THESIS
for the Degree of Doctor of Philosophy,
University of Edinburgh.

STUDIES ON AGRIOLIMAX AGRESTIS L., THE GRAY FIELD SLUG, AND OTHER FIELD SLUGS OF ECONOMIC IMPORTANCE.

PART I
The Life-history and Development of Agriolimax agrestis L.

PART II
An Ecological Study of Agriolimax agrestis L., the Gray Field Slug, and Related Species.

PART III
The Control of Slugs attacking Potatoes.

With an additional paper:
"THE SPERMATOGENESIS OF THE AXOLOTL (AMBLYSTOMA TIGRINUM)."

Presented by
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PART I

THE LIFE-HISTORY AND DEVELOPMENT OF AGRIOLIMAX AGRESTIS I.

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1. Introduction.

Among Gasteropod Molluscs the life-histories and development of the most highly evolved members, the terrestrial Pulmonates, have been less intensively studied than those of more primitive aquatic forms. This has been due in part to the technical difficulties involved in the preparation of material for study, and in part to the fact that the investigator seeking for primitive features of phyletic interest is more likely to find these among more generalised species of Gasteropods than among those which are obviously adapted, both during the early stages of their development and in adult life, to a habitat far removed from the ancestral one.

It is the purpose of this paper to present data bearing upon the life-history of a single species of land Pulmonate, Agriolimax agrestis L.; to enlarge certain aspects of the embryology of this species; and to demonstrate the structural and functional changes of the larva which have accompanied its transition from an aquatic to a terrestrial breeding habit.

The material for this investigation was chiefly obtained from fields planted with potatoes, in the vicinity of Edinburgh. Field observations were made continuously between November 1934 and September 1936. Adult slugs were brought to the laboratory and housed in closed glass vessels containing moist earth taken from the same field as the slugs; oviposition occurred freely so long as the soil was kept sufficiently moist and fresh plant food was provided. The soil was examined each morning for egg-masses laid during the previous night; these were washed clean of soil and kept on moist filter-paper in separate petri-dishes, covered to prevent evaporation.

The eggs already contain zygotes when laid, and throughout the entire course of their development it is possible to observe the living embryo by transmitted light in eggs immersed in water, for the surrounding albumen and jelly are quite clear and the embryonic tissues also remain translucent until a few days before hatching. The comparative ease with which a complete series of the various stages can be obtained considerably reduces the difficulties of tracing the organism's development. In addition to the study of living material, whole mounts and sections of each stage were prepared for examination.

The inner membrane of the egg and its contained albumen render extraction of the embryo difficult, and the following technique was eventually found to give the best results. The entire egg is dropped into fixative (saturated solution
of corrosive sublimate in 5% glacial acetic acid) and is kept there for one and a half minutes, during which time the albumen becomes opaque and less fluid and the embryo is killed. The egg is then transferred to distilled water, when the albumen clears again, and the embryo is carefully removed. Two mounted needles are used, one to hold the egg in position and the other to pierce the inner membrane and liberate embryo and albumen, the latter being soluble in distilled water. In the case of the very early stages the embryo is usually liberated without damage, for the needle can be pushed well into the egg-capsule, increasing the pressure of the fluid albumen and making an aperture in the membrane through which the embryo can comfortably pass with the outflow of albumen which follows a rapid withdrawal of the needle. Larger embryos are more difficult to deal with, and it is necessary to release the albumen through a small puncture, pull the membrane and embryo out of the jelly, and carefully tease the rather tough membrane away from the embryo. All embryos damaged in the process are discarded. Freed from the egg the embryo is washed clean of the last traces of albumen by streams of distilled water from a pipette and then replaced in the corrosive-acetic solution to ensure adequate fixation. Dehydration follows in the usual way.

Whole mounts were made by prolonged staining with dilute borax carmine, alum carmine, Delafield's haematoxylin and Ehrlich's haematoxylin. The third of these stains, diluted one in twenty with 25% alcohol, gave the best results. The
clearest preparations of older embryos resulted from the use of Delafield's haematoxylin which had lain diluted for several weeks and become stale, so that instead of the blue colour which is usually differentiated by subsequent treatment of the embryo with 70% ammonia alcohol the tissues remained brown. It was found useful to keep stained embryos of all stages in clove oil in solid watch-glasses so that they could be readily manipulated and studied in any desired position.

Sections, varying in thickness from 6μ to 9μ, were made of embryos from the gastrula stage onwards and stained either with Ehrlich's haematoxylin alone or counter-stained with eosin. All the specimens sectioned were previously embedded in paraffin.
3. Life-history.

Information concerning the life-histories of injurious species of British slugs and snails falls far short of expectation when the universal occurrence and economic importance of these animals are borne in mind. The precise data which have been obtained relative to the breeding and other habits of insect pests, both in the field and under controlled conditions, have no counterpart in reference to the land Mollusca which attack crops. Taylor (1907) reviewed the facts published prior to the date of his monograph and included a description of the act of conjugation of A. agrestis as well as some observations on fecundity, growth and longevity. Miles (1921) dealt briefly with A. agrestis and (1924) described the hatching of the same species, while Miles, Wood and Thomas (1931) included short accounts of the life-histories of A. agrestis, Milax sowerbii and Arion subfuscus. Abroad, Bouchard-Chantereaux (1857) gave an account of the breeding habits of A. agrestis in France, and Felseneer (1935) compiled most of the data upon the biology of Molluscs in general. Kunkel (1916, 1928, 1929) discussed the breeding habits of A. agrestis and several species of Arion in Germany, and Lovett and Black (1900) investigated the life-history and control of the former species in Oregon, U.S.A.

a) Seasonal Activity.

The activities of slugs have been found to be governed not by any definite seasonal cycle but by the prevailing condition of weather. An annual aestivation occurs only when conditions through the summer are too dry and warm for slug activity, a
state of affairs which seldom persists for any length of time in this country. During the unusually dry summer of 1935, however, it was extremely difficult to find slugs in the Lothian fields where they were ordinarily known to be plentiful, but the first period of wet weather which interrupted the drought immediately caused their appearance in large numbers. The impression was formed that instead of descending deeply into the soil to undergo complete dormancy, the slugs had migrated from the open field to the surrounding shelter belts and grassy headlands. Slugs were continually in evidence throughout the wetter summer of 1936. There is no true period of hibernation in the course of the life-cycle, and hard frost is necessary to induce complete cessation of activity. Conflicting statements prevail with regard to the breeding season of A. agrestis as is evident from the following list:

Bouchard-Chantereaux (1837) .......... April to November.
Van Beneden (1876) ................. Throughout the winter months.
Pol (1880) .......................... Beginning of winter.
Taylor (1907) ........................ All seasons of year, even January.
Künkel (1916) ...................... October to January.
Lovett and Black (1920) ............ All seasons, but particularly spring and early summer.
Hawley (1922) ...................... Autumn.
Ellis (1926) ........................ Throughout the summer.
The reproductive period is not confined to one particular season of the year, but breeding takes place whenever conditions are suitable. It is readily induced in the laboratory at any time. The eggs of *A. agrestis* have actually been found in the field during every month of the year except July, and it was judged that egg-masses found during the first week of August 1936 were so far developed that they must have been laid in July about two weeks previously. They are most plentiful, however, when weather is most favourable to slug activity, and late August to November has been found to be the period of greatest reproductive activity. In the summer, previous to August, conditions are generally too dry and warm, and during the winter low temperatures tend to retard development, except in situations which provide protection from the cold. An increase of reproduction in spring corresponding to that of autumn was not observed, and eggs and young slugs are less plentiful during April and May than in late winter.

b) Pairing.

The complete act of pairing was observed in the field on only one occasion, 3 Oct. 1935, during a mild damp evening, at 8.30 p.m. Miles (1921) states that mating takes place between 4 a.m. and 6 a.m., a fact which has not been verified during the present investigation since observations were seldom made after midnight. The prolonged fondling and excitatory gestures with the tentacles such as Taylor (1907) describes did not take place. The two slugs, coming from opposite directions, approached each other on the surface of the soil. Neither
seemed to sense the other until they were almost touching, and then both pairs of tentacles were used as each explored the mantle and head region of the other. They took up a position facing in opposite directions with the right sides of their heads, where the genital aperture is situated, close together. Each everted the conical, pointed penis and almost at once found the aperture of the other, pairing being reciprocal. The period of union lasted for just over a quarter of an hour, during which time little movement took place and the anterior tentacles were held half retracted. This does not agree with Taylor's (1907) observation that the act of pairing occupies usually not more than a few seconds. When they separated the slugs moved apart with the penes well extended and kept them so for some time. The entire process occupied less than half-an-hour, a much shorter period than the "several hours" given by Heath (1916) for the same species and 4½ hours by Kunkel (1916) for Limax. The slugs were (collected and kept) for records of oviposition.

The lapse of time between pairing and oviposition is difficult to determine. Taylor (1907) has quoted records of the interval lasting from 5 to 20 days in the case of A. agrestis, and Kunkel (1916) has stated 6 to 8 weeks for Arion and Limax. In the present case one individual laid its first mass on 13 Oct. 1935, i.e. after ten days, and the other commenced to oviposit after sixteen days. There is no proof, however, that the egg-masses were those associated with the observed pairing and were not the result of fertilization by sperms previously stored in the spermatheca. Further, the occurrence of self-fertilization in several land Pulmonates has been
demonstrated by Simroth (1887), Luther (1913) who bred two successive generations of *A. agrestis* in this way, and Künkels (1916) who obtained several generations of Arion, so that in view of these observations the time which elapses between conjugation and the oviposition which results from that conjugation cannot be stated with certainty.

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c) Oviposition and Fecundity.

The deposition of eggs has been observed only in the laboratory and takes place under conditions which stimulate slugs to activity, that is in dim light and high humidity. Invariably eggs were laid at night and the following account of the process on 27 Feb., 1935 is typical. Oviposition commenced at 5.30 p.m. and prior to laying the first egg the slug made no attempt to excavate a "nest" in the soil, as stated by Pelseneer (1935) for *Limax* and Arion. The first egg was pushed into a fissure less than an inch below the soil surface. The eggs were passed out singly through the genital aperture and each was pressed into the fissure as it emerged. Throughout the process the tentacles were kept evaginated and there was little movement on the part of the slug (Fig.1). Nineteen eggs were laid at regular intervals with short pauses between each, and the total time taken to lay the complete mass was eight-and-a-half minutes. This is a much shorter period than Bouchard-Chantereaux (1897) gives for Stylommatophora in general; the laying of each egg is stated to occupy two to three minutes, with four- or five-minute intervals between each, the total time required being twenty to forty hours.
Agriolimax agrestis during Oviposition.
(Drawn from life. x 3).

Agriolimax agrestis var. albitentaculata Dumont and Mortillet.
The body is pure white and there is no optic pigment.
(Photograph from life. Nat. size).
In the field egg-masses are placed in any damp situation. They have been found in damp soil, at the roots of grass, potatoes, turnips, etc., occasionally inside tubers, and abundantly in the folds of wet sacks and inside masses of farmyard manure strewn on winter stubble.

The fecundity of individual slugs under natural conditions is a difficult matter to determine and it varies with climatic and other factors. Bouchard-Chartereaux (1837) states that A. agrestis lays six to eight masses of eggs at intervals of three or four weeks, the deposition of each mass being preceded by a separate coitus. The relative size of successive (layings) has been found by this writer and by Künkel (1916) to decrease as time goes on, and the first mass was by far the largest. The former author also found that the number of eggs constituting a mass varied from 28 to 70, figures which are considerably higher than were encountered in nature during the present investigation. The number of eggs in a mass was found to vary from 9 to 49 eggs, and there apparently exists a seasonal variation of numbers, larger masses occurring in late autumn. The number of eggs per mass and the situations in which they occurred are given in Table I; in some cases it is probable that a few eggs were overlooked with the disturbance of the soil as the plants were uprooted or when the soil was sifted.
Egg-mass of *Agriolimax agrestis* at roots of potato plant. The eggs are translucent and separate.

(Photograph. x 2).

Egg-mass of *Arion circumscriptus* Johnston, laid in laboratory. The eggs are semi-transparent, and joined by a sticky mucus.

(Photograph. x 2).
Under laboratory conditions larger egg-masses were obtained and more than eight were laid at intervals of a few days without renewed coitus. A larger egg-mass than usual is laid following a period of unfavourable conditions. Five slugs which had been kept for two weeks in soil too dry for oviposition laid masses numbering 66, 58, 42, 37 and 29 eggs during the first night after being transferred to moist soil. This seems to indicate that the smaller masses which the slugs would normally have laid at intervals but for unsuitable conditions were accumulated with retention to form a single large mass. The degree of development of the egg at the time of laying did not vary. In the field direct evidence has not been forthcoming of an increase in the size of egg-masses after a period of unfavourable conditions unless the autumnal maximum following upon the lessened reproductive activity of the summer months be an expression of the same process.

Tables II and III are the oviposition records of slugs kept out-of-doors in glass vessels containing moist earth and a constant supply of fresh food. Tables IIa and IIb give the total number of eggs laid each day by six slugs, and in Table III individual records are given of slugs which were housed in separate vessels. In the latter case the second and third columns refer to the pair of slugs taken in coitus on 3 Oct, 1935 as described previously.
### Table II. Records of Oviposition.

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**Notes:**
- One slug died on each of the specified dates.
- The table records the oviposition of two groups of Agriolimax agrestis over a 30-day period, showing the number of eggs per egg-mass and the total number of eggs laid.
### Table III. Oviposition Records of Seven Individuals of *Agriclimex agrestis*

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**Totals** | 132 | 292 | 164 | 116 | 102 | 77 | 177

**Gross total** | 1080 eggs.

**C**... slug collected and experiment begun.

**D**... slug died.

... no eggs laid.
These figures indicate the capacity of slugs for reproduction under favourable conditions. The periods which elapsed between pairing and deposition of the first egg-mass in the case of the second and third individuals in Table III were ten days and sixteen days respectively, and 292 eggs and 184 eggs were laid without further coitus. The total number of eggs laid by each of the other five individuals over the same period was less than this; they had evidently laid a number of egg-masses in the field after pairing and before being brought into the laboratory. The Tables emphasise the marked reduction in the number of eggs in later masses, and the fact that oviposition continues until very shortly before death. The intervals between layings are very variable, and there is a tendency, which may be significant, toward repetition of the same size of egg-masses laid by the same individual.

When the gross number of eggs in each of these Tables is divided by the number of slug-days, an average of 2.7, 2.1 and 2.7 eggs per slug per day is obtained for Tables IIa, IIb and III respectively. If conditions permitted this rate of reproduction to be maintained throughout the year each slug would produce almost 1000 eggs. Taylor (1907) records 776 eggs laid in one season by a pair of A. agrestis, and Miles, Wood and Thomas (1931) state that an estimation of 1000 eggs per functional female has been made. The latter figure would seem rather high, and when reduction of reproductive activity during summer is taken into account it is probable that each individual may lay upwards of 500 eggs in the course of a season.
d) Time of Development.

Previous estimations of the duration of the egg stage of *A. agrestis* are not consistent, due perhaps to the slugs having been studied under very different climatic conditions. Taylor (1907) states that the period is three or four weeks and varies somewhat according to the weather. In France, Bouchard-Chantereaux has given 18 - 30 days and Van Beneden and Windischmann (1941) have given 30 days, figures which are probably correct for a warmer climate. Künknel (1916) finds that the period of incubation in Germany is 27 - 30 days, lengthening to 45 - 50 or even 110 - 120 during cold weather.

When laid, the eggs contain zygotes which develop into the adult form before hatching takes place. The time required for development varies within wide limits according to temperature, a subject which is more fully discussed in the section on *A. agrestis* in relation to its physical environment. In nature, those eggs which are laid during late summer and early autumn, that is, at the commencement of the most intensive breeding period, hatch in the shortest time. During August and September the time of incubation is 22 - 36 days, and as conditions become colder the time lengthens. The shortest period of development of eggs kept under outside conditions was 21 days, from 15 Aug. 1935 to 5 Sep. 1935. Eggs laid early in December may not hatch until the end of March, and mortality becomes greater as the time of development increases. The longest time recorded was 96 days, from 19 Dec. 1935 to 24 Mar. 1936, and in this case only two slugs emerged from a mass of thirteen eggs. During early
winter eggs develop extremely slowly during three months or more and remain for long periods in a state of suspended animation until hatching occurs. A wide variation has been observed in the rate of development of eggs of the same mass kept under the same conditions. It practically never happens that all the eggs of a single mass hatch on the same day, and as the time of development lengthens the difference in the time of hatching increases correspondingly.

c) Hatching.

The adult form is attained before the young slug makes any effort to emerge from the egg. The hepatic lobe is withdrawn and the posterior sac has ceased to function and is reduced to a mere vestige; the mantle is evident, the heart beneath it can be observed contracting strongly at a rate of forty-eight beats per minute, and both pairs of tentacles are fully developed, with prominent pigmented eye-spots on the larger anterior pair. Movement of the embryo becomes more pronounced just before hatching takes place, and use is made of the radula to effect an escape from the tough inner membrane of the egg. As noted by Miles (1924) there is no portion of this membrane weaker than the rest but the slug rasps indiscriminately at any part of it. The action of the radula can be clearly seen in embryos within eggs immersed in water and examined under the microscope. The radula can be protruded well in front of the tentacles (Fig. 4) and it is addressed to the membrane with an upward motion, a steady stroking movement at the rate of thirty-two strokes a minute.
Fig. 4. Fully-developed Agriolimax arrestis,
just before hatching.

(Drawn from nature. x 50).
This causes the membrane to bulge at the point attacked and eventually it ruptures, though great exertion on the part of the slug is often required to accomplish this. The young slug pushes its way out, enlarging the tear as it goes. It has no difficulty in passing through the outer gelatinous coat, and once free it may often be observed to commence feeding upon the remains of the egg, if not upon an intact egg nearby.

f) Growth.

The rate of growth and the time taken to reach maturity after hatching are not easy to estimate with any degree of accuracy under field conditions; in the laboratory they have been found to vary directly with temperature and food-supply. Bouchard-Chantereaux (1937) cites a precocious case in which eggs were laid by a slug 66 days after hatching, although full growth was not attained until 82 days had passed. Lovett and Black (1920) estimate that the adult condition is reached in from 90 days to almost a year, and Fankel (1929) gives $3\frac{1}{2} - 4$ months for A. agrestis and (1916) $5\frac{1}{2} - 9$ months for Arion subfuscus.

Young slugs collected in the field began to oviposit when 2.5 cm. in length, (the measurement is taken from the front of the head between the tentacles to the hind end of the body when the slug is extended moving along a straight line) and no individuals smaller than this were ever observed to produce eggs. The time taken to reach this size is indicated by the following experiments. Slugs which hatched on 25 Oct. 1935 took 131 days to increase to 2.5 cm. and lay eggs when they were kept in a laboratory the temperature of which was approximately 17°C by day
and fell to 10° - 12°C at night. Others from the same egg-mass, exposed outdoors during a winter of continued hard frost, were 1.6 cm. after the same period. Another batch of slugs which hatched on 2 Dec. 1936 was divided into two lots; those of the first lot which were kept at a constant temperature of 15°C were 2.6 cm. and began to reproduce after 118 days, while those of the second, kept outside in less severe weather than prevailed the previous year, were 1 cm. after the elapse of the same time. These periods extend over the coldest part of the year and indicate that maturity is reached in 4 - 6 months after hatching, depending on the season. When full-grown the slug measures 3\(\frac{1}{2}\) - 4 cm.

