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STUDIES ON THE FEEDING OF THE GREY FIELD SLUG
AGRIOLIMAX RETICULATUS (MÜLLER) IN THE LABORATORY
AND IN WOODLAND.

by

Dennis Pallant B.Sc.

JUNE, 1967

A THESIS PRESENTED FOR THE DEGREE OF MASTER OF SCIENCE
OF THE UNIVERSITY OF DURHAM.



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1. INTRODUCTION

The food of slugs (Pulmonata: Testacellidae, Arionidae, Limacidae.) is known in general terms. Innumerable isolated feeding incidents have been reported, and acceptability of various foods has been determined with captive animals from unspecified habitats. Taylor (1907) gives detailed comments on the feeding of different species providing an encyclopaedic survey of the literature up to that time. Gain (1891) had investigated the acceptability of over a hundred plant species and some other materials to twenty species of pulmonate molluscs including twelve species of slugs. Field observations by many workers are scattered through the "Journal of Conchology". The destructive feeding of slugs on cultivated plants is known in detail. Ellis (1926) and Quick (1960) give brief notes species by species; Frömming (1954) has studied feeding by various methods including direct observation and the examination of faeces. Barnes and Weil (1954) observed feeding in gardens; Getz (1958) and Duthoit (1964) have investigated feeding in relation to agricultural land.

Feeding in specified natural habitats does not however appear to have been studied in detail and this would seem to be a subject of some ecological importance. It seems probable that acceptable food materials will differ in availability in different habitats,



especially in the case of so widespread a species as Agriolimax reticulatus (Müller), Quick (1960). The ecological significance of the species in a particular ecosystem might be related to the utilisation of different foods and therefore vary in different habitats also.

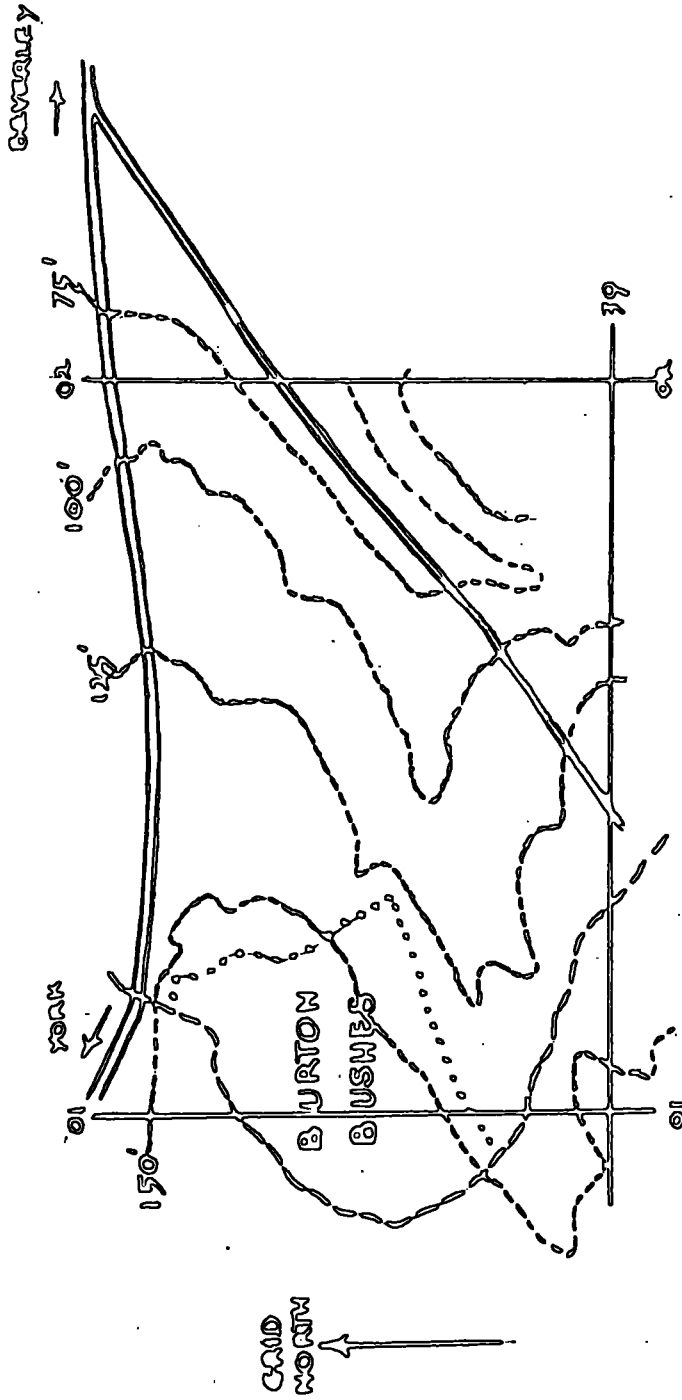
The present study attempts a limited interpretation of the feeding of Agriolimax reticulatus (Müller) in woodland which seems to provide a suitable habitat for this species. Several British species are found in woods; Quick (1960) refers to the occurrence of 12 species in woods, Limax tenellus Müller being the only one restricted to this habitat. Boycott (1934) describes woodland as providing "...exceptionally well the conditions which many Mollusca find convenient" and indicates that most common species are woodland species.

Van der Drift (1951) estimated a population of Arion subfuscus in beechwoods at 14 per square metre. South (1965) found average populations of Agriolimax reticulatus of over 60 per square metre on pasture and Hunter (1966) found over 115 of the same species per square metre of arable land; on both grass and arable land other slug species were also present. Populations of these magnitudes, corresponding to biomasses of approximately 5, 18 and 34 grams per square metre respectively, could occupy niches of appreciable importance in the economy of the habitat and might provide important

links in the turnover of materials in the ecosystem by their feeding activity.

There is some evidence that slugs and snails could provide an important stage in the conversion of plant litter, and living plant material, using substances not otherwise used directly as food materials by soil and litter animals. Of a group of soil and litter invertebrates investigated by Nielsen (1962,1963) only slugs and snails possessed the ability to digest a full range of plant structural polysaccharides. This author tentatively suggests that the ecological position of these animals may therefore be special as primary decomposers of these substances.

Food materials of Agriolimax reticulatus are investigated in the present study by the examination of the gut contents or faeces of specimens taken from woodland, and by laboratory feeding experiments on specimens from the same habitat. Quantities of food eaten and assimilated are determined in the laboratory and estimates are made of assimilation in woodland. The occurrence of cellulose digesting bacteria in the gut and faeces of A. reticulatus is investigated. The study was started in March, 1962, and has continued to February, 1967.



BURTON BUSHES : SITUATION.

TA 010394.

Scale: 6" = 1 mile.

- Westwood boundary.
- ... Open boundary of wood.
- 25' contours.

2. STUDY AREA.

A small wood, known as Burton Bushes, Beverley, Yorks., (M.R. 010394) has formed the principal study area. It is situated at the western end of Beverley Westwood, an area of rough common pasture. The wood is about a quarter mile square and is on a gentle slope at about 150 feet above sea level. The soil varies from leaf mould overlying loam to small areas where the underlying Pleistocene boulder clay reaches surface level. The base rock is Upper Cretaceous chalk, Wilson (1948).

The tree layer is mixed, but predominantly Oak, Quercus robur L., with Quercus petrae (Matuschka), Fraxinus excelsior L., Betula pendula Ehrh., Ulmus sp. L., Fagus sylvatica L., Ilex aquifolium L., Acer spp. also present. In some parts of the wood there is abundant undergrowth of Blackberry, Rubus sp. L. and Dog-rose, Rosa sp. L. The ground layer varies from none in the shade of Ilex to complete cover of grasses and other woodland herbs and mosses. Patches of nettle, Urtica dioica L. and Ranunculus repens L. are fairly frequent. Table 1 gives some indication of the nature of the ground flora in April, May and July. It is based on supervised line transects taken in 1959 and 1960 by two Sixth Form pupils of Beverley Grammar School and on 20 cm. square quadrat studies in 1964. Clapham, Tutin and Warburg (1962) is taken as the taxonomic authority on plant material.

TABLE 1.

LINE TRANSECT AND QUADRAT STUDIES OF GROUND FLORA IN BURTON BUSHES

	A	B	C
<u>Anemone nemorosa</u> L.	17.2	3	2.4
<u>Anthriscus sylvestris</u> (L.)	-	-	8
<u>Arum maculatum</u> L.	-	-	2.8
<u>Galium aparine</u> L.	3.5	2.5	0.6
<u>Geranium robertianum</u> L.	-	0.5	-
<u>Glechoma hederacea</u> L.	-	-	5.6
<u>Mercurialis perennis</u> L.	2.5	-	2
<u>Oxalis acetosella</u> L.	12.5	5.5	0.8
<u>Ranunculus ficaria</u> L.	24.2	-	32.4
<u>Ranunculus repens</u> L.	13.5	21.2	12
<u>Urtica dioica</u> L.	9	9	12.2
<u>Veronica montana</u> L.	1	3.5	2

A Average percentage occurrence in 4 line transects taken in April by P.H.Holmes and G.B.Abbott.

B Similar to A but taken in July.

C Average percentage occurrence in ten 20 cm. square quadrats taken in May.

Daytime searching, night searching and trapping showed the following species of slugs to be present in the wood:

ARIONIDAE; Arion intermedius Normand,

Arion fasciatus (Nilsson),

Arion hortensis Férussac,

Arion ater ater (L.),

LIMACIDAE; Agriolimax reticulatus (Müller).

Agriolimax reticulatus appeared much more frequently than any of the other species and this species was therefore chosen for study.

Quick (1960) is taken as the taxonomic authority for slug material.

3. DAYTIME RESTING SITES OF SLUGS IN ASSOCIATION WITH THE HERB LAYER IN WOODLAND.

(a) Methods.

Kuhnelt (1961) quotes Frömming as pointing out that slugs and snails are generally absent from broadleaf forests lacking a ground cover. It was generally observed in daytime searching during the present study that slugs were not present in areas of the wood where there was no herb layer although there was no lack of suitable refuges in the form of fallen wood and leaf litter. One survey was therefore made using a class of able 14 to 15 year-old grammar-school boys as observers. The boys were instructed to search under fallen wood and record the presence or absence of slugs and other animals and the proximity of herbs to the log. To avoid duplication of observations and to investigate the different regions of the wood each boy was given a separate section of the wood in which to search. Logs lying in areas without herbs for a distance of 1 yard in each direction were taken as being distant from ground flora (without herbs), and were compared with logs lying on or near herbs. Forty logs of each kind were investigated.

(b) Results.

Table 2 shows the total numbers of logs of each category under which the different groups of animals were found. It is seen that eight logs near herbs had slugs present and three others had slugs

eggs, whereas none of the logs distant from herbs had either slugs or eggs present. The other groups of animals appear to be roughly equally distributed between the two types.

This distribution gives a chi-square value of 10 if eggs and slugs are included showing a significant association at the 1 per cent level. A t-test gives a value of t equal to 2.1 for slugs showing a significant association between resting site and ground flora at the 5 per cent level ($t = 2.0$)

TABLE 2.

Animal type	Number of logs with animals	
	near herbs	without herbs
Earwigs	2	2
Earthworms	8	3
Beetles	10	7
Flies	23	19
Centipedes	4	3
Millipedes	7	8
Spiders	16	13
Woodlice	15	12
Slugs	8	0
Slug's eggs	3	0

4. OCCURRENCE OF *Agriolimax reticulatus* (Müller) IN ASSOCIATION WITH DIFFERENT HERBS.

(a) Method.

On a few occasions selection of search sites was made by casting at random a 20 cm. side wire square. The flora within the square was recorded and slugs occurring among the plants and litter were collected. Twenty three 20 cm. quadrats were investigated in this way on five different days.

(b) Results.

The plant and slug species present in the 23 quadrats are shown in Table 3. *A. reticulatus* was more abundant than other species of slug in these collections and this species was found more frequently in May than in October and November.

Although relatively small samples, producing "expected" distributions near the minimum required for reliability; these results are analysed by chi-square tests for association between *A. reticulatus* and the two species of herb found subsequently to provide "favourite" food of this species of slug.

TABLE 3 Contd.

	25.11.64									24.2.65
	14	15	16	17	18	19	20	21	22	23
<u>Ranunculus repens</u> L.	-	-		-	-	-			-	-
<u>Ranunculus ficaria</u> L.		-								-
<u>Glechoma hederacea</u> L.							-	-		
<u>Anthriscus sylvestris</u> (L)									-	
<u>Urtica dioica</u> L.					-		-	-		-
<u>Veronica montana</u> L.	-	-		-	-	-		-	-	
<u>Oxalis acetosella</u> L.		-								-
<u>Fagus sylvatica</u> L. leaf litter		-								
<u>Quercus robur</u> L. leaf litter	-	-	-	-	-	-				-
<u>Agriolimax reticulatus</u> (Müller)	1	E	0	0	0	0	0	0	0	1

Plant species present indicated by -,

Number of slugs found indicated by numbers,

Egg mass found indicated by E.

The association of A. reticulatus with Ranunculus repens is investigated by a chi-square analysis in Table 4. This plant is of interest as being a food plant of the slug species as is established later in sections 5 and 6. The degree of association indicated is hardly significant and it cannot be assumed that the distribution of A. reticulatus is related to that of R. repens.

TABLE 4

CHI-SQUARE ANALYSIS OF A. reticulatus AND R. repens DISTRIBUTION IN QUADRATS.

Number of quadrats containing:

	<u>R. repens</u> and <u>A. reticulatus</u>	<u>R. repens</u> without <u>A. reticulatus</u>	<u>A. reticulatus</u> without <u>R. repens</u>
Observed	8	6	2
Expected (e)	5 1/3	5 1/3	5 1/3
(Null hypothesis)			
Difference (d)	2 2/3	2/3	3 1/3
d^2	64/9	4/9	100/9
d^2/e	4/3	1/12	25/12

$$\chi^2 (= \sum d^2/e) = 21/6 = 3.5$$

p less than .20 ($\chi^2 = 3.219$)

The association of A. reticulatus with Urtica dioica L. in the quadrat collections is investigated by a Chi-square analysis in Table 5. This plant is also established as a food of A. reticulatus in sections 5 and 6. Here again however no significant association is established.

TABLE 5

CHI-SQUARE ANALYSIS OF A. reticulatus and U. dioica DISTRIBUTION IN QUADRATS.

	Number of quadrats containing:		
	<u>U. dioica</u> and <u>A. reticulatus</u>	<u>U. dioica</u> without <u>A. reticulatus</u>	<u>A. reticulatus</u> without <u>U. dioica</u>
Observed	7	5	3
Expected (e)	5	5	5
(Null hypothesis)			
Difference (d)	2	0	2
d^2	4	0	4
d^2/e	4/5	0	4/5

$$\chi^2 = \sum d^2/e = 8/5 = 1.6$$

p less than 0.30 ($\chi^2 = 1.305$)

South (1965) found A. reticulatus distribution on pasture to be associated with cocksfoot grass tufts, suggested that this could be largely due to the provision of shelter by the tufts, and quotes Barnes and Weil (1945) and Boycott (1934) as concluding that food is unlikely to be an important factor in determining the distribution of slugs other than the Testacellidae. Although in the present study no significant association is found with known food plants in the quadrat studies it is suggested that the association with the herb layer shown in the previous section could be determined by the presence of the herbs representing available food. The small size of quadrat used, and the widespread distribution of the food plants of interest could account for the inconclusive result shown by Tables 4 and 5.

5. STUDIES ON FOOD EATEN BY A. Reticulatus IN WOODLAND

(i) Leaf anatomy of possible food plants

(a) Method

Initially, starved slugs were fed in captivity with various materials to obtain faeces representative of these. This method provided some useful observations, but when natural faeces examinations showed a predominance of fresh green leaf material largely of dicotyledonous origin it seemed preferable to take reference material directly from common herbaceous plants in the wood, and to examine this more systematically. Eight herbaceous plants commonly occurring in Burton Bushes were examined.

