) showed that the lactic acid content in specimens of Pila after 6 months' dormancy was approximately 450 times that of active snails. In Helicella, snails dormant for three years contained only about twice as much lactic acid as those that were active.

On arousal from dormancy lasting 6 months, specimens of P. virens consumed approximately 1.8 times as much oxygen as did normal active snails, this oxygen debt was repaid after 6 days. Helicella virgata did not show an oxygen debt on arousal from dormancy. The consumption of oxygen in these snails was less than that of active specimens, and of the same order initially as that found in dormant specimens, indicating that there was no pool of organic acids being metabolised. Further, on crawling,

these snails showed no obvious excretion or defaecation to indicate that organic acids were being eliminated from the body.

There is good reason to believe that in Helicella virgata the overall metabolism during dormancy is aerobic, although the rate of oxygen consumption is low.

On this assumption it is possible to calculate approximately the amount of glycogen that would have been necessary to account for the observed changes in energy in the tissues of dormant snails, assuming that glycogen only is the energy source..

Since this calculation requires that no significant changes in dry weight occur during the period of dormancy, only the data from those snails at 20°C in Experiment 3.4 are useful.

From Section 3.34 the relevant quantities are

Initial live weight of samples = 14205.9 mg (Table 3.4)

Initial weight of dry tissue = 1204.35 mg (Table 3.4)

Decrease in energy (day 0 - 160) = 4.91 - 3.90 cal/mg (Fig. 3.07)

= 1.01 cal/mg

Heat of combustion of glycogen = 3.8 Kcal/gm

Hence the amount of glycogen that would have been necessary to provide the observed change in energy under aerobic conditions is

$$\frac{1.01\ (1204.35)}{3.8} \text{ mg}$$

which is $\frac{1.01\ (1204.35)}{3.8\ (14205.9)}$ x 100% of total live wgt

= 2.25% of the total live weight

This figure is interesting, as von Brand (1931) found that for Helix pomatia the glycogen content was at its greatest just before "hibernation" began. The maximum glycogen content of the animals in his study was approximately 2.5 per cent of the total live weight.

The snails in Experiment 3.4 were collected from the field just prior to the onset of natural dormancy, at a time when I would have expected the energy reserves to be maximal. It is perhaps dangerous to make too close a comparison of Helix and Helicella, but in view of the close taxonomic relationship between these snails, it does not seem unreasonable, at this stage, to suggest that there may also be close similarities between them with respect to the nature and quantity, relative to body size, of the energy stores.

In spite of the evidence to suggest that the overall metabolic processes of dormant Helicella virgata are aerobic, there is also the observation that calcium is transported from the shell to the body of dormant snails. This observation alone does not necessarily imply that an anaerobic process is involved in dormancy (the observed phenomenon might simply suggest that calcium is necessary for the formation of epiphragms), but, taken together with the observation that there is an increased lactic acid content in dormant snails, it might suggest that

short periods of anaerobiosis occur during dormancy, perhaps as an adaptation to prevent water loss (Martin 1966). If, following such a period of anaerobiosis, the lactate was metabolised in preference to further glycogen, then the overall process would appear to be an aerobic one, but would also account for the observed phenomena of calcium transport and elevated lactic acid in some snails.

In order to investigate this hypothesis it will be necessary to devise some means of measuring regularly the oxygen consumption of individual dormant snails over quite long periods of time. Further, it will be necessary to achieve constant temperature conditions in which the manometers can be read accurately, and where no vibration is present.

When snails that have been dormant are aroused to the active state, they release carbon dioxide in large quantities. The volumes and rates of release suggest that this carbon dioxide is being eliminated from some pool, accumulated within the tissues of the snail during dormancy. Clearly there must be retention of carbon dioxide if snails are not to lose dry weight during dormancy.

Meyer and Thibaudet (1937) found that at 0° to $4^{\circ}C$ specimens of Helix pomatia did not lose dry weight during dormancy, but did so at 20° to $30^{\circ}C$.

Pomeroy (1966) did not find a decrease in dry weight amongst dormant specimens of Helicella virgata during his study. He even suggested that there might have been a slight gain in dry weight during this time.

My experiments with H. virgata have shown that, during dormancy at 20°C, no significant changes in dry weight occurred. However at 30°C there was a decrease in dry weight. It is suggested that this loss was due to the fact that carbon dioxide was lost from these animals during dormancy.