The duration of life of A. agrestis is stated by Ellis (1926) to be little over twelve months and by Taylor (1907) to be probably under eighteen months in nature, but the exact period is not known. In the many instances in which slugs died during the course of breeding experiments, death was not preceded by any outward sign of senility and oviposition was always found to continue until a few days before death. Künkel (1916) pointed out that death supervenes once egg-laying has been completed.
4. The Egg.

There is considerable variation in the size and shape of the eggs of *A. agrestis*, though those of the same batch are usually uniform. The volume of the egg fluctuates with changes of the surrounding humidity, for the albuminous matter absorbs and gives up moisture readily. To maintain its turgidity the egg must remain in contact with a moist surface; the response to external changes of humidity is direct and immediate, and the egg possesses no compensatory mechanism whereby it can accommodate itself to such changes. The normal egg is 2 mm. long and not much less in width; spherical eggs 1.5 mm. in diameter commonly occur; a few normal and many abnormal eggs are over 3 mm. long and 2 mm. wide. These dimensions are those of eggs which have become turgid by being kept in contact with moist filter-paper.

The surface is roughly granulose due to the presence of small calcium concretions embedded in the outer gelatinous coat. When sulphuric acid is added to water containing eggs the concretions are replaced by bundles of calcium sulphate crystals and bubbles of carbon dioxide are liberated. At either end the coat is produced as a short tapering thread, and two masses were encountered in which successive eggs were united by these threads to form a string, as is usual in the larger species of *Limax*.

Under water the inner structure of the egg becomes clear when viewed by transmitted light. The gelatinous coat has a faintly striated appearance, as if composed of concentric layers, and the width is about 150μ. Separating the coat from the fine inner membrane is a thin layer of clear jelly which appears to be
homogeneous. It is this jelly and the tough inner membrane which render extraction of the embryo entire and undamaged difficult to accomplish. The inner membrane surrounds a mass of faintly bluish albumen, the medium in which the embryo develops, and it also contains a twisted membranous structure, the remains of the body of the sperm. Foreign inclusions which may be present in the albumen are fungal hyphae and small nematode worms.

Abnormalities. A relatively small proportion of eggs is abnormal but one type of abnormality seems to be of usual occurrence during certain phases of the sexual cycle of the slug. One of these eggs is depicted in Fig. 2. A few abnormal eggs are laid before the normal egg-masses begin to be produced by young slugs which have just reached maturity, and later their production is a sign of senility and approaching death in older individuals. Occasionally slugs which have been kept under favourable conditions of temperature, moisture and food, but for a week or more denied soil or other suitable medium for oviposition have produced a few of these large abnormal eggs before proceeding to lay the large normal egg-masses which result from such treatment. Instead of a single zygote there may be present within a single egg-capsule as many as thirty dark unfertilized (ova) which may attempt incipient division by budding off an elongate process. Segmentation (never) proceeds further than this, and if, as occasionally happens, one or two normal zygotes are present, they do not develop beyond the four-cell or eight-cell stage.

Eggs which contained more than one normal embryo were obtained (on one occasion). Successive batches laid by the same
Fig. 2. Abnormal Egg of Agriolimax agrestis.
(Drawn from nature. x 30).

Fig. 3. Quadri-embryonated Egg of Agriolimax agrestis.
(Drawn from nature. x 30).
individual are listed in Table IV, and the number of embryos per egg and records of their development are given. Fig. 3 represents a quadri-embryonated egg, and any possibility of polyembryony or similar mode of development is ruled out by the fact that without exception the eggs contained the same number of sperm remains as there were embryos, and each zygote was seen to possess polar bodies. It will be noted that eggs containing more embryos took slightly longer to hatch than others of the same mass with fewer embryos. The young slugs which hatched were markedly smaller than normal, and on dissection the parent slug was found to possess a much smaller albumen gland than usual. Simpson (1902) records two embryos occasionally and four once in a single egg-capsule.

Gegenbaur (1851) describes and figures a sort of "Siamese twin" embryo consisting of two individuals united by a common hepatic lobe, but no abnormalities of this nature have been encountered in the course of the present investigation.
### Table IV. Multi-embryonated Eggs Laid by a Single Slug (Agriolimax agrestis)

<table>
<thead>
<tr>
<th>Date of Oviposition</th>
<th>Size of Egg-mass</th>
<th>Number of Embryos</th>
<th>Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 Aug. 1936</td>
<td>16</td>
<td>6 with 1 embryo</td>
<td>5 hatched 27 Aug. 1936</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 died</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 with 2 embryos</td>
<td>6 hatched 27 Aug. 1936</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 with 3 embryos</td>
<td>7 hatched 28 Aug. 1936</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 with 4 embryos</td>
<td>11 hatched 29 Aug. 1936</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 died</td>
</tr>
<tr>
<td></td>
<td>10 Aug. 1936</td>
<td>11 with 1 embryo</td>
<td>All died</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>6 with 2 embryos</td>
<td>All died</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 with 3 embryos</td>
<td>All died</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 with 2 embryos</td>
<td>2 hatched 29 Aug. 1936</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 with 3 embryos</td>
<td>3 hatched 31 Aug. 1936</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 with 4 embryos</td>
<td>3 hatched 31 Aug. 1936</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 died</td>
</tr>
</tbody>
</table>
5. Embryology.

Previous work upon the embryology of Stylommatophora deals chiefly with the development of particular internal systems and not much attention has been devoted to external features. The earliest accounts of Laurent (1835, 1837) and Van Beneden and Windischmann (1838, 1841) of A. agrestis are necessarily superficial, and structures are misinterpreted, yet the figures drawn by the latter authors are remarkably accurate when the limitations of the optical equipment of their day are borne in mind. Gegenbaur (1851) has little to add to the accounts of his predecessors, but Pol (1880) describes and figures in greater detail the development of Limax maximus from gastrula to adult. Simpson's (1902) account of the same species is very similar, and Schmidt (1895) deals with the development of tentacles, foot and mantle in A. agrestis, etc., but does not figure the stages in detail. Segmentation and gastrulation are dealt with in the cell-lineage studies of Kofoid (1895, A. agrestis) and Meisenheimer (1897, L. maximus), and the latter author (1898) describes fully the organogeny of L. maximus.
a) Segmentation and Formation of the Blastocoeloe.

At the moment of oviposition the ovum has not yet undergone the maturation divisions, although the presence of the tail of the sperm in the albumen indicates that the male nucleus has already penetrated the ovum. The metaphase of the first maturation division is the stage reached by ova fixed as they were laid (Fig. 6), and the remaining eggs of the mass were found to be in metaphase and early anaphase of the second maturation division thirty minutes later. Thereafter the male and female nuclei approach and become prominent in the resting stage (Fig. 7), remaining thus for several hours before fusion and the first segmentation division ensue (Fig. 8).

The rapidity of the divisions depends upon temperature, as does the whole course of development which follows. In the laboratory at about 17°C five or six divisions take place during the first twenty-four hours and gastrulation is complete after three days. Eggs of the same mass fall out of step very soon after segmentation begins, for which Kofoid (1893) suggests the explanation that the time of fertilisation has varied. Observation does not support this, however, for eggs of the same mass which produce polar bodies at the same time and undergo the first segmentation division together soon begin to show differences which increase as development proceeds. As is shown later, there is a wide variation in the rate of development of eggs of the same mass kept under the same conditions, a variation which can only be explained by differences in the
Fig. 6. Egg at Time of Laying, in Metaphase of First Maturation Division.

Fig. 7. Male and Female Prometaphase in Resting Stage before Fusion.

Fig. 8. Telophase of First Segmentation Division. (Figs. 6-8. Fixed in corrosive-acetic, stained with borax-carmine. x 480).

Fig. 9a-e. Formation of Temporary Vacuole at 2-cell Stage. (Drawn at intervals from the same living embryo. x 250).
vitality of the eggs.

As the first segmentation division nears completion the first two blastomeres become spherical and all but separate from one another. The point of contact is marked by the bridge of spindle fibres which continues to link the daughter nuclei as they become reconstituted (Fig. 8). There follows a process of close apposition of the daughter cells so that each loses its spherical shape. Fig. 9a-e is a series of drawings, made at intervals, of a living embryo, and the gradual enlargement of a fluid intercellular space between the two cells is represented. The intercellular vacuole reaches a maximum (Fig. 9) and then empties itself fairly rapidly into the surrounding albumen, leaving the cells closely apposed. The process is reminiscent of the expansion and collapse of the Protozoan contractile vacuole, though the collapse is here much more deliberate. At each division the cells undergo the same change of shape accompanied by the formation of similar temporary vacuoles. The early eight-cell stage is shown (Fig. 10) in which all the cells are rounded and the four micromeres fit so closely into the interspaces of the macromeres as to leave little or no space in the centre. The latter is soon formed and the eight cells are then disposed at the periphery of a central spherical vacuole, the primitive blastocoele (Fig. 11). From the sixteen-cell stage onwards the central cleavage cavity is persistent, and becomes now the definitive blastocoele.

Kofoid (1893) has noted the appearance of a cavity and the forcible expulsion of the contents between the first two
Fig. 10. Early 8-cell Stage.  (Whole mount; fixed in corrosive-acetic, stained with borax-carmine. x 480).

Fig. 11. Advanced 8-cell Stage.  (Whole mount; fixed in corrosive-acetic, stained with borax-carmine. x 480).

Fig. 12. 16-cell Stage.  (Whole mount; fixed in corrosive-acetic, stained with Delafield's haematoxylin).
blastomeres of *A. agrestis*, and MacBride (1914, Fig. 263) draws attention to Meisenheimer's description of the intermittent appearance of the blastocele in the Pelecypod *Preissensia*.

As the cells of the early embryos of *A. agrestis* are nourished by the albumen which bathes them, their cytoplasm is constantly flushed in the same manner as the Protozoan cell, and the spherical blastocele originates by expulsion of the fluid into the centre of the cell-mass, which leads to the formation of a single cell-layer on the periphery (Fig. 12).

The early stages in the development of the embryo, prior to gastrulation, are very susceptible to drying and freezing, since these conditions render the albuminous medium which surrounds the embryonic cells more viscous, retard osmotic exchange between embryo and albumen, and prevent formation of the blastocele. As a result the cells form a loose cluster, like a morula, instead of giving rise to a spherical blastula. As it ages the embryo develops an increasing resistance to low temperatures and dessication; once the tissues are differentiated and the internal organs are formed it acquires greater independence of external changes, but so long as the external medium is in direct contact with every cell, changes in the consistency of the former react at once on the ability of the latter to continue normal development.

b) Development of External Features.

Successive stages in the development of *A. agrestis* are not sharply defined. There is no metamorphosis, but rather a gradual assumption of adult features, and the distinctive
larval types which characterise marine Gasteropods tend to become obscured in a form which reaches the adult condition within the shelter of the egg-capsule. Owing to lack of uniformity in the rate of development it is not possible to define progress in terms of time, hence it has been found convenient for purposes of description to choose a series of stages which are clearly recognisable by the development of prominent external features.

Stage I. Blastula (Fig. 13).
Stage II. Advanced Gastrula (Fig. 14).
Stage III. Rudiments of body and mantle appear (Figs. 15a, 15b).
Stage IV. Rudiments of tentacles and posterior sac appear (Figs. 16a, 16b).
Stage V. Anterior and posterior sacs at maxima (Figs. 18a, 18b).
Stage VI. Hepatic mass retracted; posterior sac atrophied; adult form assumed (Fig. 19).

Intrusion of the macromere 3D into the blastocoele to form mesoderm and part of mid-gut takes place during Stage I, while the embryo is still a spherical blastula. There follows the formation of a shallow invagination, the blastopore, the lips of which are gradually withdrawn until a narrow orifice is formed. Coincident with this the spherical form is lost, and two lateral projections, the "velar" projections of Kofoid, appear. In Patella (Patten 1885) gastrulation is effected by inflowing of the macromere 3D followed by 3A, 3B
Figs. 13-19. Series of Successive Stages, I to VI, showing Development of External Features. (Drawn from unmounted embryos, fixed in corrosive-acetic and preserved in alcohol).

Fig. 13.
Stage I. Blastula.
× 460.

Fig. 14.
Stage II. Advanced Gastrula.
× 460.
Fig. 15a. Stage III, ventral view. x 320.

mouth

hepatic mass

external opening of right larval nephridium

mantle rudiment

body rudiment

hepatic mass

Fig. 15b. Stage III, right view. x 320.
and 36. The advanced gastrula, Stage III, is spindle-shaped when viewed from the ventral side, and the small external openings of the larval nephridia can be seen on each side of the blastopore. The spindle shape is soon lost as the cells forming the anterior wall of the archenteron absorb large quantities of albumen and enlarge to form the rounded hepatic lobe. At the same time the rudiment of the body appears in the posterior region and dorsally the first indication of the mantle is seen (Fig. 15b). In the living embryo slight ciliary action may be observed on each side of the stomodaeum and sections show a few short cilia in this region. At this stage movement of the embryo commences; it rotates slowly and continuously inside the egg-capsule.

Further development consists in enlargement of the hepatic lobe and modification of the covering ectoderm as a thin membrane sparsely beset with contractile cells. At the posterior end of the body a bud of similar tissue is the rudiment of the posterior contractile sac. The body has a slight ventral furrow (Figs. 16a, 16b), the sides of which soon coalesce to form the foot. In more primitive Gasteropods such as Patella and Trochus the foot is at first double. At this stage the mantle clearly shows a similar dual formation, more marked posteriorly, which is soon lost. The rudiments of the tentacles have now appeared in the ventral anterior region of the body, and almost from their inception a furrow demarcates the anterior from the posterior tentacles. A ridge along the dorsal side of the mouth gives the opening the shape of a blunt crescent.
Fig. 16a.
Stage IV, right view.
x 200.

Fig. 16b.
Stage IV, ventral view.
x 200.
Stage V is characterised by maximal development of the hepatic lobe anteriorly and of the posterior sac. In the living embryo the enlarged hepatic cells show clearly through the membranous anterior sac, and the path of the larval nephridia which lie between the hepatic cells and the sac is marked at the inner end by crystals of excretory matter (Figs. 34a, 34b). The mantle is larger and sits upon the back of the embryo like a saddle, while a deep cleft on its right side is the future pulmonary orifice. Fig. 17 represents a stage intermediate between Stages IV and V in which subdivision of the posterior tentacles has just commenced. For a time there are three distinct sets of tentacles, as figured already by Schmidt (1875), but eventually fusion of the third pair forms the lower lip. The anterior tentacles show the optic invaginations (Fig. 18a). Posterior to the third tentacles a blunt lobe of the anterior part of the foot covers the entrance to the pedal gland. The embryo is now able to move about inside the egg-capsule by muscular contraction, and the lines of the insertion of the muscles on the body-wall produce the characteristic sculpturing of the adult slug.

The adult form is attained when atrophy of the posterior sac and retraction of the hepatic lobe are complete. While this takes place optic pigment is deposited and also pigment on the head and tentacles, and the first movements of the radula are made preparatory to hatching.

The newly hatched slug (Figs. 5a-c) is 3.8 mm., transparent and faintly pink in colour. Pigment is present in the eye-spots and usually in fine speckles on the mantle, and
Fig. 17.
Stage IV+.  x 100.
posterior tentacles

Fig. 18a.
Stage V, right view.  x 100.
posterior sac
hepatic mass
optic invagination
mantle
tentacle 1
left side
tentacle 2
tentacle 3
pulmonary orifice

Fig. 18b.
Stage V, ventral view.
x 100.
tentacle 1
right side
tentacle 2
tentacle 3
Fig. 19a. Stage VI-, right view.  x 75.

mantle
hepatic mass almost retracted
optic pigment
atrophied posterior sac

Fig. 19b. Stage VI-, anterior view.  x 75.

right anterior tentacle
right posterior tentacle
fused third tentacles
orifice of pedal gland
hepatic mass
Fig. 5a-c.
Young Agriolimax agrestis, just hatched. × 25.
a) dorsal view.
b) right view.
c) dorsal view - retracted.
the dark retractors of the tentacles and head are clearly visible. The surface of the body is sculptured by longitudinal and transverse furrows arranged in a net-like fashion and the mantle surface is covered by indefinite wavy ridges. The fringe is usually rather more strongly lineolate than in the adult slug, the extreme posterior end of the body only is slightly keeled, and the white raised ring which surrounds the pulmonary aperture of the adult is not yet present.

c) Fate of the Blastopore.

Animals higher in the evolutionary scale than the Coelenterata and Platyhelminia possess two openings, mouth and anus, by which the endodermal tube communicates with the exterior. During development they all pass through a Coelenterate-stage, the gastrula, in which a single primitive opening, the blastopore, leads into the endodermal pouch. In different phyla, or even within the single phylum Mollusca, the fate of this blastopore in forming mouth, anus or both is a matter of little agreement.

The annelida (Wolterekck, Polygordina 1903) and primitive Arthropoda (Sedgwick, Peripatus 1865-1822) demonstrate how the slit-like blastopore may become constricted across the middle to form an anterior stomodaeum and a posterior proctodaeum. In Echinodermata (Field, Asterias 1824) the blastopore persists as the anus, and the mouth is a separate invagination of the ectoderm some distance in front of it.

In Mollusca, on the other hand, the balance of evidence goes to prove that the blastopore becomes the mouth and that the anus is a new perforation. The Pelecypod Dreissensia (Meisenheimer, 1901) and the lower Casteropod Patella (Wilson 1904) are similar
in that there is a temporary closure of the blastopore, the mouth appears just where this closure took place, and the anal invagination appears later. In the case of *Viviparus*, Erlanger (1891 to 1896) holds the view that the blastopore persists as the anus and that the stomodaeum forms as a separate ectodermal invagination in front of it. The same explanation is given for *A. agrestis* (Kofoid, 1893), but Heisenheimer (1897) has shown that in *Limax maximus* the blastopore becomes the mouth and the anus is a subsequent development. Simpson (1902) finds a temporary closure of the blastopore in the latter species, the mouth appearing at this spot.

In *A. agrestis* the blastopore becomes transformed directly into the stomodaeum without the intervention of a temporary closure. A close series of embryos has been examined alive, as whole mounts and as sections, and the transition of the blastopore into the mouth-opening is readily followed. The position of the blastopore is never in doubt for it is defined exactly by the nephridial tubes and their openings on either side of it. A series of sagittal sections of Stages II and III is shown in Figs. 20, 23, 24, and 28.