Epidermal scrapings of leaves, leaflets and petioles were taken from the common herbs by scraping the surfaces with a small scalpel with the blade held vertical to the surface. Fragments obtained in this way were mounted without further treatment in Puri's medium or latterly in Gurr's "Aquamount". Separation of fragments of epidermis by maceration in 50% nitric acid was also used, but the necessity for staining such preparations made the process of mounting longer and although this method would be essential for a quantitative specification of species differences it was considered that the scrapings gave sufficient information for the present purpose.

Material from the following plants was examined:

Anemone nemorosa L., Glechoma hederacea L., Mercurialis perennis L.,
Oxalis acetosella L., Ranunculus ficaria L., Ranunculus repens L.,
Urtica dioica L. and Veronica montana L.

Preparations were examined at magnifications of approximately 100 and 400 diameters for features likely to be useful in determining the food species from fragments occurring in faeces and crop contents. The form, size and frequency of occurrence of epidermal hairs was particularly noted as these were found to be prominent features in the faeces of Agriolimax reticulatus produced after feeding in the field. Drawings and photographs were made of the interesting features. Measurements were made with an ocular micrometer scale calibrated with a stage scale at the magnifications used.

(b) Results

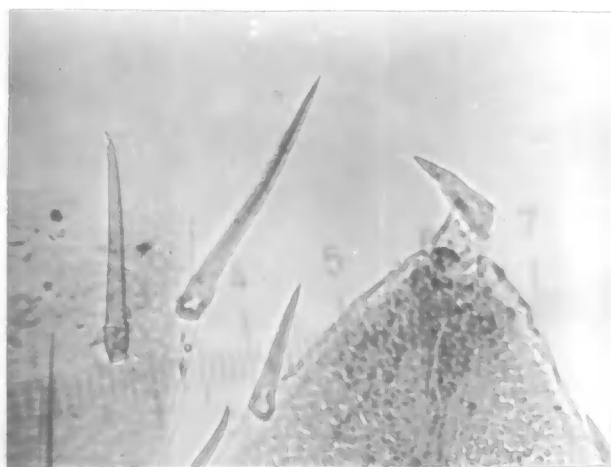
Epidermal cells from the herbaceous plants investigated are shown in the plates on pages 19 to 42.

Unicellular hairs of characteristic shapes were found on the leaf epidermis of the following species: Anemone nemorosa L., Mercurialis perennis L., Oxalis acetosella L., Ranunculus repens L., and Urtica dioica L. Multicellular hairs were found in Glechoma hederacea L., Urtica dioica L., and Veronica montana L.

Epidermal cell shape and size were found to be variable as between small and large leaves and in different regions of the leaf. Some characteristic shapes and sizes of these cells are indicated in the

illustrations as are the arrangements of epidermal cells round stomata for some species. Metcalfe and Chalk (1950) found four main types of arrangement of these last mentioned cells and the types found are referred to this classification.

In identifying material in crop contents and faeces of Agriolimax reticulatus epidermal hair types were taken as the only sure guide to species of food plant and only in rare cases were other features used as corroboratory evidence.



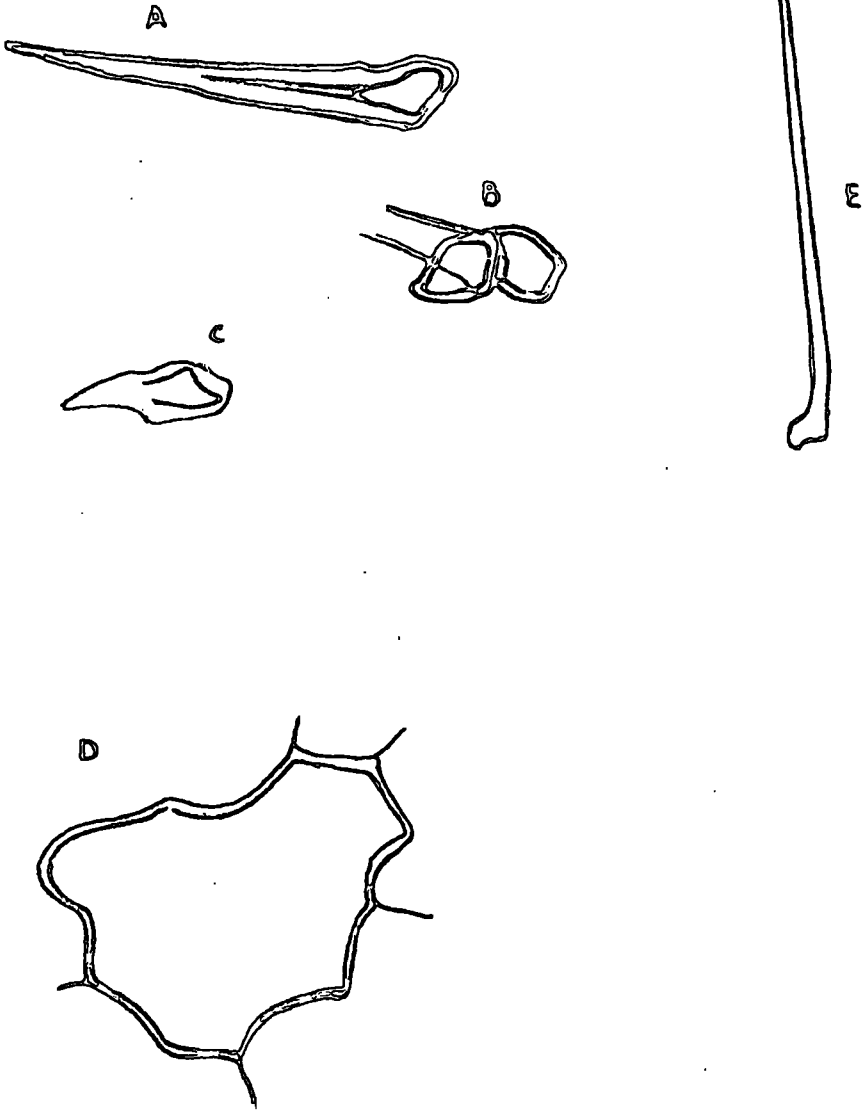
Anemone nemorosa L.

Leaf scraping showing unicellular
hairs (X 400 approx.)

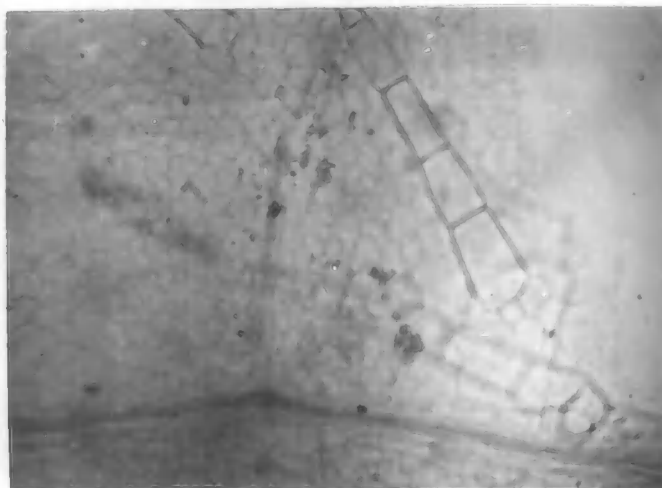
20.

Anemone nemorosa L.

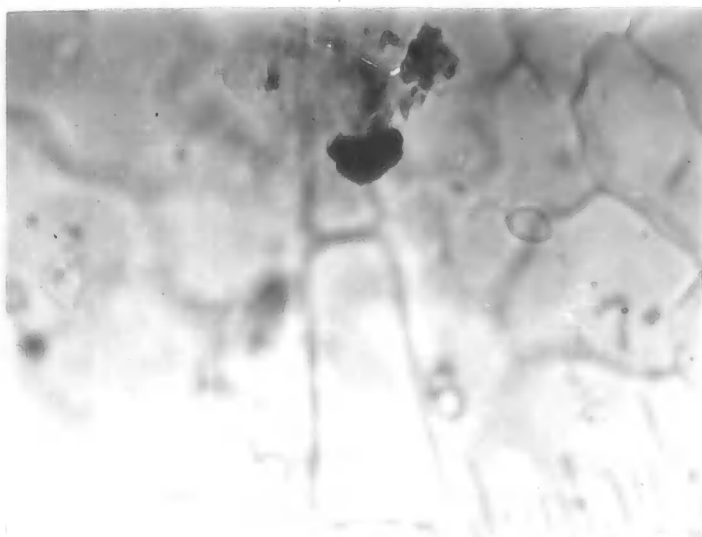
- A. Unicellular hair from epidermis of leaf lamina length 180 microns.
These hairs occur along the edges of the leaflets, about 4 per mm.
Shapes of bases of hairs variable as shown in B. (X 300 app.)
- B. Base of hair as in A (X 300 app.)
- C. Unicellular hair from epidermis of leaf lamina length 100 microns.
(X 200 app.)
- D. Typical epidermal cell showing rather irregular outline 58 microns
long (x 800 app.)
- E. Unicellular hair from epidermis 625 microns long, distribution
about 2 per sq. mm. (x 120 app.)



Leaf lamina epidermal features of Anemone nemorosa L.



A.



B.

Glechoma hederacea L.

Leaf scrapings showing multicellular
hairs and epidermal cells.

(A. X 400 app. B. X 800 app.)

23.

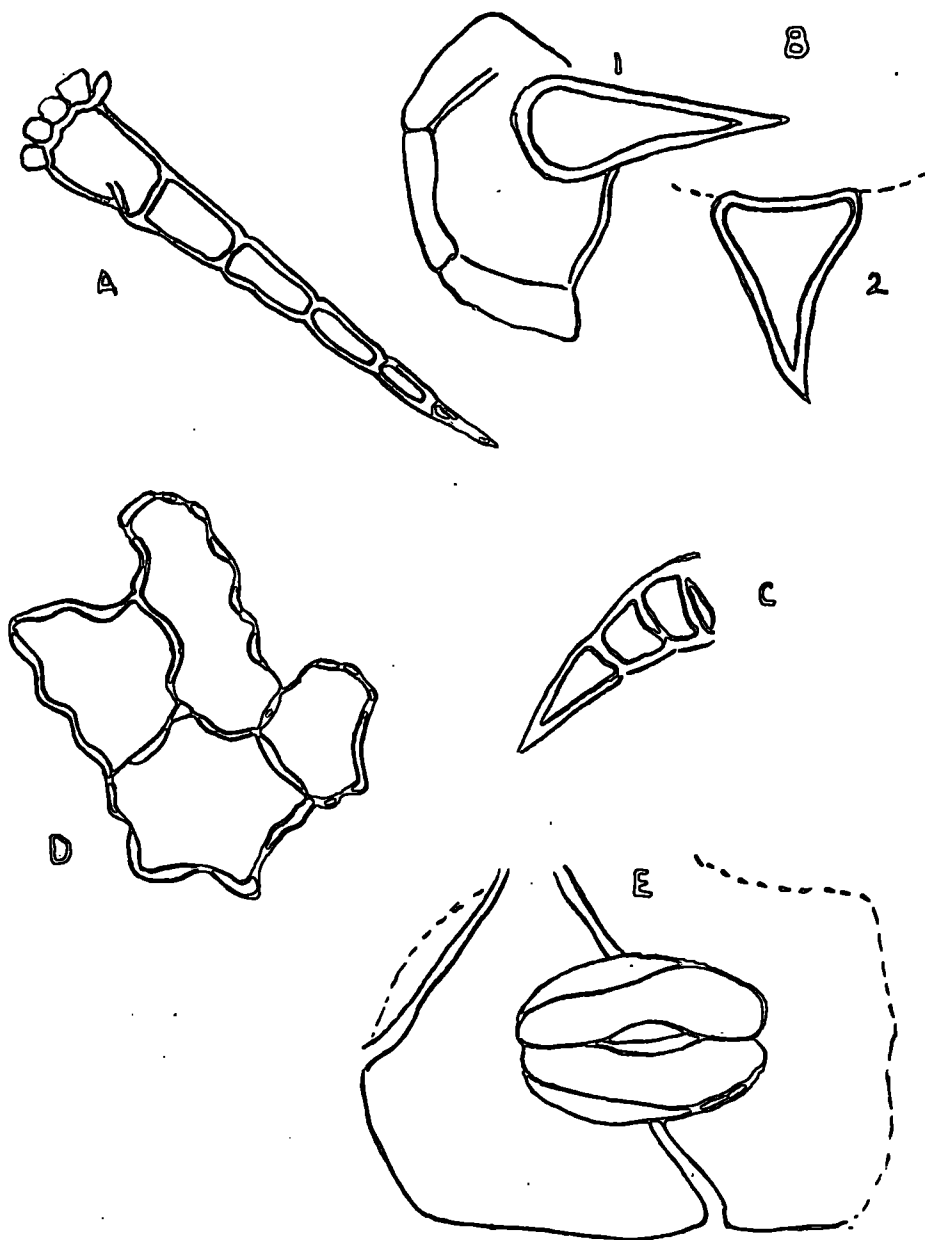
Glechoma hederacea L.

- A. Multicellular hair from leaf lamina epidermis. Similar hairs with seven cells also occurred. Distribution approx. 2 per sq. mm. Length approx. 390 microns. (X 200 app.)
- B. Unicellular hairs, length about 30 microns. In B2 dotted line indicates surface of neighbouring cells. (X 1000 app.)
- C. Small multicellular hair. Length 110 microns. (X 300 app.)
- D. Epidermal cells, length about 62 microns. Variable in shape, but all with wavy outlines as seen in surface view. (X 600app.)
- E. Stoma and guard cells.

This is not the typical textbook diagram of the arrangement of cells round a stoma in this species, although the typical arrangement has also been observed.

Metcalfe and Chalk (1950) Type D (Caryophyllaceous type).

Length of guard cells about 16 microns. (X 2000 app.)

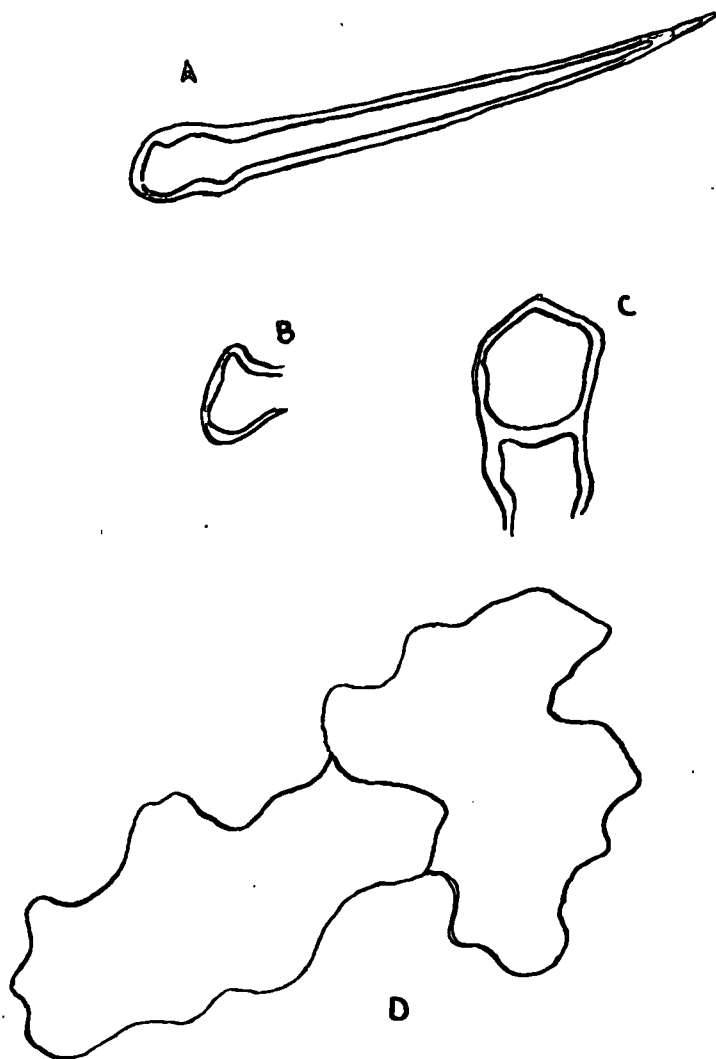


Leaf lamina epidermal features of Glecnoma neureracea L.