The ability of dormant snails, to retain within their bodies all the carbon dioxide produced from metabolism, would clearly favour the retention of water by these animals. This ability would enable H. virgata, at moderate temperatures, to overcome the problem facing most animals in a dry environment, where water may be lost in association with expired carbon dioxide. It is not clear how carbon dioxide was lost from the snails that were dormant at 30°C, but this loss might have occurred by diffusion through the surface of the mantle, or by expiration through the pneumostome. It has been suggested above that no carbon dioxide was lost from snails dormant at 20°C. It seems unlikely that the permeability of the mantle to carbon dioxide would change such that the loss of weight (presumably CO_2) observed in snails at 30°C, could be accounted for solely in terms of a diffusion of CO_2 from the tissues. It is more likely that, whereas in snails dormant at 20°C the pneumostome was kept tightly closed during the whole period of dormancy, in those animals at 30°C it was not.

When dormant snails were aroused to the active state, it was seen that many of these produced a "burst" of carbon dioxide. From the time

at which this "burst" occurred, and the speed with which the gas was excreted, it seems likely that this carbon dioxide was voided by way of the pneumostome.

In several species of insects, the consumption of oxygen has been shown to be more or less continuous, but the release of carbon dioxide is cyclical. The carbon dioxide is released in "bursts" during periods when the spiracles are opened widely. The "bursts" are followed by long periods during which the spiracles are restricted, and little or no carbon dioxide is excreted (Schneiderman and Williams 1953, 1955; Punt et al. 1957). Buck (1962) has pointed out that this phenomenon is most developed in forms that spend long periods without water, such as diapausing lepidopterous larvae and pupae. Buck (1958) has also shown that, in theory, less water would be lost from the insect in this way than if the spiracles were set permanently at the minimum opening that would suffice to rid the animal of carbon dioxide by diffusion alone.

The relevance of these insect studies to the situation in H. virgata is possibly slight. However, if it is assumed that most of the carbon dioxide, lost from a dormant snail, is excreted by way of the pneumostome, then one can speculate on the reasons for the observation that snails at 30°C lost dry weight, while those at 20°C did not.

The work of Ysseling (1930), Wit (1932) and Maas (1939) has shown that carbon dioxide concentration, oxygen tension, temperature and humidity, all influence the rate of ventilation in active land pulmonates. Carbon dioxide concentrations were found to have little effect on the rate of

respiratory movements, but at low concentrations carbon dioxide was found to stimulate the opening of the pneumostome. Dahr (1927) showed that the pneumostome would remain open when snails were kept at carbon dioxide tensions of 3 to 5 per cent. Increasing the carbon dioxide concentration above 5 per cent caused a decrease in the amount of oxygen utilized by the snails.

It is not clear how these observations relate to the situation in the dormant snail, however, if the opening of the pneumostome was controlled by the presence of certain concentrations of carbon dioxide in the lung, then whether or not it opened would depend on the relative rate at which carbon dioxide was produced and bound in the tissues. If, at 30°C, the speed of the processes, whereby carbon dioxide is fixed, lagged behind the production of carbon dioxide from metabolism, then one might expect the concentration of this gas to increase in the lung and the pneumostome might then be opened. By analogy with insects, if Buck's (1958) conclusion is correct, it might be of advantage for a snail in a dry place to expel the carbon dioxide rapidly and to close the pneumostome tightly again for long periods.

4.0 APPENDICES

4.1 Method of constructing snail-proof pens

The need to prevent snails from leaving an experimental area necessitated that they be enclosed by some form of snail-proof fence. This is difficult to achieve since snails both burrow and climb.

The method used to construct the pens was similar to that used by Pomeroy (1966), but some minor modifications were adopted.

The size of the pens was five feet by ten feet. Within such an area it was possible to examine the ground without having to step into the pen to do so.

The fences were made from lengths of 24 gauge galvanized iron strip, 10 gauge galvanized trellis wire (a welded product consisting of lengths of 10 gauge wire laid to form six inch grids), and galvanized fly-wire mesh (approximately 10 strands to the inch).