Considerable doubt must rest upon Erlanger's interpretation of *Viviparus*, that the blastopore becomes the anus, a condition found in no other *Mollusca*. It has to be borne in mind that in this viviparous species it is not easy to obtain large numbers of the early stages of development; and once obtained these must be arbitrarily arranged in series by the investigator.
SERIAL SECTIONS. Drawings have been made by means of the Abbé camera lucida. The primary germ-layers are distinguished by differences of shading; ectoderm is dark, endoderm is medium, and mesoderm is lightly shaded. All the material has been fixed with corrosive-acetic mixture, and most of the sections are stained with Ehrlich's haematoxylin.

Fig. 20.
Sagittal Section of Gastrula,
Stage II—. x 600.

Fig. 21. Transverse Section (slightly oblique), Stage II—. x 600.
(Borax-carmine).

Fig. 22.
Horizontal Section,
Stage II.
x 600.
d) Origin of the Mesoderm.

The formation of the mesoderm in Mollusca, especially Gastropods, has been the subject of much controversy. The method by which the middle body-layer is constituted appears to be totally different in different species, but even for the same species opposing views are held.

The Pectinibranch Viviparus viviparus (=Paludina vivipara) is the Gastropod which has received most attention in this connection. Buttelli (1876), before the study of cell-lineage had given an exact nomenclature to blastomeres, supposed that the mesoderm appeared first as two primary mesoderm cells. Erlanger (1891, 1894) made the discovery that gastrulation is followed by proliferation of the endoderm in the form of two ventral coelomic sacs, in other words exactly the condition which obtains in Echinodermata, Chaetognatha, and certain Protochordata (Balanoglossus and Amphioxus), but in no other Mollusca. The sacs lose their cavities and become irregular lateral bands of mesoderm, which divide to form anteriorly the larval kidneys and posteriorly the coelomic organs (pericardium, kidney and gonad) of the adult. Tonniges (1896) found that the mesoderm of Viviparus originated from small cells budded off from the ectoderm, and there ensued a controversy in which neither author yielded. MacBride (1914) accepted Erlanger's account but the matter was taken up recently by Dautert (1929) who agreed with Tonniges and failed to find any evidence of endodermal coelomic sacs. This brought forth a reply from MacBride (1929) and Hernando (1931) who confirm Erlanger's observations and
discredit Dautert on the grounds that vital early stages immediately subsequent to gastrulation had been overlooked. When this happens the solid mesodermal bands only are seen in close contact with the ectoderm, and their previous hollow nature and archenteric connection pass unobserved. Evidence certainly favours the endodermal origin, but it is unfortunate that in this species these stages are obtained from the oviduct, are scarce, and the relative ages of embryos are not known exactly.

In marine and terrestrial Casteropods, as well as in freshwater forms, eggs laid within egg-capsules can be fixed at any required stage. This applies particularly to Agriolimax agrestis, the clear eggs of which can be observed under water and a plentiful series of embryos in close succession can be easily obtained. There is complete agreement with regard to origin of mesoderm in Prosobranchia (Wilson, Patella, 1904; Robert, Trochus, 1902), Opisthobranchia (Carazzi, Aplysia, 1905) and Pulmonata (Wierzejski, Physa, 1905; Wofiod, A. agrestis, 1893; Meisenheimer, L. maximus, 1897). Origin of the mesoderm is traced to macromeres of the fourth quartette, the parent cell of which enters the blastococele prior to gastrulation. The mesoderm is thus essentially endodermal. Two lateral mesodermal bands are formed, similar to those derived from the coelomic sacs of Viviparous, and they appear to have a close connection with the ectoderm which, according to MacBride (1914) has caused Meisenheimer (Limax maximus, 1898 and Dreissensia polymorpha, 1901) and Harms (Unionidae, 1909) to mistake the origin of derivatives of these mesodermal bands and term the resultant
structures ectodermal. Further reference will be made to this question in the following section dealing with larval nephridia.

Origin of the mesoderm in _A. agrestis_ is found to conform to what may be termed the normal method in _Casteropoda_. Fig. 20 represents a sagittal section of a gastrula at a stage when the mother cell of the mesoderm has undergone several divisions. Few mesoderm cells are present in the sagittal plane, wedged between ectoderm and endoderm, and more are present in the lateral regions. The bands which they form (Fig. 21) are not well defined and are more closely in touch with the ectoderm than with the gut.

e) The Larval Nephridia.

The definitive kidneys of _Casteropods_ are not formed until a comparatively late stage of development and are not functional until the adult mode of life is assumed. The temporary excretory organs of the larvae differ widely in different groups, and even within the same group there is disagreement regarding the nature and origin of larval nephridia.

Of marine forms such as the _Aspidobranchs Patella_ and _Trotus_ the only special excretory organs during larval life are groups of external ectodermal cells behind the velum, which fill with waste products and are cast off. The _Tectibranchs Philine aperta_ (Brown, 1936) and _Aplysia punctata_ (Saunders and Poole, 1910) are provided with unicellular primitive kidneys and four-celled secondary kidneys or black excretory bodies, both of which lie close to the anus and are undoubtedly ectodermal in origin.
These types bear no relation to the larval nephridia of freshwater and terrestrial Gastropods. The freshwater Pectinibranch Viviparus viviparus has been extensively studied by Erlanger (1891 to 1896) who ascribes to the larval nephridia a mesodermal origin from the anterior parts of the mesoblastic bands. The inner ends are not open to the haemocoel. Larval nephridia reach their highest development in Pulmonata, more especially amongst terrestrial Stylommatophora, and accounts of their origin and structure differ somewhat. Basommatophora which have been studied include Planorbis (Rabl 1875, 1879), Limnacea (Wolfson, 1890; Erlanger, 1896) and Physa (Wierzejski, 1905). The nephridium of Planorbis is stated to arise from the same large cell as gives rise to the mesoblast; a pair of invaginating ectodermal cells of the velum in Limnacea are given by Wolfson as the ancestors of the nephridia, which Erlanger describes as being composed of a single giant solenocyte; and in Physa the origin is essentially similar to those of Viviparus, namely three cells at the anterior end of the outer mesodermic teloblast.

Larval nephridia were first discovered in Stylommatophora in 1851 by Gegenbaur and O. Schmidt. De Meuron (Helix, 1884) decided upon an ectodermal origin except for the internal end, about which he was uncertain. The inner end of the tube is open and there are vibratile cilia. Fol (L. maximus, 1880) and Jourdain (A. agrestis, 1884) describe paired multicellular tubes which curve over the surface of the hepatic lobe and which arise
Fig. 23. Sagittal Section, Stage II. x 480.

Fig. 24. Sagittal Section, Stage III. x 480.

Fig. 25. Stage III- x 480. Fig. 26. Stage III- x 480.

External Opening of Larval Nephridium cut Longitudinally. Sagittal Section of Mantle.
as ectodermal invaginations between the mouth and the shell gland. In the latter case the internal surface is lined with very fine vibratile cilia and the inner end is open to the haemocoel. Meisenheimer (1898) describes in detail the development and structure of the larval nephridia of Limax maximus. The entire organ is ectodermal and when fully developed shows three histological regions. The greater part of the tube forms a duct to the exterior; internal to this there is a region in which granules of solid excretory matter are deposited in the cells; and the cells at the inner extremity are differentiated as amoebocytes which bear large tufts of cilia. These amoeboid cells are in close contact with the mesoderm and may migrate a considerable distance from the end of the tube, but always maintain a connection by means of extremely fine membranes which are the only barriers between the lumen of the tube and the haemocoel.

The first indications of the larval nephridia of A. agrestis are seen in the early Stage III embryo. A rather oblique transverse section of such an embryo is shown in Fig. 21. Two irregular bands of mesoderm are present lying close under the ectoderm, which is slightly invaginated at two points. These invaginations are further developed when Stage II is fully attained (Fig. 22) and an indefinite line of mesodermal cells is attached to the inner extremity of each. Externally, the orifices of the ectodermal invaginations lie on either side at the level of the blastopore and open toward the posterior (Fig. 14).
In addition to the mesoderm associated with the invaginations a more compact mass of mesoderm is collected between the endoderm and the region of dorsal ectoderm which shortly becomes the mantle (Fig. 22).

That the larval nephridia are not constituted from the lateral bands of mesoderm alone is clearly evident. The rapidity with which they form precludes the possibility of their being entirely ectodermal, for the invaginated pockets are not regions of exceptionally rapid cell-division and multiplication. The mesoderm cells aligned at the inner end of the ectodermal invaginations form tubes composed of a single layer of cells and the lumina of ectodermal and mesodermal tubes become confluent. Thus the larval nephridium is essentially the same as the adult one, an external ectodermal duct leading into a mesodermal excretory tube the cavity of which represents true coelome. The junction of the two tissues is still recognisable in the Stage V embryo (Fig. 33b). The ectoderm forms a duct which is the outer dorsal half of the nephridium; the cells are small, compact and completely filled with cytoplasm (Fig. 33a). The inner mesodermal part is the functional excretory organ. The cells are large, vacuolated, and contain excretory granules (Fig. 33c). They are in close contact with the enlarged cells of the hepatic lobe, round which the nephridium curves ventrally to terminate in the mesoderm just dorsal and internal to the bases of the anterior tentacles. At the inner extremity a number of large mesoderm
Fig. 33a-d. Four Sections in Different Regions of the Same Larval Nephridium of a Stage V Embryo. x 480.

a) Near the external opening under the mantle.

b) At the anterior curve of the tube, where ectoderm meets mesoderm.

c) At the inner extremity.

d) The inner extremity, showing cilia.
cells are grouped loosely round the tube and the long brushes of cilia which they carry fill the lumen (Fig. 33d). These are not true solenocytes for the cilia are borne externally.

In the living embryo, viewed by transmitted light, the nephridial tubes are not discernible until about Stage III. As Stage V is approached the deposition of crystals of excretory matter, probably uric acid, indicate the path of the inner half of the tube (Figs. 34a, 34b). It has been shown (Baldwin and Needham, 1934) that the hepatopancreas of the snail Helix pomatia synthesises uric acid from the ammonia of protein deamination, and the same process probably occurs in A. agrestis in which the sole food of the embryo is protein in the form of albumen. When examined by incident light these crystals are a bright golden-brown colour, in marked contrast to the larger white granules of the internal shell. At first few in number, they increase and are not got rid of as they are formed. By transmitted light the flickering action of the cilia at the inner extremity of the tube can be seen.

f) Derivatives of the Ectoderm.

Apart from the ectodermal invaginations which give rise to the ducts of the larval nephridia, the first organ to be derived from the ectoderm is the shell-gland. In the majority of Mollusca it appears as early as gastrulation commences and takes the form of a dorsal invagination just behind the prototroch. There follows an evagination which brings the shell-secreting surface to the exterior. In
the Pelecypod Dreissensia (Meisenheimer, 1901) the whole process of invagination and evagination is over before the trochosphere stage is reached. In Patella (Fatten, 1885) the same process takes place, but the shell is not laid down until the veliger stage, and the same is true of Viviparus (Erlanger, 1891).

In Pulmonata which possess an internal shell, evagination of the ectodermal rudiment does not take place. Meisenheimer (1897, 1898) describes an invagination in Limax maximus which is present as early as that of Patella and which becomes cut off from the ectoderm to form an internal sac inside which the shell is formed. In A. agrestis no actual invagination takes place, but proliferation of the ectoderm in the region of the mantle during Stage II (Fig. 23) is followed by the appearance of a cavity in this bud so that the Stage III embryo (Fig. 24) has an ectodermal sac lying dorsal to the stomach. Cells at the anterior end of the sac are undergoing active division (Fig. 26) and as yet the dorsal and ventral walls are similar. In Stage IV (Fig. 31) differentiation has taken place and the outer wall is a thin membrane close to the ectoderm, while the inner one is composed of larger cubical cells which secrete granules of calcium carbonate inside the sac.

The external development of the mantle and the foot have already been described. Both of these organs develop internal cavities, lined with ectoderm, which retain permanent communications with the exterior. The pedal gland is first evident between Stages IV and V as a ventral transverse fissure at the anterior end of the foot (Fig. 31). The cleft deepens
Fig. 27.  
Stage III, Transverse Section.  
\( x \times 480 \).  

Fig. 28.  
Stage III,  
Sagittal Section.  
\( x \times 480 \).
Fig. 29.

Stage III+, Horizontal Section. x 480.
Fig. 30.

Figs. 30 & 31. Stage IV+, Sagittal Sections of Same Embryo. x 200.

Fig. 31.
running parallel with the sole of the foot, and eventually it reaches half-way to the hind end of the body. The ectodermal lining develops numerous mucus glands, especially on the ventral side, the clear glassy secretion from which is poured out at the anterior opening to assist the movement of the slug over dry surfaces.

Just before invagination of the pedal gland takes place an ectodermal pocket forms under the mantle fold on the right side toward the posterior end. This is the pallial cavity, the lung of the adult, and in relation with it there develops the adult ureter (Fig. 31). The manner in which the pallial cavity enlarges and the ureter becomes secondarily connected with the true kidney is fully described by Weisenheimer (Limax maximus, 1898) and Heyder (Aron empiricorum, 1909), and A. agrestis has been found to undergo a similar process.

A very modified form of torsion brings the respiratory orifice and the rectum round to the right side of the mantle.

During Stage V, when about three-quarters of the period spent within the egg has been passed, the anterior and posterior contractile sacs attain their fullest development. These are variously referred to as "kopfblase," podocyste, caudal vesicle, and contractile sinus. They are ectodermal and histologically similar. Each consists of an exceedingly thin, transparent membrane, the scattered cells of which have long processes which give them a stellate appearance. Contractile cells are present passing from the anterior sac
Fig. 34a.
The posterior sac is distended with body-fluid.

Fig. 34b.
Contraction of the posterior sac has filled the anterior one.

(Diagrammatic, drawn from life).
to the hepatic lobe and linking anterior and posterior walls of the posterior sac. The latter is hollowed out anteriorly and communicates directly with the haemocoel of the body. At normal temperatures, about 17°C, the posterior sac contracts forcibly nine times per minute, and at each contraction a rush of body-fluid is sent through the spaces of the haemocoel so that the anterior sac fills out (Figs. 34a, 34b). When the temperature is lowered to 5°C or less the number of contractions per minute decreases to four or five and only the tip of the posterior sac contracts. The greatest number of beats which could be induced was fourteen per minute, at 20°C. As Stage VI is approached, degeneration of the posterior sac is comparatively rapid and the anterior sac also breaks down on withdrawal of the hepatic lobe under the mantle.

The central nervous system in Mollusca, as in other animals, is ectodermal in origin. *Limax maximus* has been studied by Henchmann (1890) and Meisenheimer (1898), who have shown that the cerebral ganglia arise from invaginations, while pleural, pedal, visceral, abdominal and buccal ganglia are formed from proliferations of the ectoderm. In *A. agrestis*, which is similar, the cerebral ganglia are the first to appear, but not until late Stage IV, and the cavity of the invagination is quickly obliterated. All the ganglia and commissures of the central nervous system are laid down during Stage V.

The organs of sense are also late in developing. The otocysts are small spherical sacs invaginated by the ectoderm of the sides of the foot at Stage IV, and the optic rudiments
do not appear until Stage V and do not develop pigment until almost Stage VI.

g) The Alimentary Canal.

The transition of the blastopore directly into the stomodaeum has already been noted. Almost as soon as gastrulation commences the first sign of differentiation of endoderm from ectoderm is seen in the formation of large vacuoles in the cells of the former layer (Fig. 23). This becomes more pronounced in the anterior endodermal wall during Stage II (Fig. 22) and in the following stage it gives rise to a large hepatic lobe with enormous vacuolated cells (Fig. 24). At the same time the posterior region of the endoderm forms the stomach which sends out posteriorly a short, almost straight, tube, the rudiment of the intestine. Meisenheimer (1893) derives the intestine of Limax maximus from the ectoderm by means of an invagination which sinks below the surface near the hind border of the mantle, and becomes secondarily fused with the stomach. It is more likely, however, that in all adult Gasteropods the endoderm is represented by oesophagus, stomach, hepatic gland and intestine. The first of these regions is seen as a very narrow tube in a sagittal section of a Stage III embryo (Fig. 23), and the posterior wall of the stomodaeum has formed the radular pouch at this stage. During Stages III and IV the hind end of the gut continues to remain a blind caecum, and active cell-division in this region (Fig. 29) gives rise to the convolutions of the intestine. It is not until Stage V, when the posterior region of the mantle has been
brought over to the right side, that the anal perforation is 
formed.

A stream of albumen is sent down the oesophagus by the 
action of cilia on the longitudinal ridge in the roof of the 
buccal cavity. This structure first appears during late 
Stage III and in its final form consists of a few rows of 
vacuolated cells which bear cilia on their outer edges (Fig. 30). 
The albumen completely fills the hepatic lobe and stomach, 
and can be seen to pass directly into the large vacuoles in 
the cells of the former (Fig. 32). As Stage VI is approached 
the hepatic lobe is withdrawn until it practically fills the 
body, and the intestine forms the five loops which are the 
adult condition.

h) Derivatives of the Mesoderm.

In adult Mollusca the structures which can be traced 
to the middle body-layer are pericardium and heart, kidney and 
gonad, but not the ducts of the two latter organs. No matter 
how the mesoderm may arise in Gasteropods, observers are agreed 
that a stage is reached not long after gastrulation when the 
mesoderm is present in the form of two indefinite mesoblastic 
bands along either side of the endoderm. Thereafter, fusion 
of the posterior parts of these bands in Viviparus to form the 
pericardiac rudiment which subsequently develops into heart, 
kidneys and gonad has been described by Erlanger (1891 to 1896) 
and supported by Drummond (1902). The later stages of the 
development of these organs in Limax maximus are essentially
similar to *Viviparus*, and *A. agrestis* has been found to agree with this, but Meisenheimer (1896) derives the rudiment of the pericardium from the ectoderm, hence ascribing an ectodermal origin to the coelomic organs of other writers. According to this author, proliferation of the ectoderm on the posterior dorsal wall of the mantle fold forms a solid bud, which soon develops a cavity and which is the cardio-renal rudiment.

In *A. agrestis* the lateral bands of mesoderm go in part to form the inner ends of the larval nephridia and the remainder collects on the dorsal side of the body between the endoderm and ectoderm (Fig. 22). Development of the mantle, shell-gland and intestine causes this mass of mesoderm to be wedged between these structures and closely opposed to the posterior wall of the mantle (Figs. 26, 28). It is never lost sight of, it occupies the position in which Meisenheimer found the ectodermal proliferation, and in the advanced Stage IV embryo it develops a lumen which is the pericardiac cavity. It is inconceivable that *Limax maximus* should differ from all other Mollusca in a matter so fundamental as the origin of pericardium and kidney, and it is evident that Meisenheimer's ectodermal bud is really the mass of mesoderm cells derived from the lateral bands of the gastrula.
6. Discussion: Development with relation to Environment,

Immature forms which pursue a mode of life markedly different from the adults into which they develop are no less suitably adapted to the peculiarities of their environment than are the adults to theirs. The Pulmonate Mollusc, the development of which has just been described, passes its embryonic stages within the shelter of an egg-capsule, an environment which is far removed from the ancestral one. A consideration, then, of the special features which fit the developing embryo of A. agrestis for its very specialised habitat, and a comparison of this type with the mode of development in related marine forms, may throw some light on the modifications which have enabled the most recent products of Gasteropod evolution to forsake the original aquatic habitat and colonise a terrestrial one, even for breeding purposes. The necessities which environment imposes upon function may be no less marked in the negative than in the positive direction. The omission or suppression, in the development of the higher Gasteropod, of structures found in more primitive forms are as important as the assumption of new features.