25.

Mercurialis perennis L.

- A. Unicellular epidermal hair from leaf lamina, length 400 microns.
Distribution 3 per sq. mm. These cells are sharply pointed and
have thin walls. (X 200 app.)
- B. Base of hair (X 200 app.)
- C. Hair base (X 480 app.)
- D. Epidermal cells in outline very irregular in shape. (X 150 app.)



leaf lamina epidermal features of Mercurialis perennis L.

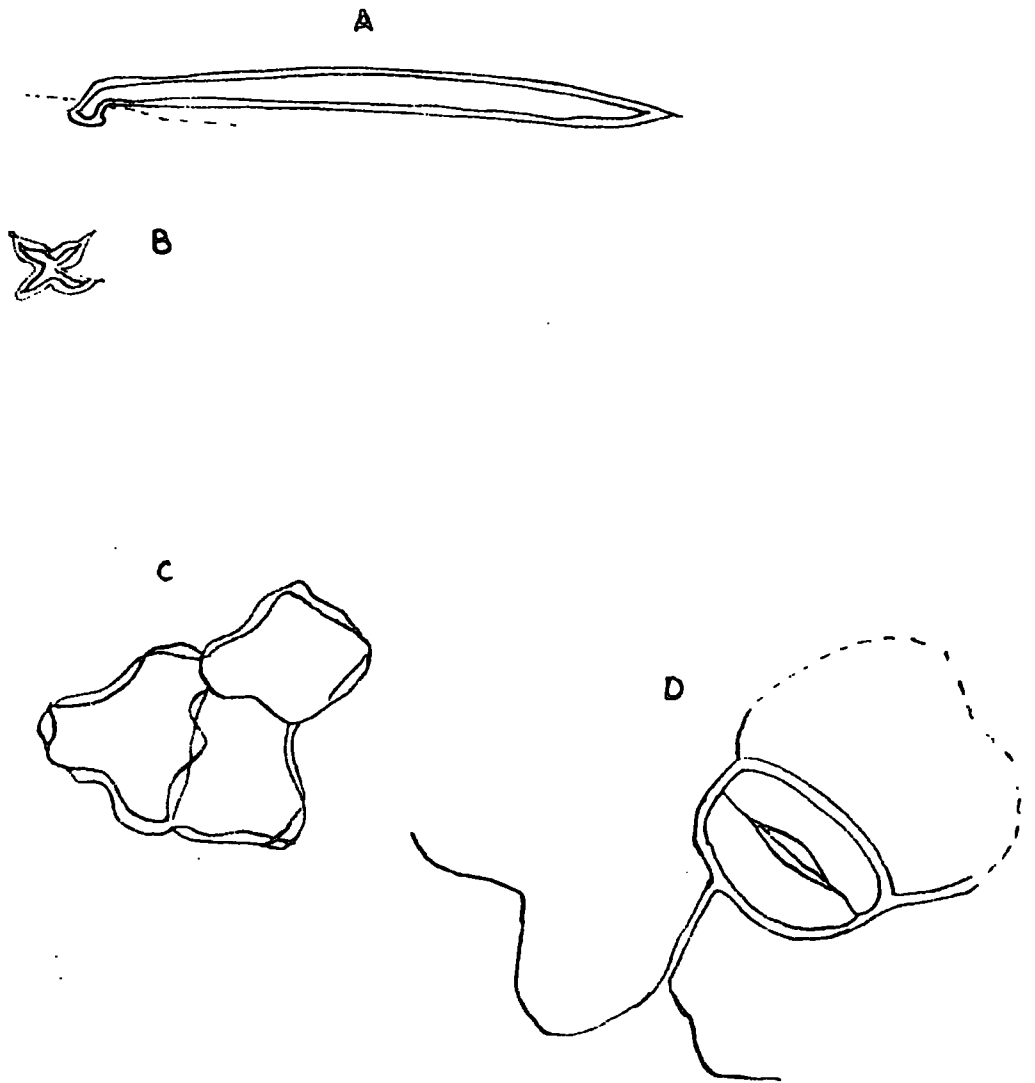


Oxalis acetosella L.

Leaf scraping showing unicellular
hair. (X 200 app.)

Oxalis acetosella L.

- A. Unicellular hairs from leaf lamina epidermis. Dotted line represents surface of normal epidermal cells. Four hairs per mm. occurred along an edge of a leaf fragment in the preparation, the hairs were uniform in length being approximately 520 microns. (X 200 app.)
- B. Stellate hair from leaf lamina epidermis. These hairs were evenly distributed on the fragments with a density of 6 per sq. mm. (X 600 app.)
- C. Stoma and guard cells with associated epidermal cells indicated. Type B. of Metcalfe and Chalk (1950), (Cruciferous Type). (X 300 app.)



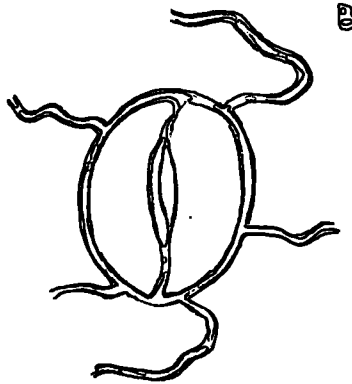
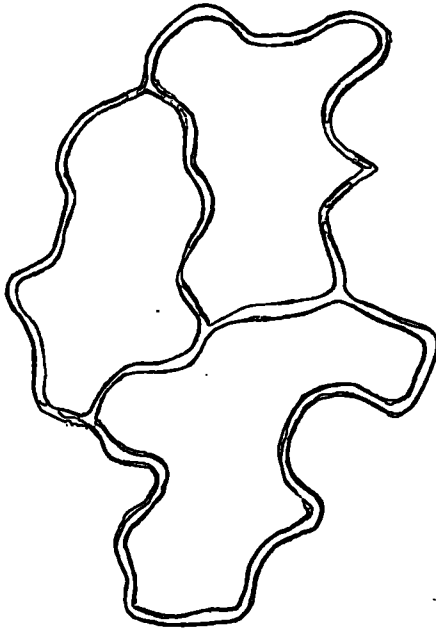
Leaf lamina epidermal features of Oxalis acetosella L.

Ranunculus ficaria L.

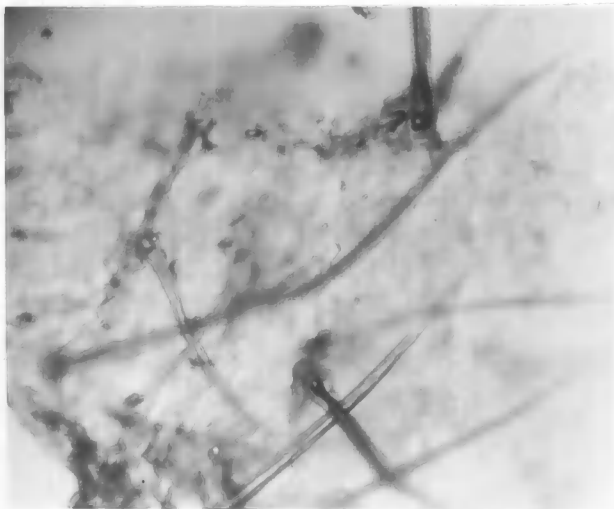
- A. Epidermal cells of leaf lamina (X 750 app.)
- B. Stoma and guard cells. Guard cells 41 microns long; together with stoma, 31 microns wide.

Distribution about 43 per sq. mm.

Type A (Ranunculaceous) of Metcalfe and Chalk (1950) (X 700 app.)



Leaf lamina epidermal features of Senecio jacobaea L.

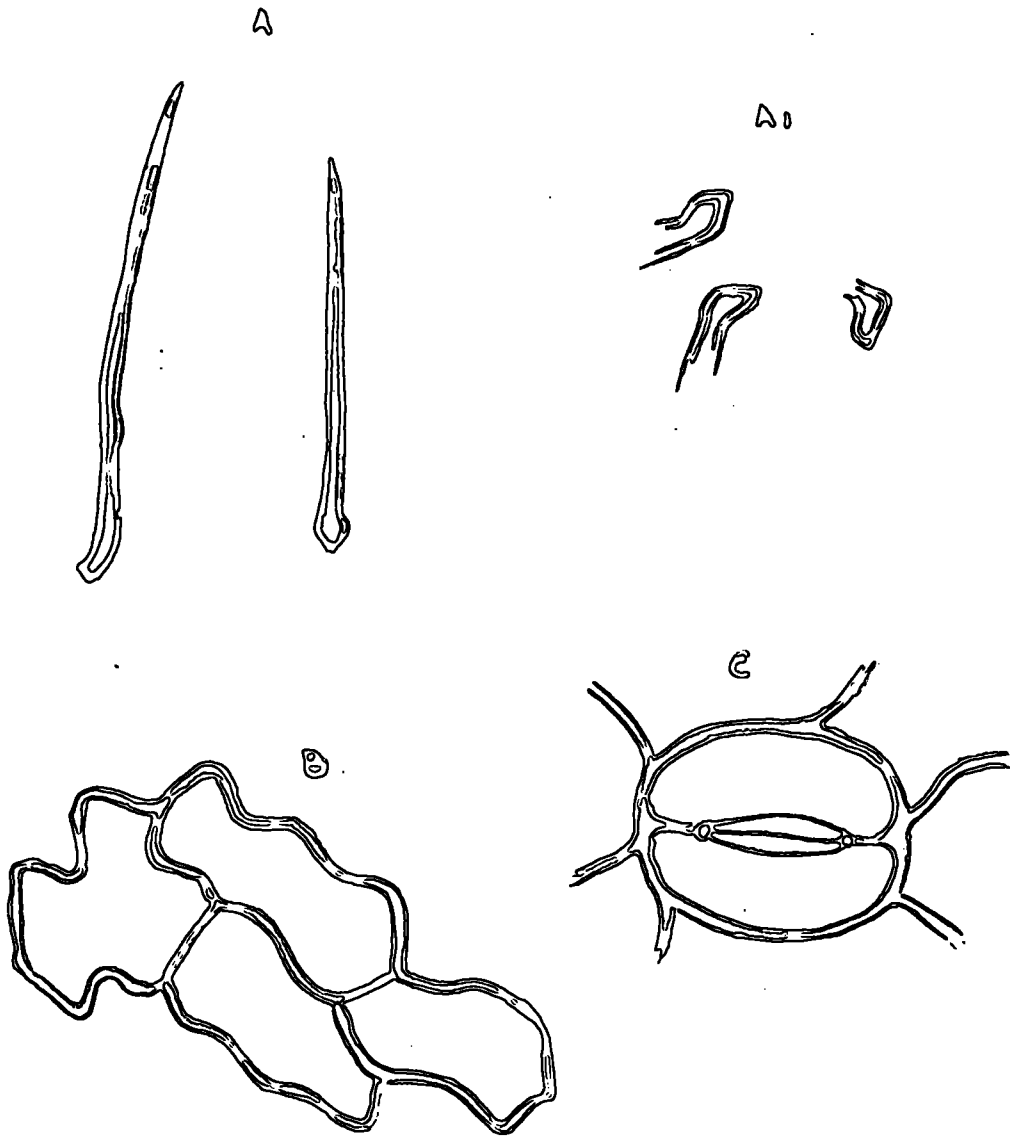


Ranunculus repens L.

Leaf scraping showing unicellular
hairs and epidermis. (X 200 app.)

Ranunculus repens L.

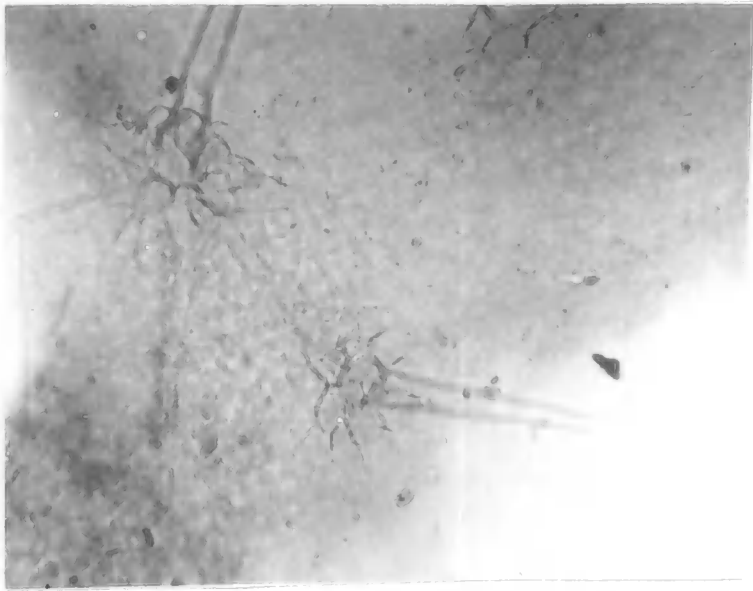
- A. Unicellular hairs from lower epidermis of leaf lamina. Length of hairs in this preparation of mature leaf varied from 420 to 760 microns with an average length of 540 microns. The hairs are slender and finely pointed with slightly swollen and angled bases inserted into the epidermis. (X 85 app.)
- A1. Common shapes of bases of unicellular hairs (X 200 app.)
- B. Epidermal cells from lower epidermis of leaf lamina, 40 to 60 microns in length with two to three curves in each wall perpendicular to the leaf surface. (X 600 app.)
- C. Stoma and guard cells. Guard cells 30 microns long and with stoma 25 microns wide. These units were surrounded by a number of normal epidermal cells: Type A of Metcalfe and Chalk (1950) (Ranunculaceous Type). (X 1000 app.)



Leaf lamina epidermal features of Ranunculus repens L.

Urtica dioica L. Photographs of features of epidermis of leaf.

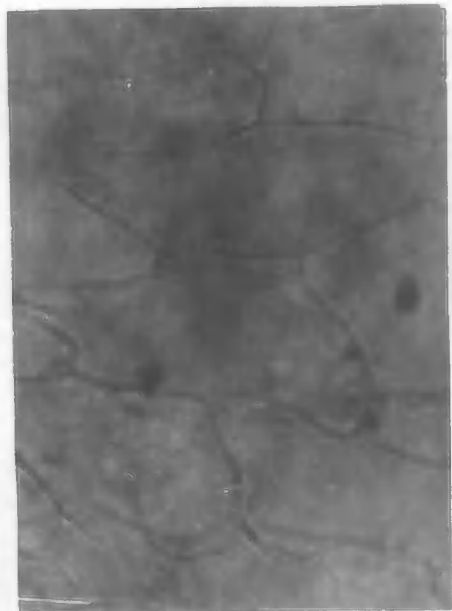
- A. Section of lower epidermis of leaf lamina showing arrangement of cells at the bases of unicellular hairs (X 166 app.)
- B. Base of stinging hair (X 100 app.)
- C. Epidermal cells (X 1000 app.)



A.



B.



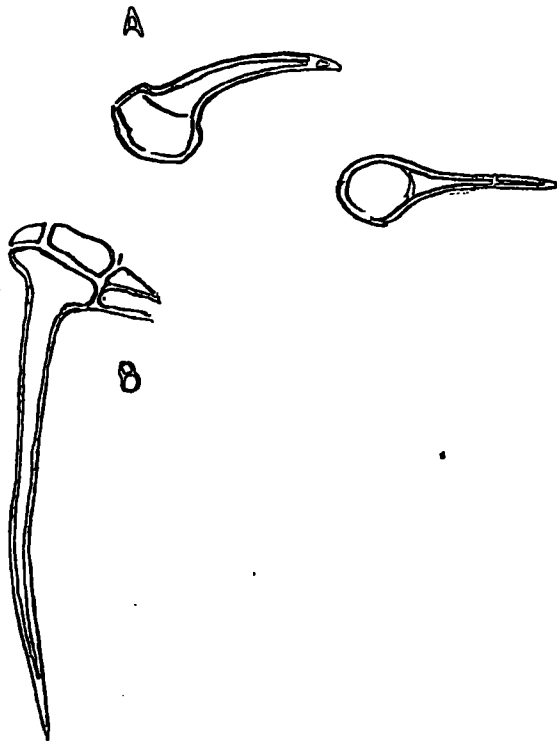
C.