Fences were assembled in the laboratory. The galvanised iron was cut into lengths of four inches by ten feet. To this was soldered a length of trellis wire, one foot high by ten feet long, and the whole structure was clad in galvanized fly-wire in the manner shown in Figure 4.01A

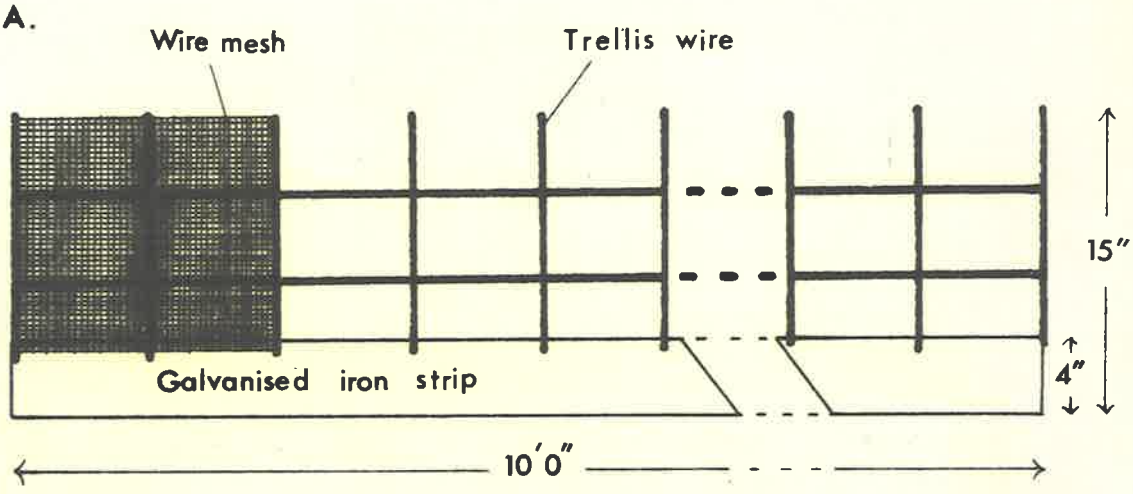
A fold was made in the trellis wire, opposite the galvanized strip, so that three inches of recurved edge was obtained along the upper edge of the fence. To the edge of the bent fence a three inch strip of "Sarlon" shade cloth was glued, using contact cement. This material was woven from plastic fibres. When the contact cement had hardened, the fibres running

FIGURE 4.01. Method of constructing snail pens.

A. (above) Lateral view of the components of a fence before bending.

B. (below) Top of a fence after bending and fringing with plastic fibres.

A.


Wire mesh
Trellis wire
15"
4"
Galvanised iron strip
10'0"

B.