A common larval type, the trochosphere, links the diverse groups included within the phylum Mollusca, with the exception of the Cephalopoda. The distinctive features of this free-swimming larva are the pear-shaped body, the prototroch or pre-oral bands of cilia used in locomotion, the apical sense-organ and tuft of cilia, and the stomodeum opening into the stomach. It is generally the case that primitive types
of larvae are confined to the more primitive members of their class, and this is true of the Gasteropod trochosphere. Only in Patella, Acmea and other closely related species which occur at the foot of the Gasteropod scale, does hatching take place at this early stage of development. The general trend within the Prosobranchia is toward an ever later emergence from the egg, with consequent restriction of free larval life. Trochus emerges as a veliger which has already undergone torsion, and Fissurella actually creeps out of the egg-shell and makes no use of its rather poorly developed velum and prototroch. Finally, Bucinum and allied whelks remain within their brood-capsules until the adult form is attained.

The same trend can be followed in the Opisthobranchia and Pulmonata, but here the starting-point is the veliger and the trochosphere stage is always passed within the egg. The Tectibranchs Aplysia and Philine emerge as free-swimming veligers, and all Pulmonata emerge in the adult form.

In such a case as the last the degree in which larval characters common to more primitive Gasteropods are discernible during ontogeny depends upon the calls which the new embryonic environment makes upon the individual. In terrestrial Pulmonates, as exemplified by A. agrestis, it is evident that the egg-capsule on land has introduced a condition so totally different from the marine habitat of its more primitive relatives, that secondary adaptations tend to obscure any hereditary recapitulation and the contrast between the two types of development is marked. It is a matter of economy
of tissue and energy; structures which are useless in the new environment do not persist but give place to new requirements.

The embryo of *A. agrestis*, from the commencement of development until it finally hatches as a small adult, lives singly, bathed by nutrient albumen, within the limitations and shelter of a capsule which is no larger than is necessary to contain it at the time of hatching. Hence the functions for which its structures are likely to differ from those of free-living Cesterozoe larva are feeding and digestion, respiration and contact with the external world.

The presence of so easily accessible a food supply as the surrounding albumen obviates the necessity for locomotory apparatus to go in search of food. The prototroch and the velum enable marine trochospheres and veligers to progress through the water, and their food consists of minute planktonic organisms which are swept into the stomodaeum as they pass. The development of these ciliary organs in the *A. agrestis* is meagre and the two larval stages are not recognisable. Some small cilia are present on the anterior edge of the stomodaeum in the region of the prototroch of other Mollusca, and some are scattered sparsely over the anterior end of the body, but no sign of the velum ever appears. Albumen is passed up the oesophagus into the stomach and hepatic lobe by means of the special ciliated ridge in the roof of the buccal cavity. Since protein in the form of albumen is the only food material available the cells of the hepatic lobe become differentiated at a very early stage, immediately after gastrulation, and there
forms a much enlarged diverticulum capable of undertaking enough digestion to satisfy the requirements of advanced embryos. And since no solid food is ingested, and there are no solid excreta to be got rid of, there is no necessity for the anal aperture possessed at an early stage by free-swimming Molluscan larvae. As has been noted, the anal perforation of the ectoderm is not established until in an embryo as advanced as Stage V.

On the other hand, fluid excretion must be possible from a very early stage in development, hence the first larval organs to appear are the temporary nephridia. These reach their highest development in land-breeding Pulmonates and they function in two ways. Ciliary action at the inner extremity sends a stream of excretory fluid to the exterior, and the small amount of metabolic end-products which cannot be conveniently voided in solution is stored in the form of concretions in the cells of the nephridial tubes. Guenot (1892) has shown that Limax maximus forms uric acid crystals, and Baldwin and Needham (1934) have demonstrated the synthesis of uric acid from the results of protein catabolism in Helix pomatia. The circumscribed environment of the egg-capsule, in which the embryo lives for so long a period and reaches so advanced a stage of development, must be kept free from the poisonous end-products of metabolism and the large larval nephridia of terrestrial Pulmonates seem to serve a function similar to that of the avian allantois.

The physiological process with which closure within an
egg-capsule on land most seriously interferes respiration, and consequently the most striking adaptation of the embryo of A. agrestis is directed toward this end. The anterior and posterior contractile sacs are derived from the primitive respiratory tissue, the ectoderm. The eggs of A. agrestis show a quick response to changes in the humidity of the environment, and the albumen readily surrenders or absorbs water. In this way small quantities of oxygen in solution can be conveyed to the neighbourhood of the embryo, but there remains the problem of transferring this oxygen sufficiently rapidly to the tissues of a relatively large embryo. The easily permeable membranes of the anterior and posterior sacs carry out this function. At full expansion the posterior sac fills with body-fluid and clear corpuscles can be seen moving in the stream of fluid. The rapid contraction sends a current of oxygenated fluid through the haemocoelic spaces of the body, foot, mantle and head, and fills the space between the hepatic lobe and its covering membrane. This forward and backward movement of the body-fluid is a simple arrangement which effectively flushes all parts of the body, and which at the same time brings the fluid into intimate contact with the cells of the larval nephridia. Thus the waste product of respiration, carbon dioxide, may be passed out with the nephridial fluid, or the contractile sacs may also perform part of this function. It has been shown by Schuurmans (1930) that respiration in the adult A. agrestis diminishes by 43 - 30% when the pallial cavity is filled with paraffin, hence there is a certain amount
of cutaneous respiration. Were the same degree of cutaneous respiration to exist during development it could hardly be sufficient for the needs of the growing embryo, particularly when it is borne in mind that the amount of oxygen available in solution is probably normally very low and there exists no rapid means of conveying it from the general body-surface to internal organs. Degeneration of the posterior sac and larval nephridia, and retraction of the hepatic lobe, are coincident with development of the pallial cavity and definitive kidney.

The calcareous shell is one of the most characteristic features of molluscs. At first cuticular and uncalcified, it becomes strengthened by the deposition of calcium carbonate, a process which is carried out by the mantle. Free-living marine veligers have ready access to the calcium salts of the sea in order to build up their shell of considerable proportions. Atkins and Lebour (1923) have shown that freshwater snails with calcareous shells are limited to alkaline waters and are most numerous at pH 7-8. The conclusion reached by Boycott (1936) with regard to the distribution of freshwater mollusca in Britain is that "the richest places are calcareous rivers, lakes and canals, the poorest rapid streams, mean ponds and mountain lakes". In the case of land mollusca (Boycott, 1934) the most important features of the environment are moisture and lime. While A. agrestis and other Limacidae require a much smaller amount of calcium to form the internal shell, they do require it at a time when they have no access to the soil or natural waters, and the provision which is made for the calcium requirements of
the developing embryo is yet another expression of the means whereby this group of Molluscs has been enabled to forsake the ancestral habitat. The first granules of the shell are laid down in the shell gland during Stage IV, and when hatching occurs the shell covers the lower epithelium of the shell gland. The surface of the new-laid egg is studded with concretions of calcium which effervesce when the egg is placed in sulphuric acid. No quantitative estimation has been made, but if the empty egg-capsule from which a young slug has just emerged is placed in acid, the bubbles of carbon dioxide which are liberated are noticeably fewer. The conclusion is that the outer coat of the egg is the source of calcium supply to the embryo, just as the egg shell furnishes the calcium requirement of the developing bird. The eggs of land snails which develop large spiral shells are provided with an opaque white external coat of calcium carbonate.

The final contrast which the development of A. agrestis affords to that of lower Casteropods concerns the relations between the embryo and the outside world. Trochospheres and veligers which live an active, motile life require sensory receptor mechanisms, hence the former possesses an apical plate with sensory cilia, and the latter has in addition velar cilia which Carter (1926) has shown to be directly controlled by the central nervous system. The eyes of free-living veligers such as Patella (Patten, 1885) and Philine (Brown, 1936) appear at a relatively early stage. The embryo of A. agrestis has no need of such sense-organs during its residence in the egg-capsule, consequently the apical plate and the sensory cilia
Classification of Gasteropoda.

GASTEROPODA.

Prosobranchia.


Trochus. Fissurella.

Pectinibranchia: Viviparus. (=Paludina).

Littorina.

Buccinum.

Opisthobranchia.


Nudibranchia.

Pulmonata.


Ancylus.


Arion.
Summary.

An account is given of the life-history of *Agriolimax agrestis*, Linné, the Grey Field Slug, from material collected in the vicinity of Edinburgh, Scotland. The processes of pairing, oviposition and hatching are described, and data relating to fecundity and growth are given.

The embryology of this species is discussed, and points of interest which emerge include the appearance of a third pair of tentacles which fuse to form the lower border of the mouth, the development of large contractile sacs, the formation of the blastocoele, the direct transition of the blastopore into the mouth, the composite structure of the larval nephridia from mesodermal bands and ectodermal invaginations, and the derivation of the internal shell gland from a proliferation of the ectoderm of the mantle.

The larval structure is discussed in relation to the habitat of the albuminous egg-capsule, and the comparison of structure and function is made between this mode of development and that found among less specialised Cestodopods.
Literature.

   The Hydrogen Ion Concentration of the Soil and of Natural Waters in Relation to the Distribution of Snails.

   Action de la Temperature sur le Coeur et les Sinus Contractiles Embryonnaires des Gasteropodes Pulmones.
   Comptes Rendus de la Societe de Biologie, Tome LXXXIX, pp 788-790.

   Problems of Nitrogen Catabolism in Invertibrates.
   I. The Snail (Helix pomatia).

4. Beneden, P.J. van, 1876.
   Animal Parasites and Mammals.

   Sur le Developpement de la Limace Grise (Limax agrestis Linn.).
Recherches sur l'Embryogenie des Limaces.
Müller's Archiv für Anatomie und Physiologie, 1841, pp 176-195.

Catalogue des Mollusques Terrestres et Fluviales dans le Département du Pas-de-Calais.
Boulogne.

The Habitats of Land Mollusca in Britain.

The Habitats of Freshwater Mollusca in Britain.

Die Entwicklung des Geschlechtsapparates der Stylommatophoren Pulmonaten, nebst Bemerkungen über die Anatomie und Entwicklung einiger anderer Organsysteme.
A Study of a Tectibranch Casteropod Molluse, Philina aperta, (L).

12. Butschli, O., 1876.
Mittheilung über Entwicklungsgeschichte der Paludina vivipara.
Zeitschrift für wissenschaftliche Zoologie, Bd. XXVII, pp 513-521.

L'embriologia dell' Aplysia.
Archivio Italiano di Anatemia e di Embriologia, Vol. IV.

Observations Physiologiques sur les Embryons des Casteropodes Pulmones.
Journal de Physiologie et de Pathologie Generale, Tome XXII, pp 575-586.

15. Carter, G.S., 1926.
On the Nervous Control of the Velar Cilia of the Nudibranch Veliger.

Etudes Physiologiques sur les Gasteropodes Pulmones.
Archives de Biologie, Tome XII, pp 633-740.

17. Deutert, E., 1929.

Die Bildung der Keimblatter von Paludina vivipara.
Zoologische Jahrbucher (Anatomie und Ontogenie),
Bd. L, pp 433-496.

18. Drummond, I.M., 1902.

Notes on the Development of Paludina vivipara with special
Reference to the Urinogenital Organs and Theories of
Gasteropod Torsion.
Quarterly Journal of Microscopical Science,
Vol. XLVI, pp 97-143.


British Snails.
Oxford.


Zur Entwicklung von Paludina vivipara.
Morphologisches Jahrbuch, Bd. XVII, pp 337-379.


Beiträge zur Entwicklungsgeschichte der Gasteropoden, i Thiel,
Mittheilungen aus der Zoologischen Station zu Neapel,
Bemerkungen zur Embryologie der Gasteropoden, I.
Biologisches Zentralblatt, Bd. XIII, pp 7-14.

Bemerkungen zur Embryologie der Gasteropoden, II.
Biologisches Zentralblatt, Bd. XIV, pp 491-494.

Etudes sur le Développement des Gasteropodes Pulmones.
1 partie: Etude du rein larvaire des Basommatophores.
Archives de Biologie, Tome XIV, pp 127-138.

25. Fernando, W., 1931.
The Origin of the Mesoderm in the Gasteropod Viviparus.

The Larva of Asterias vulgaris.
Quarterly Journal of Microscopical Science,
Vol. XXXIV, pp 105-126.

27. Fol, H., 1880.
Sur le Développement des Gasteropodes Pulmones.
Archives de Zoologie Experimentale et Generale,
Tome VIII, pp 103-232.
   The Origin and Evolution of Larval Forms.
   British Association, Glasgow. Address to Section D.

29. Gegenbaur, C., 1851.
   Beiträge zur Entwicklungsgeschichte der Landgastropoden.
   Zeitschrift für wissenschaftliche Zoologie,

30. Goodrich, E.S., 1895.
   On the Coelom, Genital Ducts and Nephridia.
   Quarterly Journal of Microscopical Science,

31. Hanitsch, R., 1838.
   Contributions to the Anatomy and Histology of
   Limax agrestis.
   Proceedings of the Liverpool Biological Society,

32. Hawley, 1922.
   Cornell Agricultural Experiment Station, Memoir 55.

   The Conjugation of Ariolimax californicus.
34. Henchmann, A.P., 1890.

The Origin and Development of the Central Nervous System in Limax maximus.


Zur Entwicklung der Lungenhohle bei Arion.
Zeitschrift fur wissenschaftliche Zoologie, Bd. XCIII, pp 90-156.

36. Jourdain, M.S., 1884.

Sur les Organes Segmentaires et le Podocyste des Limaciens.
Compte Rendu de l'Academie des Sciences, Tome XCVIII, pp 308-310.

37. Jourdain, M.S., 1884.

Sur le Developpement du Tube Digestif des Limaciens.
Compte Rendu de l'Academie des Sciences, Tome XCVIII, pp 1553-1556.

38. Jourdain, M.S., 1885.


On the Early Development of Limax.


Zur Biologie der Lungenschnecken.
Heidelberg.


Zur Biologie von Pulota fruticum Muller.
Zoologische Jahrbucher (Anatomie und Ontogenie), Bd. XLV, pp 317-342.

42. Kunkel, K., 1929.

Experimentelle Studie uber Vitrina brevis Ferussac.
Zoologische Jahrbucher (Anatomie und Ontogenie), Bd. XLVI, pp 575-630.

43. Laurent, J.J.M., 1835.

Observations sur le Developpement des Oeufs de la Limace Grise et de la Limace Rouge.
44. Laurent, J.L.M., 1837.
Comptes Rendus de l'Academie des Sciences,
Tome IV, pp 295-297.

45. Laurent, J.L.M., 1837.
Faits pour servir a l'Histoire Generale du Developpement des Animaux.
Annales Francois et Etranges d'Anatomie et de Physiologie,
Tome I, pp 17-27.

46. Laurent, J.L.M., 1838.
Faits pour servir a l'Histoire Generale du Developpement des Animaux.
Annales Francois et Etranges d'Anatomie et de Physiologie,
Tome II, pp 133-159.

The Gray Garden Slug, with notes on Allied Forms.
Oregon Agricultural College, Station Bulletin 170.
49. Luther, A., 1915.
Zuchtversuche an Ackerschnecken (Agriolimax reticulatus und Agriolimax agrestis).

50. MacBride, E.W., 1914.
London.

The Development of Mesoderm in Gasteropods.

52. Meisenheimer, J., 1897.
Entwicklungsgeschichte von Limax maximus L. I Theil.
Purchung und Keimblatterbildung.
Zeitschrift fur wissenschaftliche Zoologie,
Bd. LXII, pp 415-468.

53. Meisenheimer, J., 1898.
Entwicklungsgeschichte von Limax maximus L. II Theil.
Die Larvenperiode.
Zeitschrift fur wissenschaftliche Zoologie,
Bd. LXIII, pp 573-664.
54. Meisenheimer, J., 1901.
   Entwicklungsgeschichte von Dreissena polymorpha.
   Zeitschrift fur wissenschaftliche Zoologie,
   Bd. LIXIX, pp 1-137.

55. Meuron, P. de, 1884.
   Sur les Organes Renseaux des Embryons d'Helix.
   Comptes Rendus de l'Academie de Paris,
   Tome XCVIII, pp 693-695.

56. Miles, H.W., 1921.
   The Grey Field Slug (Agriolimax agrestis, Linn.).
   Journal of the Ministry of Agriculture,
   Vol. XXVIII, pp 451-455.

57. Miles, H.W., 1924.
   Observations on the Hatching of the Field Slug,
   Agriolimax agrestis, Linn.
   Scottish Naturalist, No. 149, pp 131-154.

   On the Ecology and Control of Slugs.

   Untersuchungen uber die Entwicklung von Paludina vivipara.
   Zeitschrift fur wissenschaftliche Zoologie,
   Bd. LXXX, pp 411-514.
60. Patten, W., 1888.

The Embryology of Patella.

Arbeiten aus dem Zoologischen Institut der Universität Wien,
Bd. VI, pp 149-174.


Publications Fondations Agathon de Potter, Tome I.


Die Ontogenie der Süßwasserpulmonaten.
(Jenaische) Zeitschrift für Naturwissenschaften,
Bd. X, pp 310-393.

63. Rabl, C., 1872.

Ueber die Entwicklung der Tellerschnecke.

64. Robert, A., 1902.

Recherches sur le Developpement des Troques.
Archives de Zoologie Experimentale, Tome X, pp 269-538.

65. Robson, G.C., 1922.

Self-fertilisation in Mollusca.

The Development of Aplysia punctata.

67. Schmidt, F., 1885.

Beiträge zur Kenntniss der Entwicklungs geschichte der Stylommatophoren.
Zoologische Jahrbucher, (Anatomie und Ontogenie), Bd. VIII, pp 318-341.

68. Schmidt, O., 1851.

Über die Entwicklung von Limax agrestis.
Müller's Archiv für Anatomie und Physiologie, 1851, pp 278-290.


Über die Atemung der Schnecken Limax agrestis L. und Helix pomatia L.
Tijdschrift der Nederlandsch Dierkundige Vereeniging, Bd. XVIII, pp 1-43.

70. Sedgwick, A., 1885-1888.

The Development of the Cape Species of Peripatus.
Quarterly Journal of Microscopical Science, Vols. XXV-XXVIII.

The Anatomy and Physiology of Polygyra albolebris and
Limax maximus, and the Embryology of Limax maximus.
Bulletin of New York Museum of Natural History,

72. Simroth, H., 1887.

Über die Genitalentwicklung der Pulmonaten und die
Fortpflanzung des Agriolimax laevis.
Zeitschrift für wissenschaftliche Zoologie,
Bd. XLV, pp 643-663.