Urtica dioica L.

37.

Urtica dioica L.

- A. Unicellular hooked hairs with simple bases (X 200 app.)
- B. Unicellular epidermal hairs with multicellular supporting base as in photograph A above (X 100 app.)



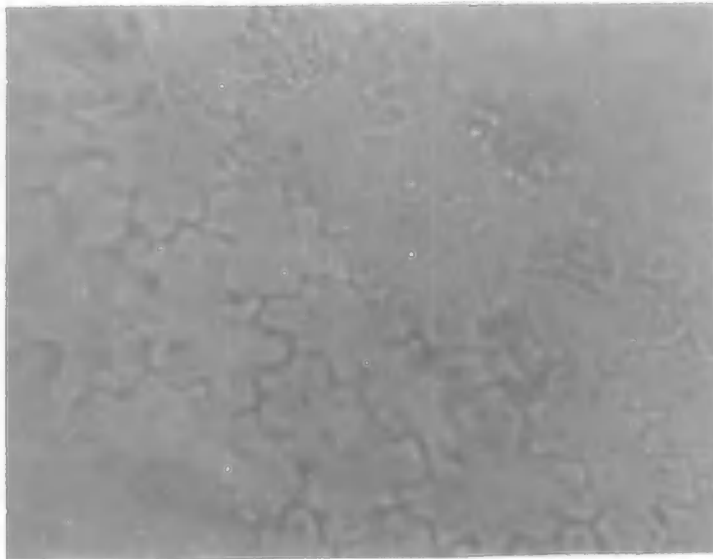
Leaf lamina epidermal features of Urtica dioica L.

Veronica montana L. Photographs of leaf epidermal features.

- A. Multicellular hairs (X 100 app.)
- B. Epidermal cells and stomata. Type A (Ranunculaceous) of Metcalfe and Chalk (1950) (X 800 app.)



A.



B.

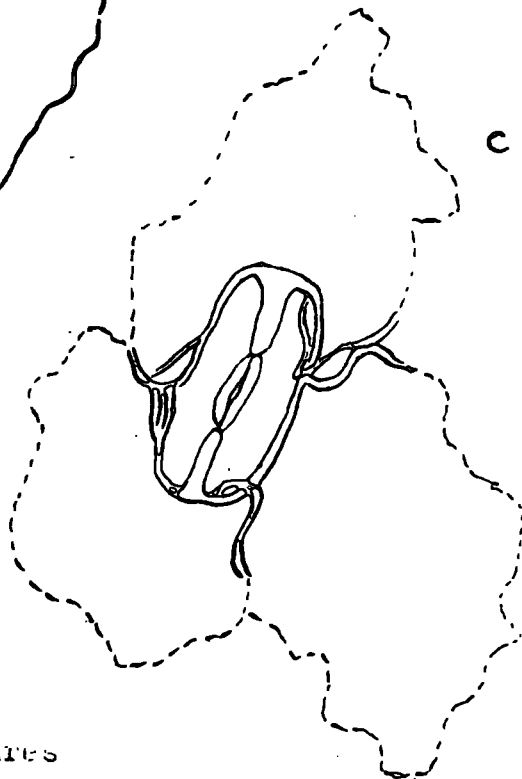
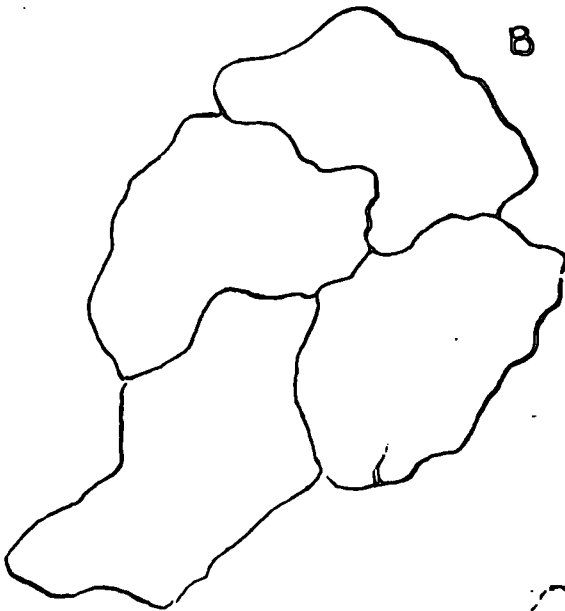
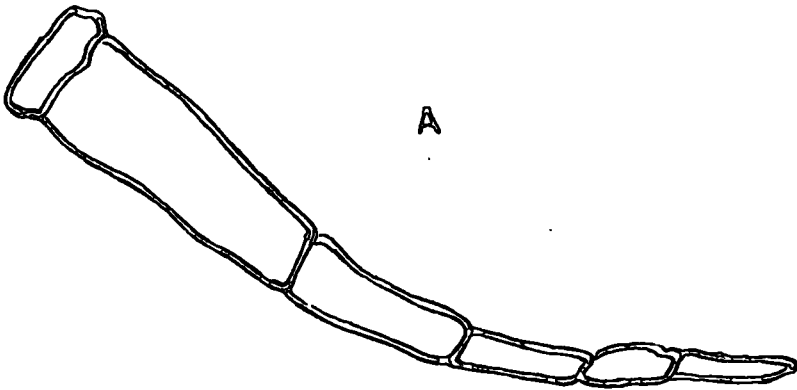
Veronica montana L.

Veronica montana L.

- A. Thin walled multicellular hair. Most hairs found to have at least one cell collapsed. Length of hair 470 microns.
Frequency of occurrence 8 per sq. mm. (X 250 app.)

- B. Outline of epidermal cells. Average width of cells 46 microns.
These cells had very thin walls and appeared to contain chloroplasts. (X 760 app.)

- C. Stoma, guard cells and neighbouring epidermal cells. Guard cells about 23 microns long and together 10 microns across. Metcalfe and Chalk (1950) Type A (Ranunculaceous Type). (X 1500 app.)



Leaf lamina epidermal structures
of Veronica montana L.

5 (ii) Examination of gut contents and faeces of *A. reticulatus*
collected at Burton Bushes.

(a) Methods

Animals were collected in the wood by random searching in daylight in the late morning or early afternoon. Sites likely to harbour resting slugs were searched in a walk through the wood. For gut contents examinations, animals were collected directly into 70% alcohol and the anterior part of the gut, up to the hepatopancreas, was dissected out and stored separately in specimen tubes of 70% alcohol on returning to the laboratory. For faeces examinations, slugs were taken alive into individual specimen tubes and transferred, wiped clean, to clean vessels on return to the laboratory.

Faeces were collected from the individual vessels after 24 hours and gut contents were obtained by cutting the gut tube. Both types of material were teased out on slides in Puri's medium or Gurr's "Aquamount" and squashed under a cover slip. Care was taken to clean instruments between the preparation of material from different animals so that there was no transfer of material. Slides were examined at a magnification of about 100 diameters with a field of view of about 1 mm. Detail was examined at higher magnification as necessary. Any type of material recognised was recorded once for each animal with a special note if it made up the bulk of the material present.

(b) Results

Gut contents and faeces slides contained the same types of material in general, and in particular the epidermal hairs used in identifying plant food were present in considerable quantity in both. Faeces contained more unidentifiable matter as fine particles than was present in gut contents. The general nature of both types of materials was a ground substance which was usually particulate but was occasionally amorphous. Plant fragments formed the bulk of the material, these varied from pieces about half a millimetre square, and longer pieces of smaller width, to single cells and cell inclusions such as chloroplasts. Many small fragments were always present including isolated epidermal hairs and lignin spirals from xylem vessels. Most plant material was dicotyledonous but occasionally small quantities of grass material was present. Fresh materials were recognisable by green colouration and normal cell structure. Occasionally, plant leaf material was obviously at least partially decayed.

Small quantities of an amorphous brown substance were sometimes present and rarely the majority of the material was of this nature, earthworm chaetae sometimes accompanied this. Animals fed with dead earthworm in the laboratory produced similar faeces. Earthworm chaetae were present in 1.3% of the slides. Arthropod fragments occurred in 21.2%, these were identified by Dr.L.Davies as pieces

of aphids and small diptera (Sciara sp. identified from the presence of substantial parts of wings showing characteristic venation). In other cases whole mites and collembolans were present. The arthropod constituents were however present in such small proportions of the total mass of material from any one slug that they are judged to be accidentally included in the diet, although not necessarily for that reason without nutritional significance. A few slides contained small nematodes and in examining unfixed material some of these were seen to be alive in faecal matter.

Of the plants studied in the previous section Ranunculus repens L. was present in 53.4%; Urtica dioica L. in 20.5%; Glechoma hederacea L. in 2.0%; Oxalis acetosella L. in 0.6%; and Veronica montana L. in 1.3% of cases from 146 animals. Fresh moss leaf material was present in 3.4%, Monocotyledonous material mostly recognised as from grasses in 12.3% and unidentified Dicotyledonous material in 21.2%. Thus it is clear that more than 50% of the food eaten by A. reticulatus in Burton Bushes consists of fresh green dicotyledonous herbaceous leaves and a high proportion of this is R. repens and U. dioica.

The seasonal distribution of the results for R. repens and U. dioica is shown in Table 6 and analysed for homogeneity of distribution of scores using a "t test". This shows only the September R. repens and May U. dioica scores to be abnormal and in both cases these are abnormally high, compared with the overall average. These may reflect some special element of availability

or palatability at the particular season or some unwitting bias in sampling. It seems likely however that availability may be a sufficient cause.

TABLE 6

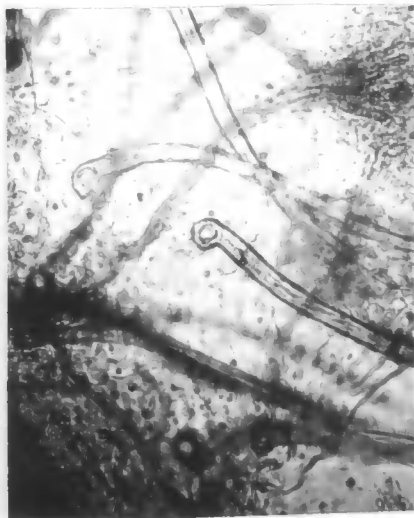
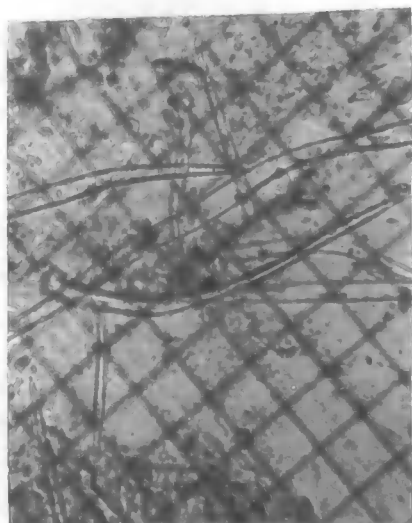
SEASONAL OCCURRENCE OF R. repens AND U. dioica IN FAECES AND GUT CONTENTS OF A. reticulatus.

Month	No. of slugs containing <u>R. repens</u>				No. of slugs containing <u>U. dioica</u>				Number of Slugs Examined
	%	t	p	%	t	p			
Feb/March	8	72	1.5	> .05	1	9	1.4	> .1	11
May	14	52	.1	> .1	14	52	3.5	< .002	27
September	11	84	3.1	< .01	5	38	1.4	> .1	13
October	13	59	.5	> .1	5	26	.7	> .1	22
November	27	47	1	> .1	5	8.7	1	> .1	57
December	5	31	2	> .05	5	31	1	> .1	16
Total	78	53.4			30	20.5			146

Examples of identifications of food materials
in preparations of crop contents of A. reticulatus.



Urtica dioica



Ranunculus repens.

6. FEEDING AND FOOD PREFERENCE EXPERIMENTS IN THE LABORATORY

(a) Methods

Various authors have shown that slugs would take some materials more readily than others when fed in captivity; Gain (1891), Fromming (1950, 1954), Getz (1959). In feeding slugs with a single type of material to obtain faeces from known food sources it was observed that some materials were more readily taken than others. This procedure, although abandoned as providing reference material for faeces, was pursued to determine which of the commonly available materials in the wood were acceptable as food in captivity.

Originally 3" x 1" specimen tubes with damp paper strips were used to house individual captives but these conditions did not favour active feeding or long life especially in the case of larger animals. Circular glass crystalising dishes of 5 cm. diameter, 3 cm. deep, were therefore used, covered with petri-dish halves with one slug to each dish. These dishes were furnished with a wet carpet of paper-towelling lining the base. The paper carpet was kept saturated by daily watering with distilled water and maintained 100% humidity over most of the dish as judged by "Protimeter DD1" strips (cobalt thiocyanate paper) attached to the lid. Slight irregularities separating dish and lid provided adequate aeration.

Slugs, Agriolimax reticulatus, were starved for 24 hours before feeding individually with test materials in a water saturated

condition. Leaves of the common herbs from Burton Bushes were soaked for about half an hour and then a single leaf was placed on the paper carpet in the bottom of each dish. In the case of choice experiments one leaf of each kind offered was placed in the bottom of the dish, care being taken to avoid overlapping to any great extent.

In the later choice experiments, when the area of leaf eaten was estimated, the leaves offered were soaked and then placed between two small sheets of glass and mounted over a 100 watt electric lamp at a distance of 4 inches. Millimetre graph paper was placed over the upper sheet of glass and the outline of the leaf was sketched quickly, i.e. a shadowgraph was made. Care was taken to expose the leaves to the lamp for as short a period as possible to avoid scorching and any seriously scorched leaves were rejected. Leaves were dipped in cold tap water before being placed in the dishes with the animals.

In many cases feeding was observed to begin within a few minutes, but feeding damage to the leaves was not recorded until a period of 24 hours had elapsed. In most cases experiments continued for a longer period and observations were made every 24 hours. The amount of feeding was estimated on a four point scale from 3, indicating considerable feeding to 0, indicating no feeding; or in the case of the later preference experiments by recording as shadowgraphs the area of leaf remaining uneaten. In these last mentioned experiments

the area of leaf eaten was estimated by a count of mm. squares within the outlines drawn. Experiments varied in length from 2 to 7 days, but are taken to be comparable in terms of daily averages since there is no obvious correlation between these and time of exposure.

The same individual animals were used repeatedly in these experiments as long as they remained obviously healthy and active, and some were kept for several weeks in this way. Results were not accepted from obviously inactive or moribund animals.

These experiments were all carried out at room temperature which was recorded twice daily and varied between 15°C and 20°C. The experimental slugs were exposed to the normal diurnal light variation but not to other variations in conditions such as could affect activity in the field.

(b) Results from offering one material

Table 7 sets out the results of 56 experiments. This shows Ranunculus repens L. and Urtica dioica L. as most readily accepted foods with Glechoma hederacea L. and Veronica montana L. fairly readily eaten. Fragments of dead earthworm were also eaten in captivity.

TABLE 7

ESTIMATES OF FEEDING BY A. reticulatus ON LEAVES OFFERED SINGLY.

Plant species	Estimate of feeding				Number of experiments
	0	1	2	3	
<u>Ranunculus repens</u> L.	-	1	1	7	9
<u>Urtica dioica</u> L.	1	-	1	11	13
<u>Glechoma hederacea</u> L.	-	-	5	1	6
<u>Veronica montana</u> L.	-	-	5	1	6
<u>Oxalis acetosella</u> L.	3	1	-	1	5
<u>Veronica chamaedrys</u> L.	-	3	-	-	3
<u>Fagus sylvatica</u> L.	6	-	-	1	7
<u>Quercus robur</u> L.	3	-	1	-	4
<u>Quercus serris</u> L.	1	-	-	-	1
<u>Aesculus hypocastaneum</u> L.	-	-	1	1	2

Scale: 3, considerable feeding; 2, fair amount of feeding;
1, slight feeding; 0, no feeding.