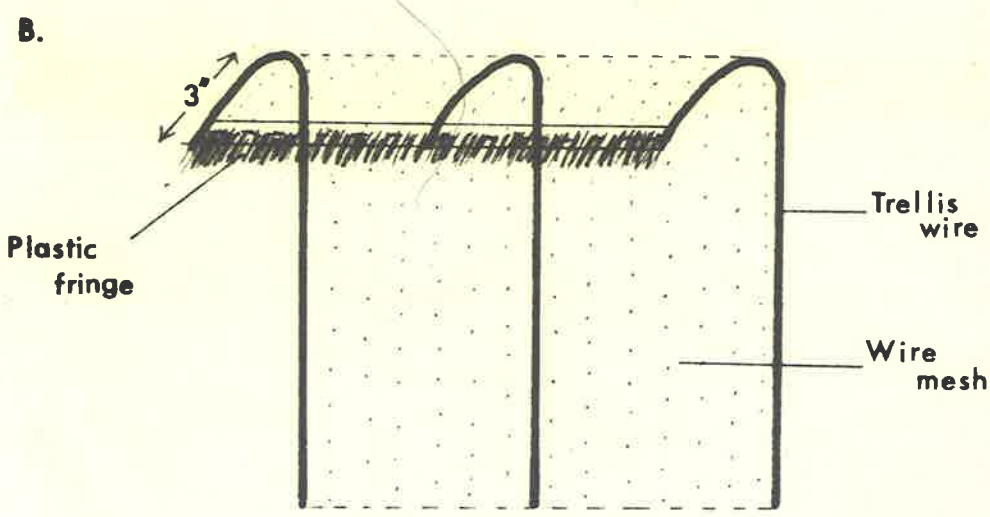
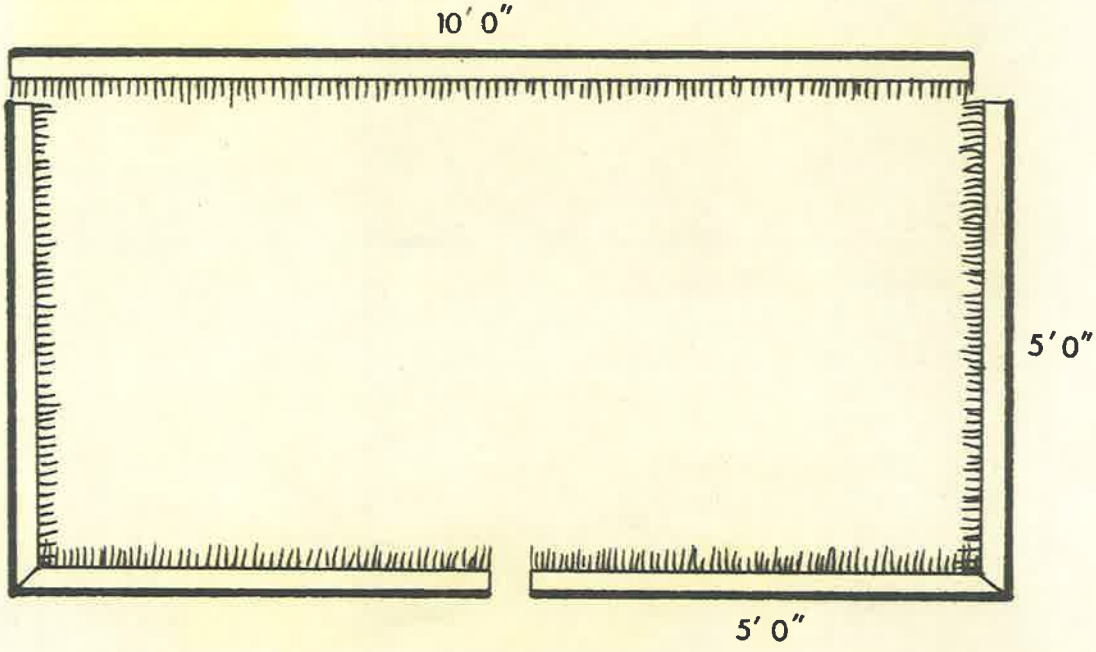

3"
Plastic fringe
Trellis wire
Wire mesh

FIGURE 4.02. Plan view of pen to show the way in which the fences were bent.



parallel to the top of the fence were pulled out so that a fringe of plastic fibres projected past the end of the bent fly-wire as shown in Figure 4.01B.

The fences were transported in straight lengths to the study area at Northfield.

A four inch trench, measuring five feet by ten feet, was cut in the soil for the erection of each pen. Three ten feet lengths of fence were bent in the manner shown in Figure 4.02, and placed so that the galvanized iron strip lay in the trench. The lengths were overlapped for about half an inch and wired together. All overlaps were sown together with waxed string and the earth was then tamped down around the galvanized iron, holding the whole structure rigid.

These fences appeared to be successful, no escapes were recorded from them. The pens did not prevent snails from entering.
Thus for any experiment in which it was desired to prevent snails from entering as well as leaving, it would probably be necessary to build fences with outward projections as well as those pointing into the pen.

4.2 Methods for measuring snails in the field

For the experiments reported in Sections 2 and 3 of this thesis it was sometimes necessary to collect large numbers of snails of a given size-class. It was necessary that the method used to measure the snails should be rapid but accurate.

A sliding measuring block was used; this is shown in Figure 4.03A. Both the fixed and the sliding jaws were made from half inch clear perspex. The measuring faces were ground flat. A section of millimeter graph paper was inserted under the fixed jaw, and located with one scale line immediately beneath the measuring face on the fixed jaw. The tightening screws were then done up to prevent the graph paper from moving.

The snails were placed between the jaws of the block in the manner indicated in Figure 4.03B. The aperture of the shell was placed downwards in the corner of the fixed block, with the umbilicus of the shell against the inner wall of the fixed block. The sliding jaw was pushed up against the shell and the width was read to the nearest 0.5 mm from the graph paper beneath the end of the sliding jaw.