73. Taylor, J.W., 1907.

Monograph of the Land and Freshwater Mollusca of the
British Isles. Vol. II.
Leeds.

74. Tomidges, C., 1896.

Die Bildung des Mesoderms bei Paludina vivipara.
Zeitschrift für wissenschaftliche Zoologie,
Bd. LXI, pp 541-605.

75. Wierzejski, A., 1905.

Die Entwicklungsgeschichte von Physa fontinalis.
Zeitschrift für wissenschaftliche Zoologie,
Bd. LXXXIII, pp 502-706.
76. Wilson, E.B., 1904.
Experimental Studies in Germinal Localization.
(2) Experiments on the Cleavage-Sclioic of Patella
and Dentalium.

77. Wolfson, W., 1880.
Embryologie du Hymaneaus stagnalis.
Bulletin de l'Academie Imperiale des Sciences de
St. Petersburg, Tome XXVI, pp 79-98.

78. Woltereck, R., 1903.
Beitrag zur praktischen Analyse der Polygordiuss-
Entwicklung nach dem Nordsee- und dem Mittelmeertypus.
Archiv fur Entwicklungsmechanik der Organismen, Bd. XVIII.
PART II

AN ECOLOGICAL STUDY OF AGRIOLIMAX AGRESTIS L.,
THE GRAY FIELD SLUG, AND RELATED SPECIES.

1. Introduction.

2. Distribution and Habitat.

   a) Soil Reaction.
   b) Soil Moisture.
      (i) The Moisture-holding Capacity of Soils and the Occurrence of Slugs.
      (ii) Oviposition in Relation to Moisture Content of Soil.
   c) Organic Content.

4. Temperature.
   a) Temperature Control of Adult Activity.
      (i) Temperature Range of Adults.
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   b) Temperature Control of Development.
      (i) Development of the Egg at Constant Temperatures.
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1. Introduction.

Up to the present time most of the ecological work carried out on invertebrates which are important agricultural pests has been confined to insects. An increasing volume of precise data is being accumulated, and the principles which underlie the relation between insect pests and their physical, chemical and biotic surroundings are becoming more clear. But whether these entomological studies throw much light upon other invertebrates is a point which has not been established, and constitutes one of the reasons for the present investigation.

In structure and habit the terrestrial molluscs contrast so markedly with insects that some knowledge of the relationship which exists between them and their environment would appear to be of importance, particularly for purposes of comparison with the insect fauna of similar habitats. The former are soft-bodied and unsegmented; their progression is aided by means of a slimy exudation; they are hermaphrodite, and *Agriolimax agrestis*, at least, will breed at any season so long as conditions are suitable; and their sensory receptors, apart from the tactile one, are poorly developed. In all of these respects the land pulmonates have nothing in common with the insects.

The soil habitat is one which has not been by any means thoroughly investigated by the animal ecologist, although it is well understood from the botanical standpoint. Indeed, apart from a number of faunal surveys and species lists appertaining to particular soils, little work has been done even
upon insects in relation to the soil they inhabit. The technique of physical and chemical soil analysis is now well established, and the soil factors which govern plant growth and distribution are understood, so that the time seems ripe for detailed investigation of the soil factors as they affect the occurrence and abundance of the invertebrate fauna.

The species chosen as the subject of the present autecological study is *Agriolimax agrestis*, the Gray Field Slug, and the treatment has been limited by the end in view. The problem has been approached not as an academic study in ecology, but with a view to reaching an understanding of the environmental factors which may have a bearing upon the importance of this species as a pest of crops, in particular the potato crop. *A. agrestis* is a desirable subject for such an investigation, owing to its universal occurrence and great economic significance, and it is a suitable one, due to the ease with which it can be bred and kept alive in the laboratory.

Previous work upon the ecology of land Mollusca is scanty. Atkins and Lebour (1923) have investigated the occurrence of snails (*Hyalina, Helicella, Limnaea,* etc.) in relation to the pH of soils and natural waters, and Boycott (1934) in a comprehensive treatise has brought together the references to the habitats of the land Mollusca of Britain and has classified the 102 species on the bases of moisture and lime in the environment. Bachrach and Cardot (1924) have made the only a study of the development of the terrestrial mollusca under
controlled conditions of temperature, using *Agriolimax agrestis* and *Limnea stagnalis* for this work. Morris (1927) has shown the effect of organic manure in increasing the population of land pulmonates on agricultural land, and Miles, Wood and Thomas (1931) have dealt with the influence of climate, soil and cultivation upon the prevalence of *Agriolimax agrestis*, *Milax sowerbiil* and *Arion subfuscus*.

In the present paper an account is given of laboratory experiments upon the relation of *Agriolimax agrestis* to its physical environment. These are supplemented by field observations in the attempt to assess the practical role of the various factors as they operate under variable conditions of weather and soil to determine the status of the species in nature.
2. Distribution and Habitat.

This investigation was carried out in the Lothians of Scotland. The area covered extends from the South Queensferry district in the west to Dunbar in the east, and is bounded by the Firth of Forth in the north and approximately by the 500 foot contour in the south. The land is almost all cultivated and the chief crop is potatoes.

Geologically, the Lothians consist of carboniferous rocks, with sandstone and limestone predominating and glacial deposits frequent. A band of calciferous sandstone surrounds Edinburgh and reappears again on the coast immediately east of Dunbar. Due south of Edinburgh it is broken by the basaltic and andesite lavas of the Braid Hills and the Pentland Hills, and there are massive intrusions of basalt around South Queensferry. Coal measures occur in the Esk valley, and the mining district of Dalkeith is not included in the present investigation. The intensive market-gardening district around Musselburgh, situated on the marine alluvial deposits at the estuary of the Esk, has also been omitted. The carboniferous limestone series appears in the Haddington-North Berwick-Dunbar area and is interrupted by an extensive igneous intrusion which lies between the two first-named centres. Upper old red sandstone extends from Dunbar in a southward direction until it meets the Silurian rocks of the Southern Uplands. In general, the soils of the coastal belt below 250' where most of the potatoes of the district are grown consist of reddish or greyish, heavy, clay loams, which grade into lighter, more sandy loams as the
altitude increases to the south. West of North Berwick there is a coastal strip of light soil on which early varieties are cultivated, otherwise maincrop potatoes are grown.

Owing to the purpose of this investigation slugs were not investigated in a complete range of habitats, but attention was directed chiefly to cultivated land, and more particularly to potato fields and to hay or oat stubble and fallow ground to be followed by a crop of potatoes. The species which occurred commonly in these situations were Agriolimax agrestis, Arion circumspectus, Arion subfuscus, Arion hortensis and Arion minimus. Of these only the first two were present in epidemic proportions. Insofar as qualitative distribution is concerned, all except the second of these species were found to be well distributed throughout the Lothians. A. circumspectus, on the other hand, was markedly local in occurrence, as indicated in the accompanying map (Fig. 1).

The general characteristics of the habitats frequented by slugs are well known. It would be difficult to imagine a terrestrial invertebrate less restricted in its choice of locus than A. agrestis. Of this species Ellis writes (1926): "It is the commonest and most ubiquitous of our land molluscs, living in every possible situation, in woods, fields, gardens, pastures, downs, moors, marshes, heaths, in dry or moist places indifferently, but is especially plentiful on arable land."

It has been encountered in every conceivable situation on farm land the Lothians, except when temporary conditions of drought brought about periods of seclusion. It occurs indiscriminately on pasture and on ploughed land, regardless of the crop, and
DISTRIBUTION OF SLUGS IN POTATO FIELDS IN THE LOTHIANS 1934-1936

250 FOOT CONTOUR
500 FOOT CONTOUR

F. A. AGRESTIS
A. CIRCUMSCRIPTUS
A. SUBFUSCUS
A. HORTENSIS
A. MINIMUS

EACH SOLID SYMBOL INDICATES 6 SLUGS PER 50 YD.
CLEAR SYMBOLS INDICATE PROBABLE ABSENCE

C.5 SLUGS PER 500 YD. 

DAMAGE TO POTATOES BY SLUGS IN THE LOTHIANS 1935

04.; -.-; 16; T.VT; BERS PER 100 DAMAGED BY A. AGRESTIS
DAMAGE ABSENT OR NEGLIGIBLE

Fig. 1

Fig. 2.
its presence can be taken for granted wherever plant food is available, though even that is not essential. Where *Arian circumscriptus* was abundant it showed a tendency to occur gregariously on ground not covered by dense vegetation. Favoured situations were in the open field at the roots of potato plants, or under stones or dead leaves lying upon bare soil, but grassy banks were not much frequented and its occurrence on pasture was rare.

The qualitative distribution of the slug species bears a relation to the factors of the environment. Accurate estimations of slug populations have not been attempted for it is difficult to visualise a method of computing the numbers of these nocturnal animals which would be sufficiently reliable and at the same time make only moderate demands upon time. An experiment carried out on a humid evening early in March when slugs were abroad in numbers serves to illustrate their relative abundance in different situations. Copper sulphate powder was thickly dusted by hand over small areas. It is very unlikely that any slugs were able to escape after the dusting, for many were still found adhering to the blades of grass by the copious slime which they exuded as they died.
<table>
<thead>
<tr>
<th>FIELD</th>
<th>AVERAGE NUMBER PER SQUARE YARD</th>
<th>AVERAGE NUMBER PER ACRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Bare soil with scattered curving blades of grass; turnips during previous season.</td>
<td>6.8 A. agrestis.</td>
<td>32,912 slugs.</td>
</tr>
<tr>
<td>2. Grass meadow.</td>
<td>12.0 A. agrestis.</td>
<td>67,760 slugs.</td>
</tr>
<tr>
<td>3. Oat stubble with catchcrop, and masses of stable manure.</td>
<td>15.0 A. agrestis.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 A. subfuscus.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 A. hortensis.</td>
<td>79,360 slugs.</td>
</tr>
<tr>
<td></td>
<td>30.5 Eggs of A. agrestis.</td>
<td>147,620 eggs.</td>
</tr>
<tr>
<td>4. Barley stubble with catchcrop.</td>
<td>38.0 A. agrestis.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5 A. subfuscus.</td>
<td>186,340 slugs.</td>
</tr>
<tr>
<td>5. Thick Italian rye-grass (Lolium perenne), 6&quot; high, in a sheltered, damp situation.</td>
<td>104.0 A. agrestis.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.5 A. subfuscus.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.5 A. minimus.</td>
<td>542,080 slugs.</td>
</tr>
</tbody>
</table>
The last formidable total refers to a strip of rye-grass growing in the shelter of a wall on the site of a recent potato-pit, and represents extremely favourable conditions which would not obtain over a larger area. The oat and barley stubble-fields, harbouring respectively populations of about 80,000 adult slugs plus 150,000 eggs, and 180,000 adults per acre, can be taken as indicative of severe infestations. The quantitative distribution of A. agrestis is here seen to be governed by shelter and the available food. The rye-grass provided succulent feeding and its dense growth and protection from wind would mean that the atmospheric humidity was higher there than in the open field. The leguminous-grass catchcrops of the stubble-fields were more luscious in their growth than the older grass of pasture-land and sustained correspondingly heavier populations. The distribution of breeding activity is interesting, for at this time of year it is confined to the oat stubble strewn with stable manure. Egg-masses occurred either in the manure or on the soil directly beneath it. Morris (1927) has demonstrated the effect of farmyard manure in increasing the numbers and species of the soil fauna, and Miles, Wood and Thomas (1931) have shown that there is a close relationship between organic manuring and the slug population.

It will be noted from Table I that A. circumscriptus does not occur, for it was an uncommon species on the farm in question. This species is more retiring in its habits and appears less on open ground or foliage than other slugs. The greater part of
its feeding takes place underground, and even in a single field it is liable to be very unevenly distributed, hence the above method would give little indication of the numbers present. Counts which were made during September in a potato field which suffered severe damage from a mixed population of slugs yielded the figures:

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>AVERAGE NUMBER PER SQUARE YARD</th>
<th>AVERAGE NUMBER PER ACRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. circumscriptus</td>
<td>23.0</td>
<td>135,520</td>
</tr>
<tr>
<td>A. agrestis</td>
<td>10.5</td>
<td>50,820</td>
</tr>
<tr>
<td>A. subfuscus</td>
<td>5.0</td>
<td>24,200</td>
</tr>
<tr>
<td>Eggs of A. agrestis</td>
<td>43.5 adults.</td>
<td>210,540 adults.</td>
</tr>
</tbody>
</table>

This population of slugs was associated with one of the heaviest losses recorded. More than fifty per cent of the tubers were affected and most of these were unfit for consumption.

The contrast in distribution between A. agrestis and A. circumscriptus can probably be explained on the basis of differences in their feeding habits. The former owes its wide range largely to the fact that it will subsist upon any plant food, although when given the choice it shows a preference for fresh growing tissue. A. circumscriptus has also a wide range of food substances but it is somewhat restricted in its manner of feeding; it prefers to feed
underground or at least in well sheltered situations. It is also a much less active species than A. agrestis, and whereas the latter reacts at once to stimulation, A. circumscriptus is very sluggish in its responses. This fact, together with the gregarious tendencies which have already been pointed out, are no doubt contributory factors to its more local occurrence.

require a large amount of calcium to enable them to grow well, and the hiracinae and orsiniids still possess a terminal calcium carbonate shell. Also the eggs of slugs are either covered with a thin shell of calcium carbonate or possess concretions resting in the egg sac, and the cephalic body slugs created by A. circumscriptus is silvery white with granules of the same substance. It might reasonably be suggested that the relative constancy of the soil, or what expresses essentially the same fact, the soil reaction, would be one of the most effective limiting factors of the occurrence and abundance of slugs.

Cenoheliologists are well aware of the presence of various species and individuals in acid pastures and the contrast compared with chalk country and alkaline terrains. Therefore, Labour (1933) have shown that while the overgrowth in soils which are neutral or somewhat alkaline for example the classified the 103 British land soils into groups of moulds (1 species), calicola (2, 39 species), cephalic body slugs (1, 10 species) and those which are introduced or new at all species. In the last group are placed all the slugs which grow commonly on arable land in the benthos, and these only because with the qualification that if the moulds a species or introduced in a way they would look more hopefully in acid pastures than chalk grass.

a) Soil Reaction.

Slugs are soft-bodied molluscs and are the highest product of evolution in the class to which they belong, the Gastropoda. Less specialised members of this class which occur in marine and freshwater habitats, as well as many land snails, require a large amount of calcium to build up their large shells, and the Limacidae and Arionidae still possess an internal calcareous shell. Also the eggs of slugs are either coated with a thin shell of calcium carbonate or possess concretions embedded in the outer coat, and the copious body slime exuded by A. agrestis on irritation is milky white with granules of the same substance. Hence it might reasonably be supposed that the calcium content of the soil, or what expresses essentially the same fact, the soil reaction, would be one of the most effective limiting factors of the occurrence and abundance of slugs.

Conchologists are well aware of the paucity of molluscan species and individuals in acid peat moors and oak woodlands as compared with chalk country and alkaline beech-woods. Atkins and Lebour (1923) have shown that snails are most abundant in soils which are neutral or somewhat alkaline and Boycott (1934) has classified the 102 British land Mollusca in terms of calcifuge (1 species), calcicole (c. 20 species), those which prefer lime (c. 18 species) and those which are indifferent to lime (c. 45 species). In the last group are placed all five slugs which occur commonly on arable land in the Lothians, and about them Boycott adds the qualification that if he wanted a number of specimens of them he would look more hopefully in calcareous places than elsewhere.
Boycott's method of testing soil acidity in the field is to add some 20% HCl to the soil, which causes a perceptible fizzle if the earth contains 0.5% calcium carbonate, that being the level at which soil begins to be calcareous (from the snail's point of view). In the present survey an accurate determination of pH, such as could be obtained electrometrically, was not essential; but it was thought necessary to record the values with fair accuracy in order to distinguish clearly between the more acid soils, hence the colorimetric method with buffer solutions was employed. Samples of soil were taken in potato fields during August to November, 1935, and were brought to the laboratory in glass bottles. After being air-dried at about 18°C and ground in a mortar, 20gms. soil were added to 30ccs. distilled water and shaken at intervals for a day. When the sediment had settled the pH of the clear supernatant fluid was taken, use being made of a comparator and Clark and Lubs' buffer solutions (Fisher, 1921).

The results are given in Table II, in which the farms are listed commencing at South Queensferry and finishing at Dunbar. They are also shown graphically in Fig. 3. The soils of the area are for the most part somewhat acid, and range from pH 4.5 to pH 7.1. Eight of the forty-one samples have a pH value of 6.0 or less, and only six are neutral or alkaline. The fact that these observations refer throughout to the same habitat, namely potato fields in which cultivation had been similar and conditions of food and shelter were the same, validates the comparison of soil factors. It has to be stated, however, that the estimations of the numbers of slugs present had necessarily to be made under very different conditions of weather
and are therefore subject to a wide experimental error. The damage to the crop, stated in terms of the percentage of tubers damaged, is a more reliable index of the infestations, though here again the practice indulged in by a few growers of lifting the crop early in the season to avoid attack makes comparison difficult.

It is evident that there is little correlation between soil acidity and the slug population or the degree of damage which occurs. In the case of the six heaviest infestations, referred to as 'epidemic', the pH values of the soils ranged from 5.4 to 6.8, and in those fields in which slugs were very scarce or even absent the pH range was found to be 4.8 to 7.0. Nor does there seem to be any distinction between the two common field slugs in this respect, for infestations in which _A. agrestis_ predominated occurred in soils with a pH value of 6.9 (fields nos. 5 and 30); _A. circumscriptus_ was the dominant species present in epidemic proportions in soils with pH values of 6.6 (field no. 22) and 5.8 (field no. 3); and heavy infestations comprising both species occurred at pH 6.2 and pH 5.4. If anything, there is an indication that _A. circumscriptus_ is better able to tolerate the more acid soils, but it is not well defined.
### Table II. Hydrogen Ion Concentration and Water-holding Capacity of Soils compared with Slug Population and Damage.