TABLE 8

CHOICE OF FOOD IN LAB. BY A. reticulatus

Groups represent plants offered together in one experiment.

Each column of scores within a group represents one animal.

Scoring: 3 - considerable feeding, 2 - fair amount of feeding,

1 - slight amount of feeding, 0 - no feeding.

<u>Ranunculus repens</u> L.	3	2	3	0					
<u>Veronica montana</u> L.	2	3	0	0					
<u>Ranunculus repens</u> L.	0	3	0	1	2				
<u>Urtica dioica</u> L.	3	3	3	3	2				
<u>Ranunculus repens</u> L.	3	2	3	2	2	3	0	3	2
<u>Veronica montana</u> L.	1	2	3	0	0	3	3	0	0
<u>Glechoma hederacea</u> L.	0	3	3	0	0	3	3	0	3
<u>Ranunculus repens</u> L.	3	0	0	0	0	1	0	3	1
<u>Urtica dioica</u> L.	3	3	3	0	3	1	3	3	3
<u>Veronica montana</u> L.	0	0	0	0	2	0	2	0	2
<u>Glechoma hederacea</u> L.	1	0	0	0	0	1	0	2	0
<u>Ranunculus repens</u> L.	3	0	3	2					
<u>Veronica montana</u> L.	0	2	0	3					
<u>Glechoma hederacea</u> L.	3	0	3	0					
<u>Cretagus monogyna</u> L.	2	0	2	0					
<u>Ranunculus repens</u> L.	2	3	3	3	3	2	0	3	2
<u>Urtica dioica</u> L.	0	0	0	0	3	0	2	0	0
<u>Glechoma hederacea</u> L.	0	0	0	2	2	0	0	0	0

(c) Results from offering a choice of food

Table 8 sets out the results from the 40 experiments in which a choice of different leaves was offered to A. reticulatus in the laboratory and the amount of feeding was assessed on a four point scale. Ranunculus repens L. leaves were offered in 38 experiments in which some feeding occurred. In 16 of these, feeding was considerable; in 10, moderate; in 3, slight; and in 9 there was no feeding on these leaves. Urtica dioica L. leaves were offered in 22 experiments in which some feeding occurred. Feeding was considerable in 12 of these, moderate in 2, slight in 1, and no feeding on this material occurred in 7 of the trials. Veronica montana L. leaves were offered in 24 experiments in which feeding occurred. Considerable feeding on this material occurred in 5 of these, moderate feeding in 6, slight feeding in 1 and no feeding in 13. Glechoma hederacea L. was offered in 30 of these experiments and eaten considerably in 7, moderately in 3, slightly in 2, and not eaten in 18.

If the figures used on the scale of feeding are added as scores for each type of material average scores are as follows:

<u>R. repens</u>	1.8
<u>U. dioica</u>	1.6
<u>V. montana</u>	1.1
<u>G. hederacea</u>	0.9

This indicates a rough scale of palatability of the different materials. There is however no clear evidence of consistent choice between R. repens and U. dioica.

In 22 experiments in which leaves from these two plants were offered together U. dioica was eaten more than R. repens in 9 cases, in 7 the reverse was found and in 6 feeding was assessed as equal for both.

R. repens and V. montana were offered together in a total of 22 experiments. In 12 of these R. repens was preferred, in 7 V. montana and in 3 equal amounts of both plants were eaten. In this summary only experiments in which some of either material was eaten are counted.

R. repens was offered together with G. hederacea in a total of 24 experiments in which some of either material was eaten. In 16 of these R. repens was preferred, in 3 G. hederacea was preferred and in 5 equal amounts of each were eaten.

In only 1 case out of 11 experiments was G. hederacea eaten in preference to U. dioica and in one case there were equal scores. In 8 experiments in which U. dioica and V. montana were offered together the former was always preferred.

Thus without accounting for the occasional individual differences, which could be due to a number of causes such as previous history or undetected differences in either plant or animal materials, there seems to emerge a picture of R. repens and U. dioica as favourite foods with V. montana and G. hederacea as possible additional foods.

TABLE 9 Contd.

<u>Ranunculus repens</u> L.	223	96	369					
<u>Veronica montana</u> L.	0	0	0					
<u>Ranunculus ficaria</u> L.	0	231	146					
<u>Ranunculus repens</u> L.	4	75	0	42	89	62	11	14
<u>Urtica dioica</u> L.	59	182	92	117	116	63	133	169
<u>Glechoma hederacea</u> L.	145	98	0	31	12	104	47	89
<u>Ranunculus repens</u> L.	11	68	60	95				
<u>Urtica dioica</u> L.	0	191	218	168				
<u>Glechoma hederacea</u> L.	52	127	105	37				
<u>Ranunculus repens</u> L.	102	328	30	33	39	170		
<u>Veronica montana</u> L.	0	0	0	0	0	0		
<u>Glechoma hederacea</u> L.	26	0	0	11	221	0		
<u>Ranunculus repens</u> L.	172	0	0	26	165			
<u>Urtica dioica</u> L.	0	142	110	172	48			
<u>Veronica montana</u> L.	44	0	27	10	38			

Table 9 sets out the results of the preference feeding experiments in which the amount of feeding was estimated quantitatively by the method described on page 49. The overall average daily consumption and the number of experiments involved for each type of leaf are shown in Table 10.

TABLE 10

OVERALL AVERAGES IN QUANTITATIVE PREFERENCE FEEDING EXPERIMENTS
WITH A. reticulatus.

Plant	Average Consumption mm ² / 24 hrs.	Number of Experiments
<u>R. repens</u>	76.0	57
<u>U. dioica</u>	67.7	39
<u>V. montana</u>	6.2	20
<u>G. hederacea</u>	32.3	40
<u>R. ficaria</u>	63.6	8
<u>A. nemorosa</u>	7.0	2
<u>M. perennis</u>	10.0	7

These results again suggest R. repens and U. dioica as favourite foods, with G. hederacea as a second choice. The position of R. ficaria is perhaps uncertain because of the small number of experiments in which this material was offered.

R. repens was offered in 57 experiments, and in 14 of these more than 100 mm²/ 24 hours was eaten, less than 50 mm²/ 24 hours was eaten in 18 experiments, none was eaten in 6.

U. dioica was offered in 39 experiments, and in 14 of these over 100 mm²/ 24 hours was eaten, less than 50 mm²/ 24 hours was eaten in 19 and none was eaten in 14.

V. montana was offered in 20 experiments and in no case was more than 50 mm²/ 24 hours eaten. None was eaten in 14.

G. hederacea was offered in 40 experiments and over 100 mm²/ 24 hours was eaten in 5 of these, less than 50 mm²/ 24 hours was eaten in 32 and none was eaten in 18.

R. ficaria was offered in 8 experiments and in 2 of these more than 100 mm²/ 24 hours was eaten, less than 50 mm²/ 24 hours was eaten in 5, and none in 2.

A. nemorosa was offered in 2 experiments, none was eaten in one of these and only 14 mm²/ 24 hours in the other.

M. perennis was offered in 7 experiments and less than 10 mm²/ 24 hours was eaten in 6 of these, none was eaten in 3. In one experiment 60 mm²/ 24 hours was eaten.

Table 11 summarises, on a seasonal basis, the experiments in which R. repens and U. dioica were offered together, showing average consumption.

TABLE 11

SEASONAL CHOICE BETWEEN R. repens AND U. dioica.

	Month					Overall
	2	3	4	5	11	
Average consumption of <u>R. repens</u> (mm ² / 24 hrs.)	72.6	77.2	42	229	44	58.1
Average consumption of <u>U. dioica</u> (mm ² / 24 hrs.)	94.4	13.1	68	0	125.6	69.4

It is perhaps interesting to note that in the February results 3 of the animals concerned are known to have fed on R. repens in the wood and these same three animals preferred U. dioica in the laboratory all of them eating more than 100 mm²/ 24 hours of this material and two of them eating none of the R. repens.

A similar case occurred in the November experiments; 7 of the 12 animals involved were known from faeces examinations to have eaten R. repens in the wood and all of these ate more U. dioica than R. repens in the laboratory. However two of the slugs which ate only R. repens in the May experiments are also known from faeces examinations to have been eating R. repens previously in the field. The same applies to 3 of the slugs eating mostly R. repens in the March experiments.

However, in faeces and gut contents in May, Table 6, it was seen that a greater than average amount of U. dioica appeared to have been eaten in the field.

These summaries, p.57 onwards, include only those experiments in which a total of more than 20 mm²/24 hours was eaten.

Thus the preferential palatability of R. repens and U. dioica is unresolved by these experiments. It may be that more R. repens is found to be eaten in the field because of greater availability, this plant being more accessible due to its recumbent habit and green leaves being available throughout the year.

7. STUDIES ON ASSIMILATION BY *A. reticulatus* IN THE LABORATORY

(a) Method

Phillipson (1960) used an indirect method of assessing assimilation in the field by the phalangid *Mitopus morio* using laboratory experiments to establish a relationship between food assimilated and volume of faeces voided. A related method was used for *A. reticulatus* using wet weights of faeces under saturated conditions.

If the faeces resulting from a known intake of food can be distinguished from other faeces it is possible to determine:

1. the proportion of the food eaten which is assimilated and
2. the period over which faeces are produced from a known period of feeding.

It is therefore possible to establish a quantitative relationship between volume or weight of faeces produced in a given time and weight of food assimilated in a given time. Such results as these can only be determined by laboratory experiments but approximate estimates of assimilation in the field can then be calculated by measuring the faeces voided by animals which have fed in the field; assuming that the relationship between faeces produced and food assimilated determined in the laboratory hold good for field conditions.

A. reticulatus feeds readily in the laboratory on carrot, *Urtica dioica* and *Ranunculus repens* and these foods may be alternated to provide faeces which are distinguishable by their

colour.

Animals were taken from Burton Bushes and housed individually in the laboratory as in previous feeding experiments. Food was saturated with water by soaking and mopping off surplus water before being presented. Saturated conditions were maintained in the experimental vessels. These were weighed on a Stanton "Unimatic C L 41" balance with and without food. The weight of the animals was also determined. After 24 hours the weight of food uneaten was determined and the food was removed. The animals were at this stage removed to clean vessels and after a further 24 hours the weight of faeces produced was determined. All weights are taken to be saturated wet weights and were obtained as differences between the weight of the whole apparatus with and without the item of interest.

The water content of the food materials and faeces were investigated and found to be substantially similar. Therefore wet weights are used throughout the study.

(b) Water content of food materials and faeces

Water saturated carrot and carrot faeces from A. reticulatus were air dried at room temperature to constant weight to give comparable figures for water content. These results are shown in Tables 12 and 13; an average of 90.7% water was found in carrot pieces such as were presented in the laboratory assimilation experiments and 92.4% water was found in faeces.

TABLE 12

% WATER IN WATER SATURATED CARROT.

Wet weight (mg.)	Air Dry weight (mg.)	% water
573.2	54.9	90.5
1360.1	125.0	90.8
2211.2	197.5	91.1
762.0	72.3	90.4
797.8	74.3	90.7
	Average	90.7

TABLE 13

% WATER IN WATER SATURATED CARROT FAECES FROM A. reticulatus.

Wet weight (mg.)	Air Dry weight (mg.)	% water
18.4	1.1	94
26.3	1.6	94
11.8	0.7	94
13.0	0.8	93.9
8.9	1.0	88.8
5.0	0.5	90
	Average	92.4

Water saturated Urtica dioica and Ranunculus repens leaves were weighed and then oven dried at 110°C to constant weight. Both types of leaves show a water content of 85%, Tables 14 and 15. Faeces from animals which had fed in the field were similarly treated and found to have a water content of 85.3%, Table 16.

TABLE 14

RELATION WET/DRY WEIGHT OF Urtica dioica L. LEAVES

20 leaves	Wet wt. (g.)	Dry wt. (g.)
	1.2037	0.1863
Ratio Dry/wet wt.	0.15	% water 85

TABLE 15

RELATION WET/DRY WEIGHTS OF Ranunculus repens L. LEAVES. COLLECTED

18.10.66.

No. of leaves	Wet wt. (g.)	Dry wt. (g.)
20	8.2237	1.2904
20	6.2052	0.9071
16	5.6157	0.9018
Ratio Dry/wet wt.	.15	% water 85

TABLE 16

RELATION WET/DRY WEIGHTS OF FAECES OF A. reticulatus.

Ref.	Wet wt. (g.)	Dry wt. (g.)
553	.0180	.0034
554	.0266	.0029
555	.0253	.0023
556	.0083	.0018
557	.0275	.0040
558	.0082	.0021
559	.0100	.0017
Totals	.1239	.0182
Ratio Dry/wet wt.	.147	% water 85.3

(c) Carrot feeding.

Preliminary experiments showed that when A. reticulatus was fed with carrot over a 24 hour period all characteristically orange coloured faeces were voided within the 24 hours following the feeding period.

Table 17 shows the results obtained from feeding seven A. reticulatus with known weights of water saturated carrot. An average of 11.9 mg. wet weight of faeces were produced from eating 67.6 mg. wet weight of food in 24 hours at approximately 20°C. Thus 55.7 mg. of carrot is assimilated in 24 hours, 82.4% of food eaten. This corresponds to 11.8 calories per 24 hours using calorific value of 6 K. calories per ounce wet weight, "Manual of Nutrition" (1958).

TABLE 17

A. reticulatus FEEDING ON CARROT IN CAPTIVITY AT 20°C.

Ref.	Live Wt. of slug (mg.)	Wet weight Carrot eaten (mg./24hrs.)	Wet wt. Faeces (mg/24hrs)	Wet weight Assimilated (mg./24 hrs)	Assimilation %
553	192.7	88.5	7.2	81.3	91.8
554	231.2	80.7	6.4	74.3	92.0
555	331.7	115.2	33.4	81.8	71.0
556	176.9	45.8	5.4	40.4	88.2
558	153.0	51.6	9.8	41.8	81.0
559	148.9	48.8	14.6	34.2	70.0
540	301.7	41.3	6.8	34.5	83.5
Averages	219.4	67.6	11.9	55.7	82.4

(d) Urtica feeding.

Table 18 shows the results from feeding eight A. reticulatus with leaves of Urtica dioica L. at approximately 18°C. An average of 14.7 mg. wet weight of faeces was produced from eating 56.9 mg. wet weight of food taken in 24 hours. Thus an average of 42.2 mg. wet weight of Urtica leaf is assimilated in 24 hours by slugs of average weight 264.5 mg., a percentage assimilation of 74.

Evans (1960) gives a "starch equivalent" of 46.5% for dried nettles based on cattle feeding experiments. From this a calorific value of wet U. dioica leaf was deduced using the wet to dry weight value established in Table 15 and a calorific value of 4.2 K. calories per gram for starch, Lovatt Evans (1949). This deduced value is .293 calories per milligram wet weight. Using this rough calorific value, the average assimilation in this experiment amounts to 12.3 calories in 24 hours.

TABLE 18

A. reticulatus FEEDING ON U. dioica IN CAPTIVITY AT 18°C.

Ref.	Live Wt. of slug (mg.)	Wet wt. eaten (mg./24hrs)	Wet wt. Faeces (mg./24hrs)	Assimilation (mg./24hrs)	Assimilation %
540	180.4	66	15	51	78
541	355.3	73	16	57	78
542	226.2	68	15	53	78
543	172.7	27	15	12	44
544	190.5	40	14	26	65
545	259.5	80	20	60	75
546	225.9	53	17	36	68
547	240.7	47	6	41	87
Averages	264.5	56.9	14.7	42.2	74

Table 19 shows the calculation of the relationship between food eaten and faeces produced in the laboratory Urtica feeding experiments as the regression of the wet weight of food eaten in 24 hours on the wet weight of faeces produced in the following 24 hours. The same relationship is shown in the following graph, page 70. The relationship, expressed in equation 2, i.e. Wet weight of food eaten in mg. = 2.05 (wet wt. of faeces in mg.) + 26.25 mg., is used in section 8 to determine "Urtica estimate" of food eaten in the field.