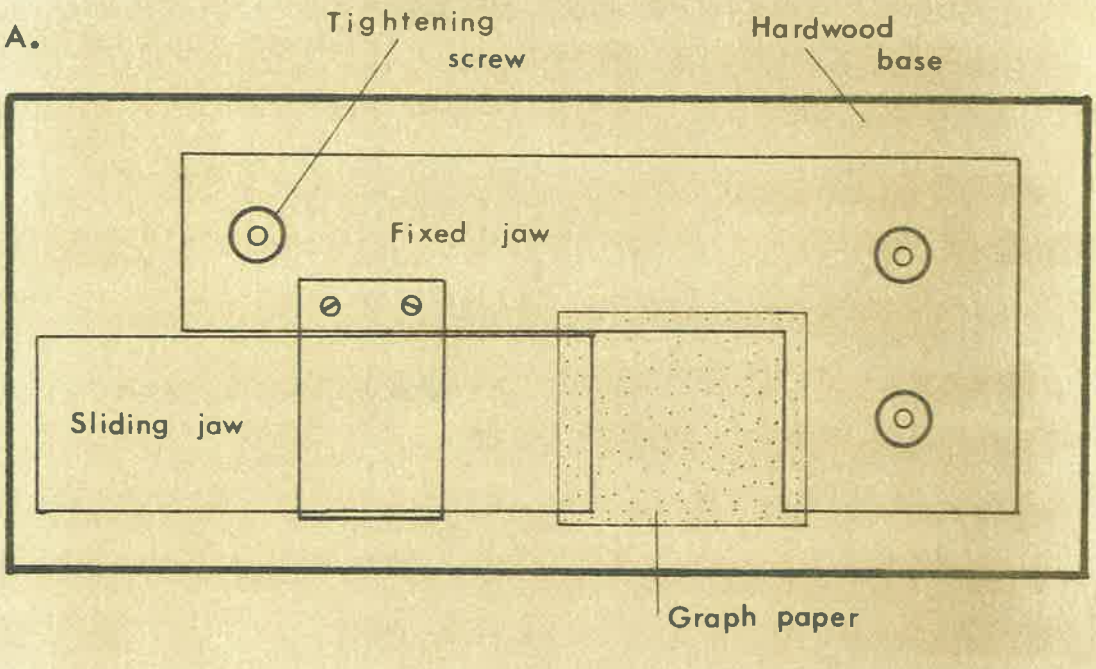
With this method it was possible to measure snails rapidly. When collections of snails of a given size-class were made, the limits of the size-class were marked in indian ink on the graph paper. It was thus simple to determine whether a particular snail was of the size-class without actually counting the scale divisions at each measurement.

FIGURE 4.03. Method of measuring snails.

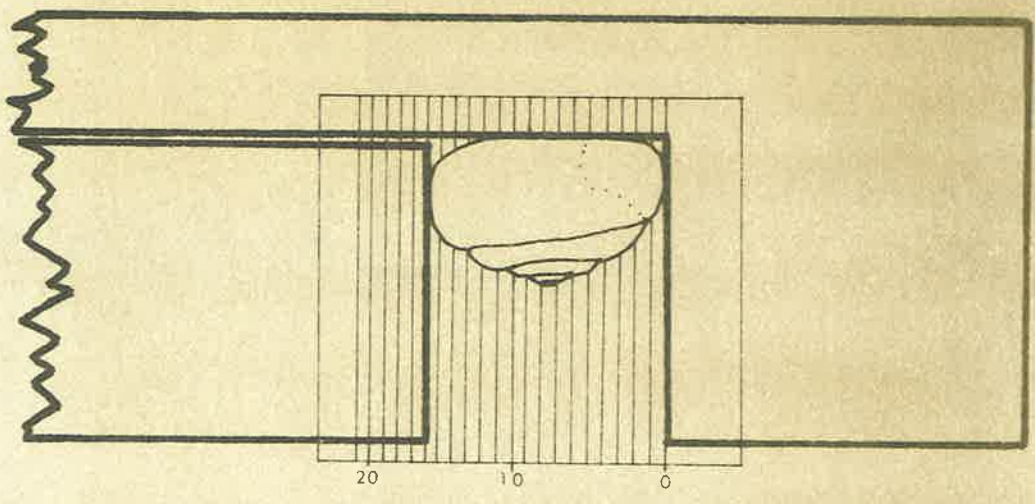
A. (above) Plan view of measuring block.

B. (below) Detail of measuring block to show the position of of snail between the jaws.
(The snail shown would have been taken to measure 15.5 mm.)

A.


Tightening screw
Hardwood base
Fixed jaw
Sliding jaw
Graph paper

B.


20
10
0

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