<table>
<thead>
<tr>
<th>FARM</th>
<th>FIELD</th>
<th>SOIL pH</th>
<th>% WATER AT STICKY POINT</th>
<th>SLUGS</th>
<th>DAMAGE</th>
<th>VARIETY OF POTATO</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>7.1</td>
<td>23.64</td>
<td>present</td>
<td>slight (crop lifted early)</td>
<td>Great Scot</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>5.8</td>
<td>23.46</td>
<td>epidemic</td>
<td>very</td>
<td>Kerr's Pink</td>
</tr>
<tr>
<td>&quot;</td>
<td>3</td>
<td>5.4</td>
<td>21.47</td>
<td>present</td>
<td>(barley stubble, not yet manured)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>5.4</td>
<td>26.23</td>
<td>epidemic</td>
<td>very</td>
<td>Great Scot and Kerr's Pink</td>
</tr>
<tr>
<td>IV</td>
<td>5</td>
<td>6.9</td>
<td>24.00</td>
<td>epidemic</td>
<td>very</td>
<td>Great Scot</td>
</tr>
<tr>
<td>&quot;</td>
<td>6</td>
<td>6.8</td>
<td>23.49</td>
<td>numerous</td>
<td>slight</td>
<td>Kerr's Pink</td>
</tr>
<tr>
<td>V</td>
<td>7</td>
<td>6.2</td>
<td>24.56</td>
<td>epidemic</td>
<td>considerable</td>
<td>Great Scot</td>
</tr>
<tr>
<td>&quot;</td>
<td>8</td>
<td>6.1</td>
<td>21.22</td>
<td>present</td>
<td>nil</td>
<td>Great Scot</td>
</tr>
<tr>
<td>&quot;</td>
<td>9</td>
<td>4.8</td>
<td>15.35</td>
<td>not found</td>
<td>nil</td>
<td>Kerr's Pink</td>
</tr>
<tr>
<td>VII</td>
<td>10</td>
<td>6.0</td>
<td>24.56</td>
<td>not found</td>
<td>not known</td>
<td>not known</td>
</tr>
<tr>
<td>VIII</td>
<td>11</td>
<td>6.1</td>
<td>20.21</td>
<td>present</td>
<td>nil</td>
<td>King Edward</td>
</tr>
<tr>
<td>IX</td>
<td>12</td>
<td>6.0</td>
<td>16.76</td>
<td>not found</td>
<td>slight</td>
<td>not known</td>
</tr>
<tr>
<td>FARM</td>
<td>FIELD</td>
<td>SOIL pH</td>
<td>% WATER AT STICKY POINT</td>
<td>SLUGS</td>
<td>DAMAGE</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>-------</td>
<td>---------</td>
<td>-------------------------</td>
<td>-------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>13</td>
<td>6.0</td>
<td>23.11</td>
<td>present</td>
<td>slight</td>
<td></td>
</tr>
<tr>
<td>XI</td>
<td>14</td>
<td>6.8</td>
<td>13.39</td>
<td>present</td>
<td>slight</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>15</td>
<td>6.3</td>
<td>16.38</td>
<td>present</td>
<td>slight</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>16</td>
<td>6.5</td>
<td>15.49</td>
<td>not found</td>
<td>nil</td>
<td></td>
</tr>
<tr>
<td>XII</td>
<td>17</td>
<td>6.4</td>
<td>14.38</td>
<td>not found</td>
<td>nil</td>
<td></td>
</tr>
<tr>
<td>XIII</td>
<td>18</td>
<td>6.1</td>
<td>17.28</td>
<td>not found</td>
<td>not known</td>
<td></td>
</tr>
<tr>
<td>XIV</td>
<td>19</td>
<td>6.1</td>
<td>11.82</td>
<td>not found</td>
<td>nil</td>
<td></td>
</tr>
<tr>
<td>XV</td>
<td>20</td>
<td>6.7</td>
<td>21.22</td>
<td>present</td>
<td>slight</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>21</td>
<td>6.4</td>
<td>19.99</td>
<td>numerous</td>
<td>slight</td>
<td></td>
</tr>
<tr>
<td>XVI</td>
<td>22</td>
<td>6.6</td>
<td>27.84</td>
<td>epidemic</td>
<td>very</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>23</td>
<td>6.9</td>
<td>25.29</td>
<td>numerous</td>
<td>considerable</td>
<td></td>
</tr>
<tr>
<td>XVII</td>
<td>24</td>
<td>6.2</td>
<td>17.70</td>
<td>present</td>
<td>slight</td>
<td></td>
</tr>
<tr>
<td>XVIII</td>
<td>25</td>
<td>5.8</td>
<td>20.30</td>
<td>numerous</td>
<td>considerable</td>
<td></td>
</tr>
<tr>
<td>XIX</td>
<td>26</td>
<td>6.3</td>
<td>21.32</td>
<td>present</td>
<td>not known</td>
<td></td>
</tr>
<tr>
<td>XX</td>
<td>27</td>
<td>6.6</td>
<td>27.84</td>
<td>numerous</td>
<td>considerable</td>
<td></td>
</tr>
<tr>
<td>XXI</td>
<td>28</td>
<td>6.4</td>
<td>25.50</td>
<td>numerous</td>
<td>considerable</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>29</td>
<td>6.2</td>
<td>23.21</td>
<td>present</td>
<td>(wheat stubble)</td>
<td></td>
</tr>
</tbody>
</table>

**VARIETY OF POTATO**

- Great Scot
- Kerr's Pink
- Arran Banner
- Majestic
- Golden Wonder
- King Edward
- Epicure
- King Edward
- King Edward
- King Edward
- King Edward
- King Edward
- King Edward
- King Edward
- Great Scot
- not known
- King Edward
- King Edward
- Golden Wonder
Fig. 3. Hydrogen Ion Concentration and Water-holding Capacity of Soils compared with Slug Population & Damage to Crop.
b) Soil Moisture.

Slugs and their eggs possess no (compensating) mechanism for changes in the humidity of their surroundings. The former frequent moist, shady places and retire into seclusion in order to avoid excessive loss of water during periods of drought. The latter become dry and are destroyed unless they can maintain contact with a moist surface. A. agrestis has been found to consist of just over 80% water and the eggs contain 85%. Arion empiricorum, according to Dahr (1927) contains 89% water. The obvious importance of this factor led to a study of the moisture-holding capacity of soils in the Lothians and to experimental investigation into the effects of moisture content of soils upon the breeding activities of slugs.

(i) The Moisture-holding Capacity of Soils and the Occurrence of Slugs.

The soil samples which were used for pH determinations were also used to estimate moisture-holding capacity. The air-dried soil, ground and passed through a sieve, was moistened with water and moulded between the palms of the hands until the sticky point was reached. It was weighed, dried overnight at 60°C, allowed to cool and weighed until constant. The percentage of water required to bring the soil to the sticky point was then calculated, and the results are shown in Table II and Fig. 5.

The close correlation between this soil factor, and the population of slugs and damage done is apparent. The lighter and more sandy soils harbour few slugs and those soils with a high percentage of clay and consequent high moisture-holding capacity
support the most severe infestations. The amount of moisture which the soils held at sticky point varied from 11.83% to 30.07%. The soils of six fields in which the populations of slugs were estimated to be greatest and damage was most severe gave an average of 25% moisture at sticky point and a range of 23.46% to 27.84%; and the nine cases in which damage was negligible and slugs very scarce showed an average of 17% and varied from 11.83% to 24.56%. Thus, practices which tend to increase the moisture-holding capacity of the soil will at the same time render it capable of sustaining an increased population of slugs.

(ii) Oviposition in Relation to Moisture Content of Soil.

It has been noted that the eggs of A. agrestis become dessicated when the surface on which they rest is no longer moist. Water in the soil is present in three forms (Cameron, 1913). Hygroscopic moisture condenses on the surface of the particles of air-dry soil, and falls so far short of the requirements of land pulmonates and their eggs as to be of no importance here. Capillary water is held against the action of gravity by surface tension, and when the amount of water increases to such an extent as to overcome surface tension the excess is termed gravitational water. It is evident that the physical nature of the soil determines the amount of gravitational water which can be held. Light, sandy soils, with large particles and a relatively small amount of pore space, allow the gravitational water to percolate quickly through them, but heavier clay soils, with fine particles and a large pore space, retain a large quantity of water. Egg-masses were most abundant in the heavy soils and were laid usually in the surface three inches of the soil, but they were
not encountered in the lighter, more sandy soil which were apt to dry out quickly on the surface during even a short period of drought.

The following experiments were carried out to determine the soil-water content suitable for oviposition. Individuals of *A. agrestis* were kept for two weeks in a container with plenty of food but no soil; they were then ready to oviposit whenever a suitable medium was available. They were placed in batches of six in closed glass vessels containing equal amounts of the same soil but with different water contents. The wettest soil was fully saturated, i.e. all the pore spaces were filled with water but there was no supernatant fluid. The soil in the other four dishes respectively was 75%, 50%, 25% and 10% saturated, and the record of oviposition which followed is given in Table III.

Table III. Oviposition by *Agriolimax agrestis* in Soils of Variable Water Content.

<table>
<thead>
<tr>
<th></th>
<th>100% saturation</th>
<th>75% saturation</th>
<th>50% saturation</th>
<th>25% saturation</th>
<th>10% saturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st. day</td>
<td>-</td>
<td>22 eggs on surface.</td>
<td>26 eggs 1&quot; deep.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2nd. day</td>
<td>-</td>
<td>35 eggs on surface.</td>
<td>59 eggs 1½&quot; deep.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3rd. day</td>
<td>34 eggs on surface.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4th. day</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total number</td>
<td>34</td>
<td>107</td>
<td>94</td>
<td>96</td>
<td>0</td>
</tr>
</tbody>
</table>

of eggs.
Oviposition did not occur in soil with a water content as low as 10% saturation, despite the fact that the slugs were ready to lay eggs. After the fourth day the water content of the soil in dish V was increased to 50% saturation and three egg-masses were laid within twelve hours. Approximately the same number of eggs was laid in dishes II, III and IV, between the limits of 25% and 75% saturation, but there were differences in the time and manner of laying. At 50% and 75% saturation oviposition took place at the earliest opportunity, on the first night of the experiment, but at 25% saturation it did not take place until the following night, and in fully saturated soil not until the third night. The depth at which egg-masses were laid varied according to the amount of moisture present; they were placed more deeply as the soil became drier on the surface. Eggs laid in dishes I and IV did not develop and hatch successfully. In the latter soil there was insufficient moisture to keep the eggs turgid and the embryos died during early stages of segmentation. In the saturated soil the eggs were immersed and, while many died at an early stage of development, a few became advanced embryos not far short of the adult condition which is attained at hatching. In other experiments which tested the ability of eggs to develop under water the same results were obtained.

The water content of soils which slugs choose naturally as a retreat and for the purpose of breeding was investigated by placing a narrow tray at a slope with the lower end immersed in water. In this way soil in the tray was flooded at the lower part of their food, and into the newly-hatched where larvae
end and there was a complete gradation to air-dry soil at the other end. Twelve individuals of *A. agrestis* and four of *A. circumspectus* were left in the closed tray overnight, and in the morning four of the former species were resting on the sides of the tray close to the water level and the other twelve slugs, along with two egg-masses of *A. agrestis*, were gathered together in a narrow band of soil which proved to be 64% saturated.

It is evident that a high water content of the soil is essential before oviposition followed by normal development can take place. The limits are approximately 40% and 80% saturation. It appears that flooding is one of the most potent physical factors for the destruction of eggs in nature. The breeding season, which is at its maximum in autumn and early winter, is determined to a large extent by the moisture content of the soil, but suitably moist situations can be found at any time of year and it is unlikely that drought is a factor of much consequence in destroying eggs in the field.

The remarkable statements by Binney (1978), to the effect that eggs of *A. agrestis* and other slugs were completely desiccated for years and were also dried eight consecutive times in a furnace, and yet developed and hatched normally, cannot be believed. The poor development of resistance to drought is a fact which has impressed itself upon all other investigators (who have used this material).

c) Organic Content.

The organic content of the soil affects slugs both directly and indirectly. Undecomposed organic matter forms part of their food, and on this the newly-hatched slugs largely
subsaist. Young slugs of the same egg-mass were separated, some being put into soil with high organic content taken from a potato field, and the rest into moist silver sand. After six weeks the former had grown from the hatching size of 3.8mm. to 6.5mm., and the latter were only 4.2mm.

Russell (1937) has shown that the retention of moisture by similar soils is to a large extent dependent upon the amount of organic matter they contain. The addition of organic matter increases the water-holding capacity of the soil, and therefore makes it a more favourable habitat for slugs (in particular), as well as for most other members of the soil fauna. This fact has been noted by Morris (1937), and Miles, Wood and Thomas (1931) have stressed the importance of large additions of organic manures to the soil as a contributory factor in causing severe infestations of slugs.

The organic content of all the soils dealt with here is high, for it is standard practice in the Lothians to apply twelve to fifteen tons of stable manure per acre during the winter previous to growing potatoes. Since potato fields are the only habitat under consideration, and since all receive this treatment it can be taken that these soils all possess a high percentage of organic matter.
4. Temperature.

Recent years have seen the production of an increasing volume of experimental work upon the effects which temperature, and temperature combined with humidity and other factors, have upon animals. Arthropods, especially insects, have featured conspicuously in this work, but soft-bodied invertebrates such as worms and molluscs are hardly represented. Indeed, the soil fauna as a whole has been rather neglected, due no doubt to the greater ease with which aerial forms may be dealt during experimentation.

The following experiments were carried out in order to form an estimate of the effects of normal weather conditions in this country upon the activities and increase in numbers of Agriolimax agrestis. There are only two stages in the life-history of slugs, the egg and the adult, the latter term being used here to connote external form and not merely sexual maturity. The egg is directly affected by every change of temperature of the soil or other medium in which it is laid, but the adult slug is able (to some extent) to avoid unfavourable temperatures by seeking shade or by descending into the soil. The range of temperature at which adult life is possible has been determined, and also the control exercised by temperature upon oviposition. The development of eggs and the rate of growth of young slugs at various temperatures have been studied.

It has been very evident in these experiments that individual slugs and eggs vary widely in their responses to similar external conditions. The times of development of
individual eggs of a single mass, kept together under exactly the same conditions, show a wide variation, and some adults were able to resist lethal temperatures for much longer than others. The circumstance of conditioning plays a large part in determining the effects of temperature, for a sudden large rise or fall in temperature is usually followed by deleterious effects. Hence rapid changes have been avoided. Slugs intended to be kept at constant temperatures below 15°C were not brought indoors; those which were to be subjected to 20°C or more were kept in the laboratory for at least a week at a day temperature of approximately 18°C; and eggs (for development) at various temperatures were selected from those laid at similar temperatures.

For constant temperatures of 15°C and over electric incubators were used in the laboratory. A temperature of 10°C was obtained by transporting an electric incubator to an outhouse during winter. And temperatures lower than this were provided by cold water or freezing mixtures in a large thermos jar, kept out-of-doors during frosty weather. The slugs or eggs were placed in test tubes surrounded by the freezing mixture, and the temperature, checked daily, did not vary more than plus or minus half-a-degree Centigrade.

a) Temperature Control of Adult Activity.

(i) Temperature Range of Adults.

Extremes of temperature control the seasonal activities of slugs and in all probability limit their geographic
range too, although _A. agrestis_ is a species almost world-wide in its distribution. The data contained in Table IV indicate the effects of a range of temperatures upon full-grown _A. agrestis_ serve to compare with the climatic conditions in computing the effects of weather upon this species.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>27.5°C</td>
<td>No movement, slight signs of activity, died</td>
</tr>
<tr>
<td>25°C</td>
<td>Slight signs of activity, died</td>
</tr>
<tr>
<td>20°C</td>
<td>Slight active, recovery after 22 hours</td>
</tr>
<tr>
<td>10°C</td>
<td>Slight after 48 hours</td>
</tr>
<tr>
<td>1°C</td>
<td>Little movement for 24 hours</td>
</tr>
<tr>
<td>0°C</td>
<td>Little movement for 12 hours</td>
</tr>
<tr>
<td>-4°C</td>
<td>Little movement for 6 hours</td>
</tr>
<tr>
<td>-10°C</td>
<td>Little movement for 3 hours</td>
</tr>
<tr>
<td>-12°C</td>
<td>After 1 hour, 3 active for 3 hours</td>
</tr>
<tr>
<td>-15°C</td>
<td>After 2 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-18°C</td>
<td>After 3 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-20°C</td>
<td>After 4 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-22°C</td>
<td>After 5 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-24°C</td>
<td>After 6 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-26°C</td>
<td>After 7 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-28°C</td>
<td>After 8 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-30°C</td>
<td>After 9 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-32°C</td>
<td>After 10 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-34°C</td>
<td>After 11 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-36°C</td>
<td>After 12 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-38°C</td>
<td>After 13 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-40°C</td>
<td>After 14 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-42°C</td>
<td>After 15 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-44°C</td>
<td>After 16 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-46°C</td>
<td>After 17 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-48°C</td>
<td>After 18 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-50°C</td>
<td>After 19 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-52°C</td>
<td>After 20 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-54°C</td>
<td>After 21 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-56°C</td>
<td>After 22 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-58°C</td>
<td>After 23 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-60°C</td>
<td>After 24 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-62°C</td>
<td>After 25 hours, 3 active for 1 hour</td>
</tr>
<tr>
<td>-64°C</td>
<td>After 26 hours, 3 active for 1 hour</td>
</tr>
</tbody>
</table>

Table IV. Effects of Constant Temperatures upon Agriolimax agrestis.

<table>
<thead>
<tr>
<th>TEMPERATURE</th>
<th>EFFECT ON ACTIVITY</th>
<th>EFFECT ON OVIPosition</th>
</tr>
</thead>
<tbody>
<tr>
<td>35°C</td>
<td>None recovered after 1 hour.</td>
<td>Eggs not laid.</td>
</tr>
<tr>
<td>30°C</td>
<td>After 4 hours, 8 slugs dead out of 10. On one occasion one slug withstood 30°C overnight.</td>
<td>Eggs not laid.</td>
</tr>
<tr>
<td>27.5°C</td>
<td>No movement. After 4 hours, all 10 slugs recovered. After 24 hours, 1 slug dead out of 20.</td>
<td>Eggs not laid.</td>
</tr>
<tr>
<td>25°C</td>
<td>Slight signs of activity; all slugs recovered after 24 hours.</td>
<td>Eggs not laid.</td>
</tr>
<tr>
<td>20°C</td>
<td>Slugs active, suffer no ill effects from prolonged exposure to this temperature.</td>
<td>Eggs laid freely.</td>
</tr>
<tr>
<td>15°C</td>
<td>Ditto.</td>
<td>Ditto.</td>
</tr>
<tr>
<td>10°C</td>
<td>Ditto.</td>
<td>Ditto.</td>
</tr>
<tr>
<td>1°C</td>
<td>Slugs show very little movement, and always recover.</td>
<td>Eggs not laid.</td>
</tr>
<tr>
<td>0°C</td>
<td>After 48 hours, 6 slugs all recovered.</td>
<td>Eggs not laid.</td>
</tr>
<tr>
<td>0-4°C</td>
<td>5 slugs frozen in solid ice for over 48 hours (3 nights) outside during 21-24 Dec. 1935, when temperature remained constantly below -2.8°C and on two nights reached -5.5°C. None recovered when thawed.</td>
<td>Eggs not laid.</td>
</tr>
<tr>
<td>-5°C</td>
<td>After 1 hour, 2 slugs thawed and recovered. Eggs not laid.</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 4. Temperature Zones in Relation to the Activities of *Agriolimax agrestis*.