TABLE 19

REGRESSION OF U. dioica EATEN ON FAECES PRODUCED.

wt. of leaf eaten - 57 (d_y)	wt. of faeces - 15 (d_x)	$d_x d_y$	d_x^2
9	0	0	0
16	1	16	1
11	0	0	0
- 30	0	0	0
- 17	- 1	17	1
23	5	115	25
- 4	2	- 8	4
- 10	- 9	90	81
	Totals	230	112

$$\begin{aligned} \text{therefore regression coefficient } & \frac{\sum d_x d_y}{\sum d_x^2} \\ & = 230/112 \\ & = 2.05 \end{aligned}$$

Wet weight of food eaten in mg. - 57 =

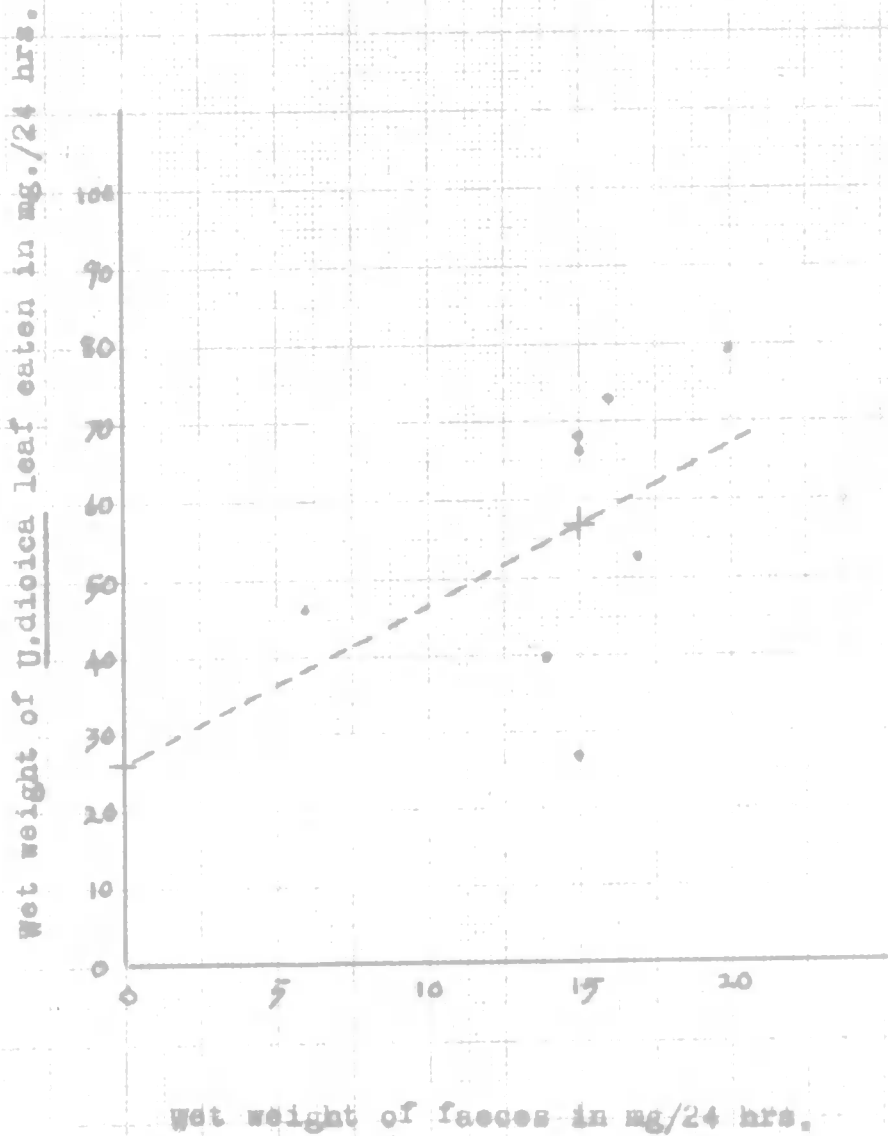
$$2.05 (\text{wet wt. of faeces in mg.} - 15) \dots\dots\dots \text{Eqn. 1}$$

or

Wet weight of food eaten in mg. =

$$2.05 (\text{wet wt. of faeces in mg.}) + 26.25 \text{ mg.} \dots\dots \text{Eqn. 2}$$

Regression of wet weight of U. dioica leaf eaten in 24 hours on wet weight of faeces voided in the following 24 hours.



(e) Ranunculus feeding.

Table 20 shows the results from feeding nine A. reticulatus in the laboratory with wet leaves of R. repens at a temperature of approximately 18°C. In this case slugs of average wet weight 314 mg. produced an average of 13 mg. wet weight of faeces in 24 hours from eating an average of 67.7 mg. wet weight of R. repens leaf. Thus an average of 54.7 mg. wet weight was assimilated in 24 hours, amounting to 80.9% of the food eaten.

TABLE 20

Ref.	Wet wt. of slug (mg.)	Wet wt. of leaf eaten (mg/24hrs)	Wet wt. of faeces (mg/24hrs)	Wet wt. of leaf assimilated (mg/24hrs)	Assimilation %
553	385.9	115	16	99	86
554	323.6	74	10	64	86
555	344.0	140	20	120	86
556	241.0	28	7	21	75
557	268.1	49	13	36	74
558	175.1	19	17	2	11
559	307.2	54	9	45	83
540	313.8	53	15	38	72
541	468.9	79	10	69	87
Averages					
	314.0	67.7	13	54.7	80.9

Table 21 shows the calculation of the relationship between food eaten and faeces produced in the laboratory Ranunculus feeding experiments as the regression of the wet weight of food eaten in 24 hours on the wet weight of faeces produced in the following 24 hours. The same relationship is shown in the following graph, page 73. This relationship as expressed in equation 4, i.e. Wet wt. of food eaten in mg. = 4.5 (wet wt. of faeces in mg.) + 9.6 mg. is used in section 8 to determine "Ranunculus estimate" of food eaten in the field.

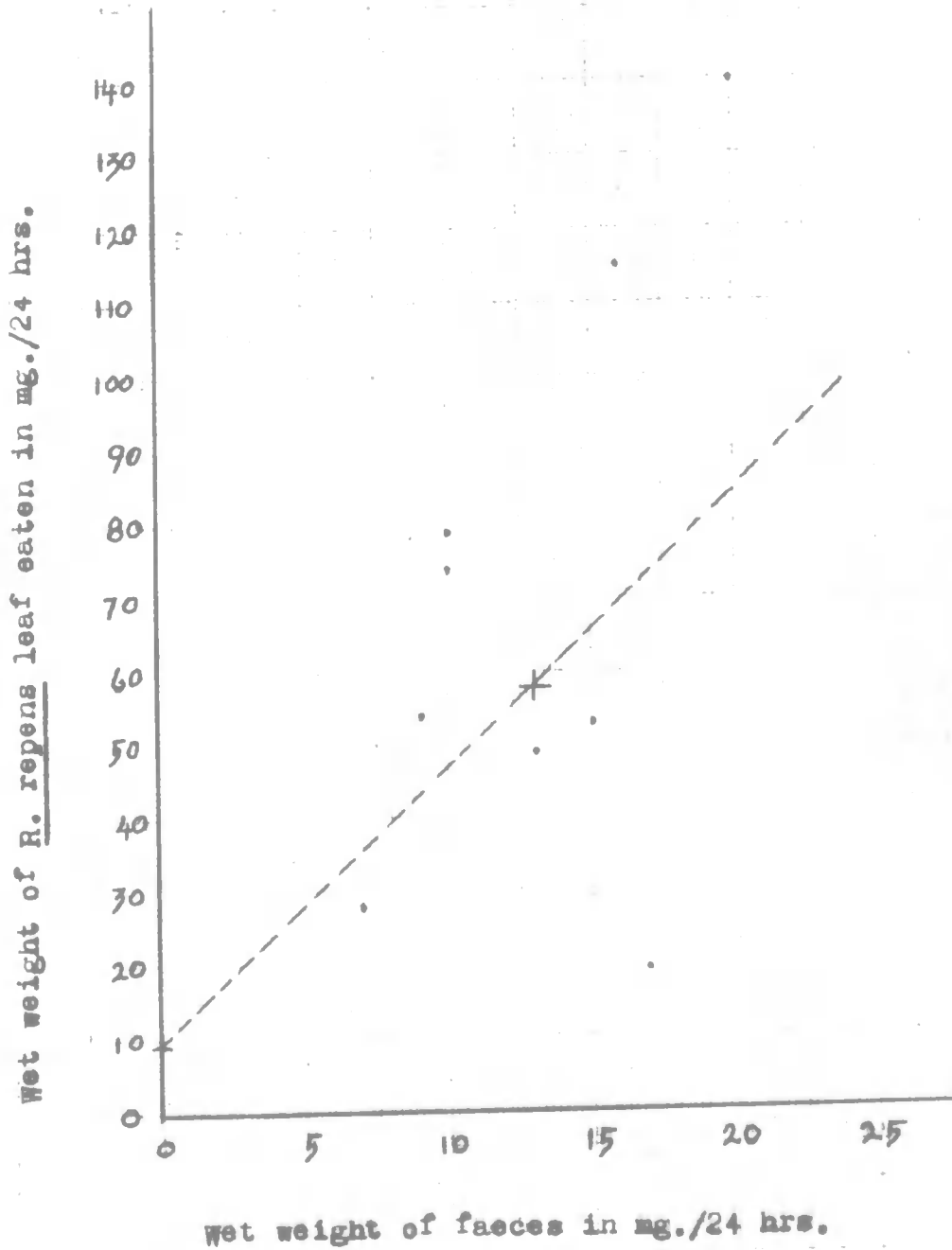
TABLE 21

REGRESSION OF R. repens EATEN ON FAECES PRODUCED.

Wt. of leaf eaten - 68	Wt. of faeces - 13	$d_x d_y$	d_x^2
(d_y)	(d_x)		
47	3	141	9
6	- 3	- 18	9
72	7	504	49
-40	- 6	240	36
-19	0	0	0
-49	4	-196	16
-14	- 4	56	16
-15	2	- 30	4
11	- 3	- 33	9
	Totals	664	148

$$\begin{aligned}
 \text{therefore regression coefficient } & \Sigma d_x d_y / \Sigma d_x^2 \\
 & = 664/148 \\
 & = 4.486 = 4.5 \text{ app.}
 \end{aligned}$$

Regression of wet weight of R. repens leaf eaten in 24 hours on wet weight of faeces voided in the following 24 hours.



Therefore :

Wet wt. of food eaten in mg. - 68 = 4.5 (wet wt. of
faeces in mg.) - 13)Eqn. 3

or

Wet wt. of food eaten in mg. = 4.5 (wet wt. of
faeces in mg.) + 9.6 mg.Eqn. 4

No figure is available for the calorific value of R. repens so an estimate is made by reference to green vegetable leaves used as human food, with known values quoted in the "Manual of Nutrition" (1958), and by comparison with the carrot and Urtica experiments. Table 22 shows assimilation energy estimates calculated from the R. repens experimental data and the known calorific values of some human foods. Using the calorific value of cabbage as a possible approximation to the unknown value for R. repens leaf the average assimilation figure for this experiment is 13.5 calories per 24 hours. This seems reasonable in comparison with the other experiments as shown in Table 23 although as indicated there it might be slightly too high.

TABLE 22

ASSIMILATION ENERGY ESTIMATES USING HUMAN FOOD VALUES

Material	Cal. per mg.	Assimilation estimate (Cal./24hrs)
Brussels sprout	.317	17.3
Cabbage	.247	13.5
Lettuce	.105	5.7
Spinach	.211	11.5
Watercress	.141	7.7

TABLE 23

COMPARISON OF ASSIMILATION ENERGY ESTIMATES PER INDIVIDUAL FROM
LABORATORY FEEDING ON CARROT, U. dioica and R. repens

Experiment	Av. slug live weight (mg.)	Wet wt. of food assim. (mg./24hrs)	Energy Assim. (cal/24hrs)	Temp. (°C)
Carrot	219.4	55.7	11.8	20
<u>U. dioica</u>	264.5	42.1	12.3	18
<u>R. repens</u>	314.0	54.7	13.5	18

It will be seen that the average weight of slugs used in the R. repens experiment was greater than that in the U. dioica experiments at the same average temperature. This may account for a slightly higher rate of assimilation. The lower assimilation

rate in the carrot experiments can be accounted for in terms of a lower average slug weight and possibly also by a reduction in activity at the slightly higher temperature. Dainton (1953) found activity decreased with rising temperature between 4°C and 20°C.

The proportion of food eaten which is assimilated in these animals, as shown in these experiments, appears abnormally high as compared with other animals. Phillipson (1960) found 47% of food eaten by M. morio to be assimilated when feeding on insects. Gere (1956) gives figures of 22.5% for Hyphantria cunea caterpillars feeding on live plant material, 76.7% for Ephestia kueniella caterpillars feeding on meal and an average of 5.7% for Isopoda and Diplopoda feeding on leaf litter from the F₁ layer.

8. STUDIES ON ASSIMILATION BY *A. reticulatus* IN WOODLAND .

(Using laboratory derived figures for assimilation and faeces production by animals which had fed in the field.)

(a) Method.

An attempt is made to estimate assimilation in the field by *A. reticulatus*. Faeces resulting from feeding in the field were collected over a period of 24 hours in the laboratory. The regression relationships, equations 2 and 4, determined in the laboratory feeding experiments were then used to estimate quantities of food eaten in the field in the 24 hours preceding collection. The percentage assimilation of food eaten as determined in the laboratory experiments was then used to estimate rate of assimilation in the field. These estimates were also converted to energy estimates using the calorific values also used in the laboratory estimates. Thus an "Urtica estimate" and a "Ranunculus estimate" is obtained for each set of data as there seems no reason to prefer one set of laboratory data. A comparison of the two sets of estimates is attempted by relating them to animal wet weight averages for each set of data and comparing these with published relationships.

A. reticulatus from "Burton Bushes" were collected into individual specimen tubes and transferred to individual dishes in the laboratory as in other experiments. Any faeces produced in the

tubes in transit were transferred to the dishes at the same time as the slugs. Saturated conditions were maintained in the dishes by wet paper carpets as in the laboratory experiments. After 24 hours in the laboratory saturated wet weights of faeces were determined. One similar sample from a school garden was also recorded and used with those from the wood to establish a relationship between the assimilation rate estimates and wet body weight of the animals.

(b) Results.

Table 24 shows the wet weights of faeces from 53 individuals of A. reticulatus collected during March and May, 1966. The overall average values from this material suggest that an individual of wet weight 269 mg. produces 11.6 mg. wet weight of faeces from 24 hours of feeding in the wood. Calculations based on equations 2 and 4 give estimates of rate of feeding in the field of 50 mg/24 hours (Urtica estimate) and 62 mg/24 hours (Ranunculus estimate) respectively, these representing wet weights of green leaf. If the same percentages of food eaten are assimilated in the field as are assimilated in the laboratory, these rates of feeding correspond to rates of assimilation of 37 mg. wet weight of Urtica dioica and 50 mg. wet weight of Ranunculus repens per 24 hours in each case. These amounts correspond with 10.8 calories per 24 hours and 12.3 calories per 24

hours respectively if energy values of the two foods are assumed as for the laboratory experiments. These estimates differ by about 12% but there seems no a priori reason for preferring one estimate to the other.

A Summary of sample averages is given in Table 25 and assimilation estimates derived from these in Table 26. A single sample from a garden is also given in Table 27. These figures are used in Section 9 to relate assimilation to body weight.