- **35°C**: Maximum Lethal Temperature.
- **29°C**: Zone of Lethal High Temperatures.
- **26°C**: Higher Zone of Inactivity.
- **0.5°C**: Zone of Effective Temperatures.
- **-3°C**: Threshold of Development.
- **< -5°C**: Lower Zone of Inactivity.
- **< -5°C**: Zone of Lethal Low Temperatures.
- **Minimum Lethal Temperature.**
The most striking features of these results are the inability of *A. agrestis* to exist at a temperature of 30°C or more, and its ability to remain active until the temperature falls almost to freezing-point. The maximum lethal temperature in this invertebrate is represented by the upper limit of effective temperature in such arthropods as *Anthonomus grandis* (Hunter and Pierce, 1912) and *Ixodes ricinus* (MacLeod, 1934). The zone of effective temperatures for *A. agrestis*, extending from 0.5°C to 26°C, is not much more extensive than that of the weevil and tick, which is 13°C to 35°C, but it occurs much lower in the temperature scale. Yet the lethal low temperatures seem to be approximately the same in the two cases and there is not much difference between the mollusc and the insect so far as resistance to freezing is concerned. It has to be emphasised that the temperatures which demarcate these zones are only approximate and cannot be fixed with accuracy to every batch of slugs subjected to similar experiments. A fairly wide fluctuation is to be expected on account of varying viability of individual slugs. Fuller reference will be made to the question of the effects of temperature upon *A. agrestis* in a subsequent section which deals with the relation of outbreaks of slugs to weather conditions.

(ii) Effect of Temperature on Oviposition.

In Table IV it will be noted that oviposition took place at 10-20°C but not outside this range. To determine the temperature zone within which oviposition will occur, a large
Fig. 5. Oviposition of *Agriolimax agrestis* compared with Minimum Night Temperatures at Aberdeen, 5 Dec. 1936 to 23 Jan. 1937.

Arrows and figures indicate date of oviposition and number of eggs laid.
number of slugs was collected and kept in the usual way with fresh food but no soil so that they would be prepared to oviposit when moist soil was accessible. Ten slugs were kept out-of-doors and ten each in vessels in constant temperature cabinets running at 15°C and 20°C. After a week, moist soil was added to the vessels containing the slugs and was sifted each morning and the egg-masses counted. The results are given in Table V, along with the record of maximum and minimum air temperatures taken beside the outdoor vessel. The latter results are also represented graphically in Fig. 5.
Table V. Oviposition Records of *Agriolimax agrestis* at Different Temperatures.
Ten individuals at each temperature.

<table>
<thead>
<tr>
<th>DATE</th>
<th>OUTDOOR, °C.</th>
<th>15°C.</th>
<th>20°C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 Dec. 1936</td>
<td>3.3</td>
<td>142</td>
<td>113</td>
</tr>
<tr>
<td>6</td>
<td>3.3</td>
<td>70</td>
<td>118</td>
</tr>
<tr>
<td>7</td>
<td>0.6</td>
<td>33</td>
<td>66</td>
</tr>
<tr>
<td>8</td>
<td>6.7</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>9</td>
<td>5.6</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>10</td>
<td>7.7</td>
<td>51</td>
<td>35</td>
</tr>
<tr>
<td>11</td>
<td>6.7</td>
<td>5</td>
<td>48</td>
</tr>
<tr>
<td>12</td>
<td>4.4</td>
<td>38</td>
<td>56</td>
</tr>
<tr>
<td>13</td>
<td>3.3</td>
<td>13</td>
<td>29</td>
</tr>
<tr>
<td>14</td>
<td>6.1</td>
<td>38</td>
<td>11</td>
</tr>
<tr>
<td>15</td>
<td>4.4</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>16</td>
<td>5.6</td>
<td>47</td>
<td>32</td>
</tr>
<tr>
<td>17</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>4.4</td>
<td>41</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>6.7</td>
<td>23</td>
<td>18</td>
</tr>
<tr>
<td>20</td>
<td>10.6</td>
<td>24</td>
<td>35</td>
</tr>
<tr>
<td>21</td>
<td>11.7</td>
<td>24</td>
<td>10</td>
</tr>
<tr>
<td>22</td>
<td>8.9</td>
<td>26</td>
<td>8</td>
</tr>
<tr>
<td>23</td>
<td>4.4</td>
<td>5</td>
<td>-</td>
</tr>
</tbody>
</table>

One slug died.
<table>
<thead>
<tr>
<th>DATE</th>
<th>MAX</th>
<th>MIN</th>
<th>EGGS</th>
<th>15°C</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 Dec, 1936</td>
<td>7.7</td>
<td>1.7</td>
<td>16</td>
<td>-</td>
<td>24</td>
</tr>
<tr>
<td>25</td>
<td>10.0</td>
<td>6.1</td>
<td>12</td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>One slug died.</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>7.2</td>
<td>5.0</td>
<td>-</td>
<td>51</td>
<td>20</td>
</tr>
<tr>
<td>27</td>
<td>2.2</td>
<td>0.6</td>
<td>-</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>28</td>
<td>5.0</td>
<td>2.2</td>
<td>-</td>
<td>25</td>
<td>9</td>
</tr>
<tr>
<td>29</td>
<td>4.4</td>
<td>2.2</td>
<td>-</td>
<td>16</td>
<td>57</td>
</tr>
<tr>
<td>30</td>
<td>7.7</td>
<td>2.2</td>
<td>59</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>One slug died.</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>10.0</td>
<td>7.2</td>
<td>43</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>1 Jan, 1937</td>
<td>8.3</td>
<td>4.4</td>
<td>-</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>5.6</td>
<td>0.6</td>
<td>-</td>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>10.0</td>
<td>3.3</td>
<td>21</td>
<td>5</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>10.0</td>
<td>5.0</td>
<td>15</td>
<td>26</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>4.4</td>
<td>2.2</td>
<td>41</td>
<td>Two slugs died.</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>4.4</td>
<td>2.2</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>5.6</td>
<td>0.6</td>
<td>-</td>
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<td>18</td>
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<td>4.4</td>
<td>1.1</td>
<td>6</td>
<td>-</td>
<td>-</td>
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<tr>
<td>9</td>
<td>7.2</td>
<td>2.7</td>
<td>19</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
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<td>7.2</td>
<td>6.7</td>
<td>10</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>11</td>
<td>8.3</td>
<td>6.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>7.7</td>
<td>6.1</td>
<td>21</td>
<td>6</td>
<td>14</td>
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<tr>
<td>13</td>
<td>7.7</td>
<td>0.6</td>
<td>-</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>14</td>
<td>4.4</td>
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<td>-</td>
<td>6</td>
<td>3</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>One slug died.</td>
<td></td>
</tr>
<tr>
<td>DATE</td>
<td>MAX.</td>
<td>MIN.</td>
<td>EGGS.</td>
<td>EGGS.</td>
<td>EGGS.</td>
</tr>
<tr>
<td>----------</td>
<td>------</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>15 JAN. 1937</td>
<td>4.4</td>
<td>0.0</td>
<td>-</td>
<td>29</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>5.6</td>
<td>3.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>17</td>
<td>5.6</td>
<td>1.7</td>
<td>-</td>
<td>5</td>
<td>6</td>
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<tr>
<td>18</td>
<td>4.4</td>
<td>0.0</td>
<td>-</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

One slug died.

19  5.0  0.6  40  -  9
20  2.7  -1.1 -  -  3
21  3.3  0.6  16  8  -
22  3.3  1.1  2   3  -
23  3.3  0.6  -   -  -

641   982   943

The most significant feature of oviposition at sub-zero temperatures is the time of deposition of the first egg mass. The slugs used in this part of the experiment received the same treatment as those kept at 15°C and 20°C, and were ready to oviposit under favorable conditions. They were supplied with suitable soil and the experiment commenced during a period of frosty weather. On the first five nights the minimum temperatures recorded varied from zero to -3.3°C, and the maximum day temperatures were only 6.6°C. Egg masses were laid on the first night on which the minimum temperature remained approximately above freezing-point, at 5.5°C. Therefore, oviposition took place chiefly when the minimum temperature was 0°C or above, although eggs were laid on 19 Jan., 1939.
At the two higher temperatures approximately the same total number of eggs was laid during the fifty days of the experiment. There is a marked inequality in the distribution of eggs throughout the period, for, apart from the larger masses and greater number of eggs which can be expected during the first few days in which the slugs are provided with suitable soil, it is clear that more prolific oviposition was stimulated at first by the higher temperature. At 20°C, 57% of the eggs were laid during the first ten days, as compared with 46% at 15°C, but this rate of oviposition was not maintained and at the end of fifty days the total was slightly lower than in the latter case. Also, the higher temperature was the more quickly lethal one.

The most significant feature of oviposition at outside temperatures is the time of deposition of the first egg-mass. The slugs used in this part of the experiment received the same treatment as those kept at 15°C and 20°C, and were ready to oviposit under favourable conditions. They were supplied with suitable moist soil, and the experiment commenced during a period of frosty weather. On the first five nights the minimum temperatures recorded varied from zero to -3.3°C, and the maximum day temperature was only 6.6°C. Egg-masses were laid on the first night on which the minimum temperature remained appreciably above freezing-point, at 3.3°C. Thereafter, oviposition took place chiefly when the minimum temperature was 2°C or more, although eggs were laid on 19 Jan. 1937 and 21 Jan. 1937 when
0.5°C was recorded. No slugs died during this period under these outside conditions, and the total number of eggs is considerably lower than the totals obtained at 15°C and 20°C. In computing the optimum temperature for oviposition alone account must be taken of the death rate at different temperatures, and it would appear that this optimum is in the region of 10°C.

b) Temperature Control of Development.

(i) Development of the Egg at Constant Temperatures.

The time which elapses between oviposition and hatching, during which development from the zygote to the adult form takes place, varies within wide limits according to temperature. The times of development at constant temperatures are given in Table VI and Fig. 6, and those obtained by Bachrach and Cardot (1934) are included for purposes of comparison. The egg-masses were kept in glass tubes on filter-paper, constant temperatures ranging from 0°C to 25°C were obtained as already described, and the tubes were opened at intervals in order to prevent oxygen deficiency from vitiating the results. The lack of uniformity in the response of similar individuals to the same conditions has already been remarked upon, and maximum, minimum and average times of emergence are given.
Fig. 6. Development of the Eggs of 
*Agriolimax asrestitis* at Constant 
Temperatures.

Solid line: curve of time of development 
at constant temperatures. 
Broken line: curve of day-degrees at 
constant temperatures.
**Table VI. Development of the Eggs of Agriolimax agrestis at Constant Temperatures.**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>0°C</th>
<th>5°C</th>
<th>10°C</th>
<th>15°C</th>
<th>20°C</th>
<th>22°C</th>
<th>23°C</th>
<th>25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum Time of Development in Days</td>
<td>Died in 6</td>
<td>9³</td>
<td>53</td>
<td>25</td>
<td>15</td>
<td>Died in 4</td>
<td>Died in 3</td>
<td>Died in 12</td>
</tr>
<tr>
<td>Maximum do. days</td>
<td>113</td>
<td>55</td>
<td>36</td>
<td>22</td>
<td>22 days</td>
<td>30 days</td>
<td>48 hours</td>
<td></td>
</tr>
<tr>
<td>Average do.</td>
<td>105</td>
<td>54</td>
<td>29</td>
<td>18</td>
<td>22 days</td>
<td>30 days</td>
<td>48 hours</td>
<td></td>
</tr>
<tr>
<td>Day-degrees</td>
<td>525</td>
<td>540</td>
<td>435</td>
<td>360</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortality</td>
<td>100%</td>
<td>0.5%</td>
<td>2.4%</td>
<td>15%</td>
<td>37%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Bachrach and Cardot:

Temperature: 6°C 14°C 17.7°C 21°C 23°C 25°C

Days: 102 48 29 20.5 Failed to hatch Failed to develop

The limiting temperatures, within which normal development is completed, are below 5°C and about 21°C. This range corresponds closely with the range of temperature at which oviposition takes place, the maximum being between 20°C and 25°C and the minimum being 0.5°C. In Bachrach and Cardot's experiments, development took place but hatching failed to occur at 23°C, a temperature which was found during the present investigation to prove fatal in three days.

During development there is a progressive increase in the resistance which the eggs of this slug show to extremes of temperature. When first laid the egg is very susceptible to freezing and to temperatures higher than 21°C. But embryos which have almost completed their development can withstand several days at 0°C and thereafter continue normal development,
a fact which helps to explain why normal winters in this country
effect only slight control of infestations of slugs.

The zones of lethal high and low temperatures on either side
of the temperature range at which normal development and hatching
occur are confined to a few degrees in the case of the egg as of
adult slug. Although certain invertebrates, e.g. *Apis mellifica*
(Firsch, 1925), have been proved able to maintain a more equable
body temperature than the surrounding air or soil, it would appear
that the slug possesses no such powers of regulation but is
directly subject to the temperature of its environment and
therefore temperatures outwith its effective range are rapidly
lethal.

The shortest time of development was 15 days at 20° C, but
in order to determine the optimum temperature for development
alone, the mortality factor has to be taken into account.
Mortality is negligible at 5° C, but there is a rapid increase
to 37° at 20° C, a fact which is due less to the direct effects
of higher temperatures on the embryo than to the encouragement
of parasitic fungi which attack the eggs.

The attempt to find a thermal constant by estimating the
number of 'day-degrees' has been tried without success in the
case of many insects. The number of degrees by which the mean
temperature each day throughout development exceeds that of the
threshold of development is summed, but in only a few cases have
the figures for different temperatures been approximately
constant. In *A. agrestis* the threshold of development can be
taken as zero, in which case a variation is found of 360
day-degrees at 20°C to 540 day-degrees at 10°C. This range is much less marked than that of the majority of insects; to take but one example, the number of day-degrees required for the development of the beetle *Sitodrepa panicea* at 20°C is 1820, a value which is doubled to 3650 for a fall in temperature of only 3°C.

The temperature-velocity curve of development, obtained by plotting the reciprocals of the time factor against the temperatures, shows the direct relation which a rise in temperature bears to the rate of growth. Theoretically a straight line, it has been found in the development of insects to incline less steeply toward the temperature axis at low temperatures approaching the zero of development. This line in the development of *A. agrestis*, Fig. 7, is practically a straight line above 10°C, and at lower temperatures tends to turn parallel with the temperature axis, a tendency rather more pronounced in the figures given by Bachrach and Cardot (1924) than in those of the present investigation. That development is thus regular in its acceleration with increase of temperature is a further indication of the direct control exercised by this factor upon *Agriolimax agrestis*.

(ii) Effects of Temperature on Hatching.

During frosty weather in winter many eggs in the field were found to contain fully-developed embryos which hatched often within a few hours of being brought into the laboratory, or at least within two days. Also, five eggs with similar embryos which had developed at 15°C were frozen for two hours
Fig. 7. Velocity of Development of Eggs of *Agriolimax agrestis*.
at -2°C and all hatched within three hours of their return to 15°C, whereas the remainder of the egg-mass did not commence to hatch until two days subsequently. This would indicate that low temperatures in the region of zero inhibit hatching, which is easily understood when it is remembered that hatching is accompanied by much muscular exertion. At the same time the change from cold to warmer conditions appeared to stimulate hatching, so that the relative temperature may be more important than the actual one, no matter how near the optimum the latter may be. The effects of fluctuating temperatures on hatching, and on development both before and after hatching, are worthy of more detailed investigation.

A mass of 23 eggs, laid on 30 Dec. 1936, developed at constant 20°C until hatching commenced on 8 Jan. 1937. Six eggs were then selected, in which the embryos were making movements of the radulae preparatory to hatching. The further development of these eggs took place outside until 24 Jan. 1937, when those which had not hatched were again placed in the incubator. The daily maximum and minimum outdoor temperatures were recorded, and the relation between hatching and temperature is shown in Fig. 8. Three of the 23 eggs failed to develop. Fourteen hatched on 8th., 9th. and 11th. Jan. 1937, having remained at 20°C since 30 Dec. 1936. Of the remaining six, which were transferred outside on 8 Jan. 1937, none hatched on the first day when the maximum and minimum temperatures were 4.5°C and 3°C respectively. One hatched on 9 Jan. 1937 and one on 11 Jan. 1937. On the latter dates the mean daily temperature was about 7°C, and from 12 Jan. onwards the mean daily temperature was consistently
Fig. 8. Hatching of Eggs of *Aegriolimax agrestis* at Low Temperatures.

Arrows and figures indicate date of hatching and number of slugs which hatched.
lower than 5°C, and no hatching occurred. As soon as the four unhatched eggs were transferred back to the incubator at 20°C the embryos emerged. Between 12 Jan. 1937 and 23 Jan. 1937 the minimum temperature was often below 1°C and on four occasions freezing-point was reached, without any detrimental effects to the fully-developed embryos.

In constant temperature experiments hatching took place at 5°C and this can be regarded as a fairly accurate determination of the minimal hatching temperature. The maximal hatching temperature is of course 21°C.

(iii) Growth at Various Temperatures.

It has already been shown that *Agriolimax agrestis* reaches maturity, as evidenced by the deposition of eggs, when about 2.5 cm. in length. The time taken to attain this size is four to six months according to the season of the year, and, other things being equal, the controlling factor of greatest importance is temperature. Two lots of ten slugs each, which hatched on 2 Dec. 1936, were kept, one at constant 15°C and the other at outside temperatures. On 24 April, 1937, the average sizes were 2.90 cm. and 1.35 cm. respectively. The number of day-degrees at 15°C was 2145, and the corresponding figure in the other case, obtained by averaging daily maximum and minimum temperatures for the period, was 643 day-degrees. In the latter case the average maximum temperature was 6.5°C and average minimum temperature was 2.5°C. A better comparison can be made when the number of day-degrees necessary for growth to the same size is taken in each case. At 15°C, a length of 1.40 cm. was attained
on 25 Jan. 1937, i.e. at 55 days old, and the number of day-degrees was 825 as against 643 at 2.5–6.5°C. The larger requirement at the higher temperature is the converse of the results given by the developing egg, but this single result does not permit any conclusion to be drawn. It is evident, however, that temperature directly controls the growth rate of the slug as of the egg, and that it must be a vital factor in determining the time which the slug takes to reach maturity and commence to oviposit in nature.

but the forces and situations of the wind are of little significance to the slug which is securely attached to the substrate, and can
diately find a suitable shelter in which to continue its activities.
Slugs have been observed on many occasions to continue feeding upon
in fairly open situations on windy nights so long as the air was
neutral. But in a few instances their failure to appear has been
associated with a dry wind, other conditions were in other respects
apparently favourable, although no slugs were observed to emerge.
This suggests that some circumstances in which slugs may be
least influenced upon the amount abundance of slugs may
be negligible. With regard to light, the negative phototaxis
of terrestrial molluscs is well known, and the fact that slugs are
not infrequently seen independent of daily light intensity,
and their daily period of activity, and the significance of these are
usually indicated by rainfall, for the two factors are in close
agreement.
5. Climate and Weather.