TABLE 24

FAECES FROM FRESHLY CAUGHT WOODLAND SLUGS (A. reticulatus)

Expt. No.	Date & Time	Min.temp. °C	Slug Ref.	Wet wt. of slug (mg.)	Wet wt. faeces (mg/24hrs)	Average faeces (mg/24hrs) per individual
1	13.3.66 14.30 -15.30	-0.5	540	279.5	4.9	7.1
			541	466.4	15.9	
			542	213.2	9.5	
			543	151.6	3.7	
			544	182.9	2.6	
			545	217.4	11.8	
			546	248.8	5.1	
			547	212.6	4.8	
			548	186.3	5.8	
2	16.3.66 9.45 -10.30	3.8	553	288.6	10.8	9.9
			554	224.4	16.5	
			555	300.3	8.4	
			556	234.3	9.9	
			557	213.3	7.3	
			558	136.9	8.5	
			559	255.8	8.3	
3	22.3.66 9.45 -10.30	0	561		7.0	8.6
			562		2.2	
			563		4.8	
			564		12.8	
			565		11.0	
			566		14.0	
4	2.5.66 14.30 -15.30	7.7	567	441.7	17.8	19.1
			568	288.2	9.4	
			569	493.9	21.3	
			570	378.3	23.2	

TABLE 24 contd.

Expt. No.	Date & Time	Min.temp. °C	Slug Ref.	Wet wt. of slug (mg.)	Wet wt. faeces (mg/24hrs)	Average faeces (mg/24hrs) per individual
			571	476.2	17.1	
			572	412.8	26.0	
			573	337.8	20.1	
			574	391.2	15.2	
			575	221.0	21.9	
5	7.5.66	5	578	309.7	5.4	
	14.30		579	419.3	19.4	
	-15.0		580	151.4	17.8	
			581	190.7	7.6	
			582	294.4	12.4	
			583	416.5	35.1	
			584	325.5	21.4	11.4
			585	174.6	2.1	
			586	237.1	9.2	
			587	132.7	2.1	
			588	326.7	11.0	
			589	188.4	1.1	
			590	57.6	4.3	
6	16.5.66	5	591	492.5	23.7	
	14.30		592	358.9	12.7	
	-15.30		593	253.1	2.2	
			594	391.1	18.5	
			595	141.4	4.3	10.8
			596	324.2	9.0	
			597	265.9	22.1	
			598	67.4	.8	
			599	367.7	14.6	
OVERALL AVERAGES:				269	11.6	

TABLE 25

SUMMARY OF GROUP AVERAGES IN TABLE 24

Expt.No.	1	2	3	4	5	6
Date of Collection	13.3.66	16.3.66	22.3.66	2.5.66	7.5.66	16.5.66
Time of collect. beginning	14.30	9.45	9.45	14.30	14.30	14.30
Min.temp. pre. 24 hrs. (°C)	- .5	3.8	0	7.7	5	5
No.of slugs in sample	9	7	6	9	13	9
Av. wt. of sample (mg.)	239.8	236.2	-	333.2	248.0	287.8
Range of wts.	151.6- 466.4	136.9- 300.3	-	221.0- 493.9	57.6- 419.3	67.4- 492.5
Av.wet wt. of faeces (mg/24hrs)	7.1	9.9	8.6	19.1	11.4	10.8
Range of faeces wts.(mg/24hrs)	2.6- 15.9	7.3- 16.5	.7- 14.0	9.4- 26.0	1.1- 35.1	.8- 23.7

Explanation of Table 26.

- a. Average wet weights of faeces from field from Table 23.
- b. Wet weight of food eaten in the field as estimated from row(a) by equation 2.
- c. Wet weight of food eaten in the field as estimated from row(a) by equation 4.
- d. Wet weight of food assimilated in the field as calculated from row (b) and % assimilation from Table 16
i.e. (d) value = (b) value x .74
- e. Wet weight of food assimilated in the field as calculated from row (c) and % assimilation from Table 19
i.e. (e) value = (c) value x .8
- f. Energy value of food assimilated using .293 calories per milligram as the value for U. dioica.
- g. Energy value of food assimilated using .247 calories per milligram as the value for R. repens.

TABLE 26

ESTIMATES OF ASSIMILATION IN THE FIELD: SAMPLE AVERAGES

Expt. No.	1	2	3	4	5	6	
Average faeces (mg/24hrs)	7.1	9.9	8.6	19.1	11.4	10.8	a
Food eaten <u>Urtica</u> est. (mg/24hrs)	40.8	46.5	43.9	65.4	49.62	48.4	b
Food eaten <u>Ranunculus</u> est. (mg/24hrs)	41.5	54.0	48.2	95.3	60.7	58.1	c
Food assimil. <u>Urtica</u> est. (mg/24hrs)	30.2	34.4	32.5	48.4	36.7	35.8	d
Food assimil. <u>Ranunculus</u> est. (mg/24hrs)	33.5	43.7	38.9	77.1	49.1	46.1	e
Energy assim. <u>Urtica</u> est. (Cal/24hrs)	8.8	10.0	9.5	14.2	10.7	10.5	f
Energy assim. <u>Ranunculus</u> est. (Cal/24hrs)	8.3	10.8	9.6	19.0	12.1	11.4	g

Estimation of assimilation by A. reticulatus in a garden.

One set of observations was made by collecting faeces produced in twenty-four hours after capture of animals from a school garden.

TABLE 27

Date	Ref.	Slug wt. in mg.	Faeces wt. in mg.
20.10.66	G1	498.8	10.2
	G2	233.3	4.8
	G3	581.1	20.3
	G4	296.5	18.0
	G5	400.9	9.6
	G6	529.5	8.3
	G7	182.7	10.9
	G8	211.0	8.3
	Average	366.7	11.3

Using the laboratory data on assimilation an estimate of 36.6 mg. Urtica or 48.3 mg. Ranunculus is given as the average assimilation in twenty-four hours.

9. RELATIONSHIP BETWEEN BODY WEIGHT OF *A. reticulatus*
AND ESTIMATES OF ASSIMILATION IN THE FIELD.

(a) Method.

Using average wet weights of slugs and estimates of assimilation in the field derived from sample average faeces in Tables 24 and 25 together with results from one garden sample, Table 27, the regression of the logarithm of assimilation per 24 hours on the logarithm of live body weight was calculated for the two estimates. Table 28 below sets out these data as used in the present section.

TABLE 28
 FIELD ESTIMATES OF ASSIMILATION

	Sample average live wt. of slug (mg.)	<u>Urtica</u> est. assimilation (mg/24hrs)	<u>Ranunculus</u> est. assimilation (mg/24hrs)
1	239.8	30.2	33.5
2	236.2	34.4	43.7
4	333.2	48.4	77.1
5	248.0	36.7	49.1
6	287.8	35.8	46.1
G	366.7	36.6	48.3

(b) Results

The regression calculations are set out in Tables 29 and 30 on pages 88 and 89, for the Urtica and Ranunculus estimates respectively. It is seen from equation 7 that the Urtica estimates are proportional to the live body weights to the power .49 and from equation 10 that the Ranunculus estimates are proportional to the live body weights to the power .53.

Prosser and Brown (1961), in summarising work relating metabolism to body weight suggest powers varying from 0.55 to 1.0 and quote an extensive survey by Hemmingsen in which this author concludes that metabolism = $k(\text{weight})^b$ with b not far from 0.73 in poikilotherms, homeotherms and beech trees. A range of values from 0.45 to 1.0 was found in pulmonate snails by Berg, also quoted by Prosser and Brown. Van der Drift (1951) and Gere (1955) working with Diplopoda and Isopoda found a power of $2/3$ fitted their data. Thus both values derived from the present study seem close to other studies.

TABLE 29

CALCULATION OF REGRESSION OF LOG. ASSIMILATION ON LOG. BODY
WEIGHT FOR Urtica ESTIMATES.

Log body wt.	d_x	Log.assimilation	d_y	$d_x d_y$	d_x^2
2.38	-.07	1.48	-.08	.0056	.0049
2.37	-.08	1.54	-.02	.0016	.0064
2.52	.07	1.68	.12	.0084	.0049
2.39	-.06	1.56	0	0	.0036
2.46	.01	1.55	-.01	-.0001	.0001
2.56	.11	1.56	0	0	.0121
Av.2.45		1.56	Total:	.0155	.0320

therefore regression coefficient, $m = .0155/.0320$

$= .49$ approx.

Therefore equation of regression line:

$$\text{Log. assim. mg.} - 1.56 = .49(\text{log. wt. mg.} - 2.45) \quad \text{Eqn. 5}$$

$$\text{Log. assim. mg.} = .49 (\text{log.assim.mg.}) - 1.2 \quad \text{Eqn. 6}$$

Therefore relation between body wt. and assimilation is

$$\text{Assimilation} = 15.8 (\text{body wet wt. in mg.})^{.49} \quad \text{Eqn. 7}$$

TABLE 30

CALCULATION OF REGRESSION LINE FOR Ranunculus ESTIMATE

Log.slug wt.	x	Log.assim.	y	xy	x ²
2.38	-.07	1.52	-.17	.0119	.0049
2.37	-.08	1.64	-.05	.0040	.0064
2.52	.07	1.89	.2	.0014	.0049
2.39	-.06	1.69	0	0	.0036
2.46	.01	1.66	-.03	-.0003	.0001
2.56	.11	1.68	0	0	.0121
Av. 2.45		1.68	Total:	.017	.0320

Therefore regression coefficient = $.0170 / .0320 = .53$

Equation of line referred to mean point as origin:

$$\text{Log.assim} - 1.68 = .53 (\text{log.wt.} - 2.45) \quad \text{Eqn. 8}$$

Equation referred to original axes:

$$\text{Log.assim} = .53 \text{ log. wt.} - 1.3 \quad \text{Eqn. 9}$$

Giving the following relationship:

$$\text{Assimilation} = 19.8 (\text{wt. of slug})^{.53} \quad \text{Eqn. 10}$$

10. CELLULOSE DIGESTING BACTERIA IN THE GUT AND FAECES OF

A. reticulatus

(a) Method.

Florkin and Lozet (1949) showed the presence of two types of cellulose digesting bacteria in the gut of Helix pomatia. One of their methods is used here to find if similar bacteria occur in A. reticulatus.

Filter paper strips were used as a source of cellulose in a liquid medium favouring the growth of cellulose digesting bacteria, Omeliansky's medium:

Ammonium sulphate	1g.
Dipotassium phosphate	1g.
Magnesium sulphate	0.5g.
Calcium carbonate	2g.
Sodium chloride	trace
Distilled water	1,000 ccs.

Test-tubes with appropriate cotton-wool plugs or metal caps were used as containers. A depth of about 2 inches of the liquid medium was placed in the tubes and the filter-paper strip nearly the full length of the tube was placed with its base in the liquid. The tubes were sterilised, set up in this way, at 15 lb per square inch for 15 minutes or longer.

In the first experiment six 6" x $\frac{1}{2}$ " soda-glass test-tubes were used with Whatman No. 1 filter-paper strips, liquid medium, and cotton-wool plugs. Three slugs were collected from a garden and

killed in 70% alcohol. Flamed scissors were used to dissect out the gut from each animal as required. Three of the culture tubes, after sterilization, were inoculated with sections of the fore-part of the gut, crop and stomach. Two were similarly inoculated with sections of the hind-part of the gut, rectum. One tube remained uninoculated as a control. The tubes were incubated at 37°C for three weeks. After this time they were observed for evidence of discolouration and thinning of the filter-paper strips.

In the second experiment nine tubes similar to those of the first experiment were set up. All, after sterilization, were inoculated with sections of the fore-part of the gut of animals taken from a garden and killed in 70% alcohol. These cultures were incubated at 27°C for 21 days and then observed as in the first experiment.

In the third experiment thirty three BS 625 rimless soda-glass, 6" x $\frac{3}{4}$ " bacteriological test-tubes were set up with Whatman No. 1 chromatography-paper strips. "Cap-o-test" aluminium caps were used to cover the tubes. Three slugs which had been taken from a garden and used in laboratory feeding experiments were used to provide inoculating material which was obtained as in previous experiments, as regards gut material. In addition some tubes were inoculated with faeces from laboratory slug culture dishes in which the slugs had been housed. Six tubes were left untouched after sterilization, sterilization controls. Two tubes were opened and a flamed loop was dipped in the medium, handling controls. One tube was opened and the

scissors were dipped in the medium after being used for dissection, scissor control. One tube was opened and the forceps were dipped in the medium after they had been used for dissection, forceps control. One tube was inoculated with the carcase of one slug after the gut had been dissected out. One tube had gut contents smeared only on the paper strip above the liquid. Five tubes were inoculated with faeces resulting from feeding on R. repens leaf in the laboratory. The faeces were transferred from the slug culture dishes with a flamed wire loop. Seven tubes were inoculated with faeces resulting from feeding on paper towel in the laboratory. Five tubes were inoculated with sections of the fore-part of the gut from one animal. Four others were inoculated with sections of the fore-part of the gut of another animal. In all the above inoculations the material was placed in the liquid medium although the paper might have been touched above the liquid level. Although bacterial counts were not contemplated it was thought possible that some indication of density of distribution might be given in this experiment by the rapidity of development of experimental colonies, early appearance of yellowing of paper strip, in the early stages of incubation. In any case it was considered of interest to discover if the bacteria present in the gut survived in the faeces, and if the gut flora was present when different foods were used. In this third experiment, tubes were incubated by accident at 37°C for four hours and then at 28°C for the remainder of the experiment. Observations were made periodically up to 43 days.

(b) Results.

In experiment 1, there was no change in the control but all other tubes showed yellowing of the paper followed by disintegration of this just above the liquid surface. This was taken to indicate the presence of cellulose digesting bacteria throughout the gut of the A. reticulatus individuals used to provide the inoculations.

In the second experiment all tubes showed yellow discolouration and disintegration of the paper just above the liquid surface.

In the third experiment the scissor control showed yellowing of the paper above the liquid surface, but all other controls did not change. This is taken to mean that there might have been transfer of bacteria from one section of the gut to another, or from one gut to another on the scissors. It seems unlikely that transfer of bacteria could have been from the skin of an animal since the tube inoculated with the carcase of one slug after dissection showed no signs of change of the paper although the liquid was discoloured and became brown. Of the 7 tubes inoculated with faeces resulting from paper feeding, 6 showed cream discolouration of the paper above the surface after 7 days incubation. Yellow colouration in the same region was apparent at 10 days and after 31 days the paper was considerably thinned in the same region. Of the 5 tubes inoculated with faeces from R. repens feeding, 1 showed cream discolouration

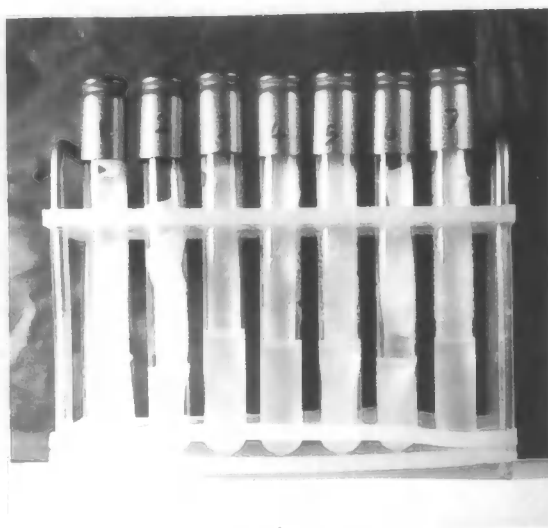
of the paper above the liquid surface after 7 days incubation, 1 showed yellow and two cream after 10 days and after 43 days, 3 tubes showed thinning of the paper. Of the ten tubes inoculated with gut material, 2 showed cream discolouration of the paper after 7 days, and after 43 days incubation, 4 showed yellow discolouration and 3 of these showed thinning of the paper. Some of the bacteria from the yellow region of several cultures in this experiment were stained and examined microscopically. These were found to be very small gram-negative cocci.

The results of the first experiment are taken to show that this species does harbour cellulose digesting bacteria in the gut, this is also shown in the other experiments. The third experiment also shows that under laboratory conditions these bacteria persist for a short time at least in faeces.

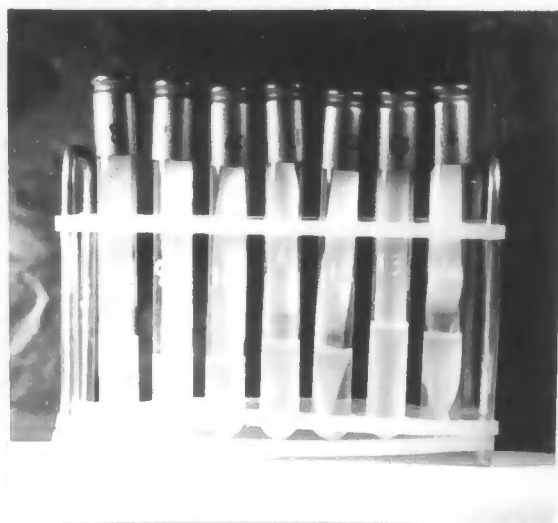
The following illustrations show the appearance of the cultures of the third experiment after 35 days incubation.

Inoculations of cultures in the third experiment
as a key to the following illustrations of the state
of the cultures after 35 days.

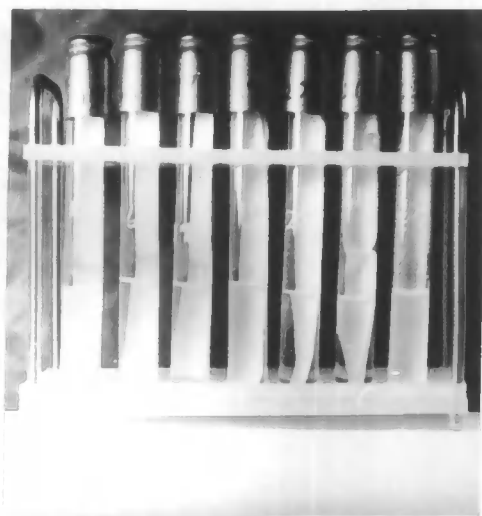
- 1 - control - sterile loop dipped in medium
- 2 - 6 inoculated with sterile wire loop dipped in faeces of
G24 resulting from feeding on Ranunculus repens leaf.
- 7 - control - left untouched after sterilization.
- 8 - control - sterile loop dipped in medium.
- 9 & 10 - controls - left untouched after sterilization.
- 11 - 17 - inoculated with faeces of G26 resulting from paper
feeding transferred to medium with sterile wire loop.
- 19 - 21 - controls - left untouched after sterilization.
- 22 - control - scissors dipped in medium after dissection.
- 23 - control - forceps dipped in medium after dissection.
- 24 - 28 - sections of foregut from G22 dropped into medium.
- 29 - carcase of G22 dropped into medium.
- 30 - 33 - sections of foregut from G26 dropped into medium.
- 34 - contents of part of foregut of G26 smeared on paper strip
above surface level of liquid medium.



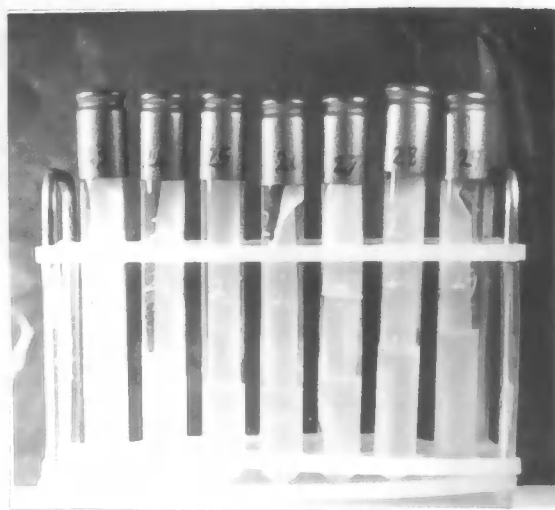
1 2 3 4 5 6 7



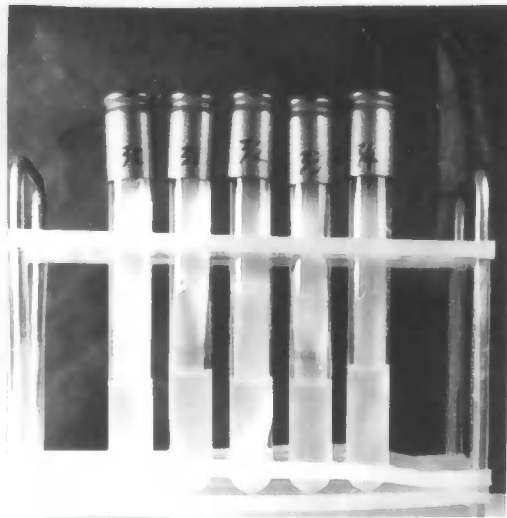
8 9 10 11 12 13 14



15 16 17 19 20 21 22



23 24 25 26 27 28 29



30 31 32 33 34

11 DISCUSSION .

(a) Food of *A. reticulatus* .

Taylor (1907) Ellis (1926) and Fromming (1954) all specify this as an omnivorous species, and indicate a range of materials eaten ranging from the tenderer parts of fresh green plants to faeces and various small animals. Barnes and Weil (1945) differ from most authors in suggesting a predominance of decaying vegetation in the diet. They observed this species on exposed tubers but considered that it only follows on after attack by other species. They considered that it selects withering and dying flowers and leaves in preference to healthy living tissues. Investigating laboratory feeding of specimens from neglected agricultural land in Michigan, U.S.A., Getz (1959) lists ten species of herbs readily accepted as food. Fromming (1954) examined faeces from nineteen animals and found: 45.1% fresh flowering plant remains, 22.5% dead plant remains (humus), 3.2% pollen, 16.1% lower fungi, 3.2% insect remains, 3.2% earthworm chaetae, 12.9% unidentifiable material. The pollen was identified as that from Alnus sp. and Sonchus sp., and in 8% of the living plant material Urtica hairs were identified but species of plant material were not otherwise stated. This author also showed that in captivity animals of this species survived longer on fresh plant material than on beech leaf litter. Pitchford (1956) observed the species feeding on damaged rabbit carcasses. Selection of food

is reported by Jones (1962) who refers to the selective feeding on acyanogenic as opposed to cyanogenic forms of Lotus corniculatus L. by this and other animals. Duthoit (1961, 1964) refers to a characteristic manner of feeding on wheat seeds, the germ being eaten before the endosperm, and has shown that grain is eaten in preference to fungi.

The present study shows the selection in captivity of one form of food in preference to another, as opposed to the view of Frömmingⁿ that they eat all before them, and supports the view of those authors who suggest that fresh living plant material forms a high proportion of the food. In the woodland studied dicotyledonous herbs form more than half of the food of A. reticulatus and green leaves of Ranunculus repens L. and Urtica dioica L. are constant and frequent elements in the diet. The former plant provides fresh leaves throughout the year and the latter does so during a period roughly from March to November. Laboratory feeding experiments have shown these two materials to be selected and most readily eaten of several offered. These results might suggest that some feature of the chemical composition of these two plant species makes them preferable as food for this species of slug in the habitat considered. These two materials would not normally be available in a well kept garden, meadow or field and other materials must be used as food in these situations.

(b) Possible importance of phosphorus.

In view of the prominent position of Urtica dioica in this study it is interesting to note that Pigott and Taylor (1964) have shown a special need of this species of plant for phosphorus. Professor Pigott (1964) has kindly supplied the figures for total phosphorus content of U. dioica leaves in Table 31. Dr. A.A. Wright (1966) has been kind enough to analyse dried leaf material from R. repens and U. dioica, Table 32. Although these figures differ from those of Pigott, they show a concentration of phosphorus in R. repens almost equal to that in Urtica.

It is perhaps possible that the relatively rich phosphorus content of these plants supply some special need of Agriolimax reticulatus or that the particular palatability of the material is due to some other feature associated with or merely coincident with the high phosphorus content. The known attraction of bran as "slug bait" may be due to the same cause. Evans (1960) lists mineral compositions of 106 common feedingstuffs of farm animals and only materials of animal and fungal origin in this list exceed or equal bran in phosphorus content at 2.8% (P_2O_5).

Using average field assimilation figures of 50 mg. R. repens per 24 hrs. and 37 mg. U. dioica per 24 hrs. and average phosphorus content figures from Table 32 to calculate the daily intake of

phosphorus, the following estimates are obtained:

R. repens estimate - 0.570 mg.

U. dioica " - 0.469 mg.

It is possible that differences in phosphorus content could account for difference of palatability of Urtica leaves found in feeding experiments. Pigott (1964) found differences of phosphorus content in different leaves from the same plant (Table 31).

TABLE 31

Total phosphorus concentration (mg /100 gm. dry matter) of leaves of Urtica dioica from the same plant in Buff Wood, Cambridgeshire. Plant shows symptoms of slight phosphorus deficiency by May. Leaves numbered from apex down (i.e. 1 is the youngest leaf). C.D.Pigott (1964)

Leaf Number	26th May	21st September
1	757.6	-
2	718.2	527.0
3	652.9	454.5
4	603.6	504.5
5	521.0	497.1
6	489.2	452.7
7	427.1	450.2
8	241.4	414.5
9	290.1	397.5
10	246.0	394.4
11	253.2	404.1
12	-	381.8
13	-	396.58
Senescent leaves	-	677.8

TABLE 32

Phosphorus content of Urtica dioica and Ranunculus repens leaves from a school garden, mature leaves collected 18.10.66 and bulked mg/g. dry matter.

<u>U. dioica</u>	13.7	10.3	12.5	average 12.7
<u>R. repens</u>	8.4	13.7	12.1	average 11.4

From A.A. Wright (1966)

(c) Assimilation and energy flow in the field.

Energy transfer and assimilation are estimated by an indirect method comparing laboratory experiments with production of faeces from feeding in the field. Phillipson (1960) suggests that such an indirect method has an advantage over measures of assimilation calculated from experiments measuring respiration rates in that it inevitably incorporates the effects of fluctuations in field temperature. The average values obtained in the present study suggest that an individual of A. reticulatus of wet weight approximately 269 mg. will assimilate between 37 mg. (U. estimate) and 50 mg. (R. estimate) fresh plant material per twenty-four hours in the field conditions investigated i.e. March and May, 1966, in woodland. 11.6 mg. of faeces are voided per twenty four hours. These figures correspond to energy estimates of between 12.3 calories/24hrs. (R. est.) and 10.8 calories/24hrs. (U. est.) assimilated and 2.9 (R. est.) calories/24hrs. and 3.4 calories/24hrs. (U. est.) deposited as faeces.

In the laboratory experiments an average of 74% of the food eaten was assimilated when U. dioica leaf was eaten and 80.9% when fresh R. repens leaf was eaten. These figures are very high compared with other animals, Phillipson (1960) Gere (1956). Gere found a high percentage assimilation in caterpillars of Ephestia kuehniella fed on meal, which he interprets as being due to a high metabolic rate prior to pupation and the oxidation of the dry plant matter to provide the

necessary amount of water. It is perhaps possible that there is some similar cause involved in the present case of A. reticulatus. The reproductive phase of the animals used was not investigated, it is possible that they were maturing reproductive structures with a consequent high metabolic activity. It is possible also that the occurrence of symbiotic cellulose digesting bacteria allows a more efficient use of food.

Although the present studies have not separated mature from immature individuals and it is not possible to allow for yearly fluctuations in temperature some crude estimates of annual levels of metabolic activity can be attempted for habitats of known population densities. South (1965) found a mean population density of 5.6 A. reticulatus per square foot of pasture ungrazed for several months, this corresponds with a density of 60 per square metre; Hunter (1966) found the mean density of the same species on adjacent arable land to be 10.7 per square foot, or 115 per square metre.

Using these population figures estimates are made for these habitats in Table 33.

TABLE 33

METABOLIC ACTIVITY OF A. reticulatus ON PASTURE AND ARABLE LAND.

Habitat	Slug density per m ²	Metabolic activity per m ²		
		Cal/24hrs.	K.cal/yr.	
Pasture	60	738	269	(R.est.)
		648	236	(U.est.)
Arable	115	1414	516	(R.est.)
		1242	452	(U.est.)

(d) Contribution of slugs to soil maintenance.

Nielsen (1962, 1963) showed the abilities of slugs (Arion ater L. and Arion circumscriptus Johnst.) to digest a wide range of plant structural polysaccharides, but suggests that there is no certain evidence that these are all used in natural feeding. The present study shows the presence of cellulose digesting bacteria in the gut of A. reticulatus and there is possibly some evidence of the digestion of cellulose under natural conditions from the frequent occurrence of isolated lignin spirals from xylem in faeces from fresh plant material. It is however possible that lignin spirals might be isolated from surrounding materials by radulation during feeding.

Nielsen (1962) showed the ability of Arion circumscriptus to digest chitin and Jeuniaux (1954) showed the same ability in three

species of slugs including Agriolimax agrestis L. (possibly A. reticulatus (Müller)). This may be used in the digestion of arthropod, earthworm and fungal materials, so that even if these are accidentally included in the diet they might provide nutrients.

It seems possible that slugs could be the only macrofauna of soil and litter which contribute to soil by the degradation of these materials. In this way, slugs may make a chemical contribution in addition to the physical contribution of reduction in particle size which they make in common with other animals.

It has been observed that a rich microfaunal development can occur in moist faeces and that living nematodes pass through the gut. Kuhnelt (1961) also refers to these phenomena, in the case of other species of slug. It is suggested that the development of microfauna in faeces would be assisted under natural conditions by the water holding capacity of the mucus content of faeces but might also be partly due to the available nutrients resulting from the feeding and digestive processes of the slug.

Macfadyen (1961) and Kuhnelt (1961) suggest the possible importance of the deposits of faeces of soil and litter animals for the nutrient enrichment and dispersal of microorganisms. It seems likely that slugs could make an important contribution in this way also.

(e) Factors affecting distribution.

South (1965) found A. reticulatus distribution on grassland to be associated with distribution of cocksfoot and suggests that adequate shelter is the factor determining distribution. The present study suggests that in a more variable habitat such as woodland the presence of food in the form of herbaceous plants may determine distribution on a broad scale, as shown by the association of resting sites under logs with the presence of a herb layer. It is probable that in rough woodland there will be adequate shelter available to slugs in the form of fallen wood, leaf litter and loose soil apart from that provided by vegetation. Therefore whereas secluded resting sites may be a determining factor in gardens and grassland, in woods food may be an important one.

12. SUMMARY.

1. Searches under fallen wood suggest that recently active A. reticulatus rest only near a herb layer as opposed to bare earth in a wood.
2. Food materials of A. reticulatus in a wood were determined by comparison of fragments in faeces and gut contents with epidermal features of leaves of possible food plants. Ranunculus repens L. and Urtica dioica L. leaf fragments appear most frequently of the recognised materials.
3. Laboratory feeding experiments showed the same two materials to be the most frequently eaten of a limited selection offered singly and together.
4. Laboratory feeding experiments established a relationship between wet weight of faeces produced in 24 hours and wet weight of food eaten in the previous 24 hours. The proportion of food assimilated was also estimated and found to be relatively high. 82.4% of carrot eaten was assimilated, 74% of U. dioica leaf and 80.9% R. repens leaf.

5. Food eaten in 24 hours in the field was estimated from the wet weight of faeces produced in the 24 hours after capture. An average of 11.6 mg. wet weight of faeces were voided corresponding to 50 mg. wet weight of U. dioica leaf or 62 mg. R. repens leaf eaten in the previous 24 hours in woodland.
6. The relationship between food assimilated in 24 hours in the field and live body weight was determined. Estimates of assimilation were found to be approximately proportional to live body weights to the power .5.
7. The presence of cellulose digesting bacteria in faeces and gut contents was shown by test-tube culture on filter paper in a liquid medium.
8. Some ecological implications of the study are discussed.

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