The individual effects of temperature, and to some extent of moisture, upon the activities of *A. agrestis* have been studied, and it remains to consider the combined effects under natural conditions of these and the other elements which constitute the weather. Other meteorological factors which may have an effect upon animals are wind, atmospheric pressure and electricity, and light. The first of these is important in one respect as a factor of evaporation, but the force and direction of the wind are of little significance to the slug which is securely attached to the substratum and can usually find a suitable shelter in which to continue its activities. Slugs have been observed on many occasions to continue feeding even in fairly open situations on windy nights so long as the air was humid. But on a few evenings their failure to appear has been associated with a drying wind, when conditions were in other respects apparently favourable, although no data were obtained to substantiate this surmise. Likewise there is no information to indicate the effects of atmospheric pressure and electricity upon slugs, but it is safe to assume that while the comparatively narrow limits within which these factors fluctuate in nature may produce slight temporary effects, their influence upon the seasonal abundance of slugs must be negligible. With regard to light, the negative phototropism of terrestrial molluscs is well known, and the fact that slugs are nocturnal by habit makes them independent of daily light intensity. Dull weather is favourable to slugs and often materially extends their daily period of activity, and the significance of light is usually indicated by rainfall, for the two factors are in close agreement.
That *A. agrestis* responds to comparatively slight changes in atmospheric conditions has been the experience on several occasions. For example, on a November night at 8.30 p.m., no slugs could be found on the surface of a field from which a badly damaged crop of potatoes had recently been taken; then, as a shower of rain decreased somewhat, they appeared with comparative suddenness, and in about ten minutes several slugs could be picked from every square yard of soil. On the other hand, the species has been observed in numbers feeding above ground during a night of heavy rain, although as a general rule it is the case that the period of actual precipitation is not a favourable time to find them. The responses of slugs to detailed changes in meteorological conditions are interesting ecologically, and become important in timing the application of insecticides and the use of traps, but the factors which are of primary importance in determining the distribution of slugs and the intensity of slug populations from season to season are rainfall and temperature.

a) Climatic Range of *Agriolimax agrestis*.

The temperature requirements of *A. agrestis* have been dealt with, and may be briefly summarised. At and below freezing point adult activity ceases, and the minimum lethal temperature, lower than -5°C, is well below normal minimum temperatures in this country. The threshold of development of both eggs and adults is within a degree of freezing-point. At the other end of the temperature range, eggs do not develop at 22°C and are lethal in twelve hours, while adults are inactive above 26°C and do not survive temperatures of 30°C and over. Thus it can be concluded that the
limiting temperatures for successful existence and reproduction are zero and 21 C, and that climatic regions in which the mean annual temperature falls outwith this range do not support *A. agrestis*.

The rainfall requirements are much more difficult to estimate, for this is a factor which cannot satisfactorily be investigated by laboratory experiment. There is some evidence to indicate that one inch per month is the approximate precipitation which provides conditions just moist enough for the activities of *A. agrestis*. During the drought in July, 1935, when the average rainfall recorded at five meteorological stations in the Lothians was 0.88 inches, slugs were forced into retirement. The same was true in May, of that year, when 1.04 inches of rain fell, but during the intervening month of June, when the rainfall was 2.48 inches, *A. agrestis* and other species were active. During winter, when evaporation from the soil is very much less than in warmer months, a lesser precipitation suffices, as witness the month of March, 1935, when only 1.01 inches of rain fell and *A. agrestis* was in evidence on the more mild nights. A mean annual rainfall of 10 inches may be taken as insufficient for *A. agrestis*, although a rather higher figure than this is probably more correct, dependent upon the temperature, the distribution of rain throughout the year, and the duration of the dry season. An annual rainfall of 30 inches has been sufficient to cause the very heavy infestations of slugs during recent years in the Lothians.

The limits of temperature and rainfall which demarcate regions favourable to the development of *A. agrestis* have been marked on the world map (Fig. 9). Where the mean annual temperature is zero there may be a short period of the year during which *A. agrestis* could
live and reproduce, but development of the eggs would be so very slow and hatching so inhibited, not to mention the fact that during the cold season lethal low temperatures must obtain for long periods, that the continued existence of the slug would be extremely precarious. The higher limit of temperature marked on the map is 80°F (26.6°C). This isotherm certainly outlines a part of the world unfavourable to A. agrestis, for liberal allowance is made for the cool season during which the temperature may fall below 22°C and permit reproduction to take place, with, however, a high mortality. And in the hot season, when the temperature reaches 32°C, it must be extremely difficult for the slugs to avoid lethal high temperatures.

The more significant records of the occurrence of A. agrestis near the limits of its geographic range are marked upon the map for comparison with the climatic data. The records for Vistrand, in the north of Norway, 70°N and well within the Arctic Circle, the Gulf of Tenisei (70°50′N) and the Stanovoi Mountains in Siberia, and Greenland are remarkable in that for the greater part of the year the temperature in these regions is below that of the threshold of development of A. agrestis, insofar as experiments with slugs in more southerly latitudes have shown. In Syria, under very dry conditions associated with a mean annual temperature of 70°F (21°C), and in arid regions of Persia and Turkestan, the presence of A. agrestis may be made possible by local differences which provide rather more moisture during the mild winter. Even more remarkable are instances of the occurrence of A. agrestis in Brazil, the West Indies and Zanzibar (the last only 6° south of the Equator), with mean annual temperatures of 80°F (26.6°C), which proved lethal to
Fig. 9. World Distribution of *Agriolimax agrestis* in relation to Climate.
eggs in this country and in which the temperature during the hot season often reaches the zone of lethal high temperatures as determined experimentally. The fact that slugs contrive to avoid the air temperature by seeking a cool retreat does not explain their occurrence where the air temperature is so far above the limit which has been found necessary for normal development, but there may be some truth in the alternative explanation that even so marked a measure of acclimatisation as this has taken place as the slugs gradually colonised new and warmer habitats. It may be the case that slugs in different climatic regions have developed very different responses to temperature, and in this connection it is interesting to note that the temperature of 23°C at which, according to Bachrach and Cardot (1924) the embryos of A. agrestis in France developed fully but failed to hatch, proved lethal within three days in Scotland.

It has not been possible to investigate the exact nature of the occurrence of A. agrestis in those parts of the world which would seem to be quite unsuited to it climatically. Nor has there been time for an exhaustive search of the literature to obtain a more complete record of the distribution of this species throughout the world. It is possible that in some or all of the cases cited above the record is based merely upon the temporary appearance of the slug as the result of transport in commerce, or, as has been indicated, there may exist fundamental differences in the temperature resistance of the same species in different parts of the world. Therefore, for the time being, the matter must rest, until there is an opportunity to investigate it more fully and
perhaps to experiment with material from distant sources.

The climatic regions which are very favourable to *A. agrestis* are less easily decided than the limits of possible existence. Records of severe outbreaks, and of localities where *A. agrestis* is fairly common, are shown in Fig. 9. The former occur in the northern and southern hemispheres along lines approximately equidistant from the isotherms which represent the theoretical extreme limits of temperature for the species. A mean annual temperature of 50°F (10°C) and a rainfall of 25 inches or more per annum represent the optimum conditions, but the greater the seasonal fluctuation of either of these factors the more the conditions recede from the optimum. The Eurasian continent shows clearly the fact that the more equable maritime climate of the west is much more favourable than the continental climate of the east, although the annual means of temperature and rainfall are similar in each case.

b) Weather and the Numbers of Slugs.

Within the climatic range the intensity of numbers of a species fluctuates according as weather conditions approach and depart from the optimum. The climate of Great Britain is near the optimum for *A. agrestis*. Rainfall is nowhere deficient, and the normal annual amount for the Lothians is 27 inches, usually evenly distributed throughout the twelve months, there being no prolonged period of drought. The mean January and July temperatures are 38.5°F (3.7°C) and 58.5°F (14.7°C), a range which approximates to the optimum of about 10°C for the species. The maximum and minimum temperatures recorded each year are never far outwith the extremes which the species can withstand, and are of so short
duration that the slugs are undoubtedly able to avoid their effects by escaping to sheltered situations. During the last decade the maximum temperature recorded in Scotland was 31°C, and in their shady retreats the slugs would not be subject to this temperature, which in any case prevailed for so short a time as to be quite ineffective in control. In the abnormally cold winters at the beginning of 1929 and 1931, -18°C was recorded, but once again the seclusion of the slugs and the temporary nature of the extreme cold make it doubtful whether any degree of control resulted. In this country a winter which is termed severe serves merely to stop the breeding of slugs and check their increase, but effects little or no reduction of the population (which already exists).

When it became evident, from printed records and as the result of verbal enquiry from farmers, that losses due to slugs had varied greatly from year to year during the past decade, a possible correlation was sought between the fluctuation and the changes of weather. In the absence of precise data relating to infestations of slugs and the resultant damage for each year, no useful purpose can be served by a very detailed study of the weather during those seasons.

In the case of many agricultural pests, particularly insect ones, to deduce the effects of weather from any but a detailed study of meteorological data taken daily at the exact scene of infestation, would be to invite criticism. Periods of intense weather conditions such as brilliant sunshine, heavy rain, sudden changes of temperature, thunderstorms, and so on, which are
limited in time and local in extent, become glossed over by
monthly means of temperature, rainfall, light intensity, etc.
They may even pass unrecorded by the meteorological stations of
the district, yet particular insect populations may be profoundly
affected by these phenomena. But the slug pest is in a different
category from the insect one. It is less exposed to and less
dependent upon the elements of the moment, and is affected only by
gross changes of weather occurring over a large area and for a
considerable period of time. Hence the standard weekly and
monthly meteorological data for the area can be used in computing
the effects of weather upon the slugs.

Since most of the potato crop is lifted during September and
October, November has been taken as the starting-point of the
season, and the average monthly rainfall at meteorological
stations in Midlothian and Haddington has been arranged in three
four-month groupings (Table VII). By this arrangement the rainfall
conditions throughout winter, the following spring, and the months
which immediately precede lifting of potatoes can be compared at
a glance with the amount of damage done that season. Monthly
rainfall figures which have a special significance are given, and
notes upon outstanding features of the weather are added from the
reports to be found in the Transactions of the Highland and
Agricultural Society of Scotland. Until the year 1927,
meteorological data are available for the stations Glencorse,
Edinburgh (University), North Berwick and Stobshiels Reservoir;
from 1928 onwards Gorebridge (Deanbank) replaces Glencorse and
Oxenfoord Castle is an additional station.
Table VII indicates the wide variation in the damage done to potatoes by slugs in the Lothians during the period 1925-1935, and presents the relevant weather data in such a manner as to make possible the interpretation of the effects of weather upon the slugs each season.
<table>
<thead>
<tr>
<th>Season</th>
<th>Weather</th>
<th>Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov-Oct</td>
<td>11.28</td>
<td>Considerable damage reported. There is a record of 1 ton potatoes per acre having to be discarded.</td>
</tr>
<tr>
<td>1924-25</td>
<td>Mild Nov. &amp; Dec.</td>
<td>10.84</td>
</tr>
<tr>
<td>1927-28</td>
<td>Cool Dec.</td>
<td>11.60</td>
</tr>
<tr>
<td>1928-29</td>
<td>Dry year.</td>
<td>9.76</td>
</tr>
<tr>
<td>1930-31</td>
<td>Cold Jan.-Mar.</td>
<td>6.98</td>
</tr>
<tr>
<td>1931-32</td>
<td>Very mild Jan.</td>
<td>10.60</td>
</tr>
<tr>
<td>1932-33</td>
<td>Mild, dryest year since 1870.</td>
<td>9.23</td>
</tr>
<tr>
<td>1933-34</td>
<td>Warm year.</td>
<td>10.56</td>
</tr>
<tr>
<td>1934-35</td>
<td>Mild Feb.</td>
<td>7.40</td>
</tr>
</tbody>
</table>

*Figures give rainfall in inches, 'Cold' & 'mild' are used relative to the time of year.
The degree of damage which was reported each autumn can be compared with the weather of the preceding twelve months (November to October):

1925: Mild preceding winter. Drought in June was of too short duration to destroy slugs; monthly rainfall figures April to August were 3.00, 3.67, 0.67, 1.64 and 2.88 inches. Wet September was one of main causes of damage this year.

1926: An average year.

1927: Preceding winter was not severe, and excessive rain in August and September was the chief cause of serious damage.

1928: Although December was severe, any effects upon the population of slugs were nullified by mild January and February and by three moderately wet months, January to March (7.32 inches). Spring attacks followed.

1929: The drought, and the very severe frosts at the beginning of the year, controlled numbers of slugs to such an extent that even a wet August did not result in much damage.

1930: The high total rainfall, but more especially the fact that 50% of rain fell in the three critical months July to September, led to serious outbreaks.

1931: Although very low temperatures and frost prevailed in first three months of the year, July (4.14 inches) and more especially August (6.45 inches) were so wet that serious attacks developed.

1932: 1933: The fact that these years were generally dry, but especially the very dry Augusts (0.42 and 0.80 inches), reduced damage by slugs to a minimum.
1934 &
1935: Rather wet years preceded by mild winters with rain
evenly distributed and consequent absence of a prolonged
dry period in the summer, resulted in heavy losses. In
1935, the dry months March (1.01 inches), May (1.04 inches)
and July (0.88 inches) were separated by wet months
February (1.79 inches), April (2.87 inches), June (2.48
inches) and August (2.64 inches), and thus the dry periods
were too short to exert any appreciable influence upon
the slugs.

Reports of outbreaks of slugs throughout England and Wales
during the same decade are contained in the Government bulletins
"Insect Pests of Crops", and in other publications. The
information given is of a general nature and damage is expressed
relatively, as "above normal", "normal", and "below normal" for
each district to which reference is made. Accordingly in Table
VIII these reports are compared with deviations of the weather
from the normal during that period, weather notes and data being
taken from the weekly and monthly weather reports of the Air
Ministry. In the table unbroken arrows connect reports of severe
local outbreaks with the heavy rainfall recorded in the same
district during that year. Unbroken arrows mark records of much
reduced rainfall associated with losses which were below normal.
It is evident from the table as a whole, as from the previous
table, that damage was severe in wet years and slight in dry
years, but the distribution of rain throughout the year is equally
important as the total rainfall. Such detail has not been
included, however, for the reports refer not only to damage to potatoes in autumn but also to attacks upon seedlings, cereals and vegetable produce throughout the season, and the times of the attacks are not given.
<table>
<thead>
<tr>
<th>Year</th>
<th>Weather Notes for British Isles</th>
<th>Temperature $T$ (deviation from normal)</th>
<th>Precipitation $P$ (normal)</th>
<th>Local Rainfall, in inches</th>
<th>Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>England &amp; Wales</td>
<td></td>
</tr>
<tr>
<td>1925</td>
<td>Normal year; drought in June.</td>
<td>40.1</td>
<td>108</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>1926</td>
<td>Mild year; rainfall average;</td>
<td>41.0</td>
<td>103</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sunny deficient.</td>
<td></td>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>1927</td>
<td>Wet year, with a dull wet</td>
<td>slightly plus</td>
<td>124</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>summer June-September.</td>
<td></td>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>1928</td>
<td>Wet year, but much sunshine</td>
<td>+0.9</td>
<td>113</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>in central &amp; eastern England</td>
<td></td>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>during July-September.</td>
<td></td>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>1929</td>
<td>Sunny; temperature &amp; rainfall</td>
<td>normal</td>
<td>normal, but dry</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>normal but pronounced</td>
<td></td>
<td>Jan.-Sept., &amp;</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>monthly fluctuation.</td>
<td></td>
<td>exceedingly</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>wet Oct.-Dec.</td>
<td></td>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>1930</td>
<td>A wet year; no prolonged</td>
<td>almost</td>
<td>117</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>spells of intense cold.</td>
<td></td>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>1931</td>
<td>Wet &amp; dull; excessive rain</td>
<td>almost</td>
<td>108</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>April-Sept. &amp; no hot periods.</td>
<td></td>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nov. &amp; normal Dec. abnormally</td>
<td></td>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mild. (The year 1931 continued</td>
<td></td>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>the remarkable run of wet</td>
<td></td>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>years which act in 1932).</td>
<td></td>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>1932</td>
<td>Dull year; mild Jan., droughts</td>
<td>49.7</td>
<td>102</td>
<td>Heavy local</td>
<td></td>
</tr>
<tr>
<td></td>
<td>in Feb. &amp; June, wet May &amp; hot Aug.</td>
<td></td>
<td></td>
<td>attacks in E. &amp; W.</td>
<td></td>
</tr>
<tr>
<td>1933</td>
<td>An exceptionally dry year.</td>
<td>+1.0 to +2.1</td>
<td>93</td>
<td>Above normal in</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>midland, Wales,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&amp; South-western.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Elsewhere, normal or</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>below.</td>
<td></td>
</tr>
<tr>
<td>1934</td>
<td>A mild year; droughts &amp; floods</td>
<td>+0.7 to +1.8</td>
<td>96</td>
<td>Below normal in all</td>
<td></td>
</tr>
<tr>
<td></td>
<td>deficient rainfall in England</td>
<td></td>
<td></td>
<td>districts.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&amp; Wales Jan.-Nov. Hot sunny</td>
<td></td>
<td></td>
<td>Exception is</td>
<td></td>
</tr>
</tbody>
</table>
From the information presented in Tables VII and VIII it is possible to formulate the general principles underlying the fluctuations of populations of slugs in response to changes of weather. In this instance, in which slugs are being considered as pests of the potato crop, conditions of food and habitat can be taken as constant from season to season, and weather can be regarded as the only variable. And, of the elements of weather, temperature and rainfall are all-important, for the other factors exercise very little control upon slugs.

First of all there is the obvious fact which has long been realised, that losses due to slugs are always more severe at the end of a wet season, and conversely that damage is much reduced in dry seasons. The high losses during the period 1925 to 1931, with the exception in Scotland of 1929, coincided with a series of years of high total rainfall, and especially of wet summers. Thereafter, in two drier years the damage was reduced considerably, and then two wet seasons resulted in extensive losses once again. But, while the total rainfall indicates the probable extent of the damage, it is a matter of less consequence than the manner in which the rain is distributed throughout the twelve months which precede the lifting of potatoes in October. The rainfall figures given in Table VII indicate how widely the amount of rain which falls during corresponding periods of the year may vary, and this variation bears an interesting relation to the damage. In the seasons 1925-26 and 1926-27 the total rainfall was very nearly the same, being about six inches above the normal for the Lothians in each case. But, at the end of June, 1926, 20.08 inches
of rain had fallen in eight months, whereas in June, 1927, only 14.62 inches fell in the same period. Thus, at that time of year, it appeared in the former season that damage would be very extensive, and in the year 1927 there was every indication at the end of June that damage would not be unduly severe. In actual fact 1926 turned out to be a year of not very serious losses, whereas 1927 was, in the Lothians as throughout Great Britain, a year in which slugs took an exceptionally heavy toll of potatoes. The circumstance which altered the case was the excessive rainfall of August and September, 1927.

In respect of rainfall distribution and damage the season 1927-28 is comparable with 1925-26; 19.00 inches had fallen by the end of June, 1925, and the total amount was fairly evenly distributed among the three four-month periods of the season. The season 1929-30 was similar to 1926-27, for fifty per cent of the rain fell in the period July to October, and August was a very wet month (5.58 inches). Somewhat similar also is the following season, 1930-31, in which 19.87 inches of rain fell during the first eight months, and July (4.14 inches) and August (6.45 inches) were exceedingly wet. Thus it is evident that the amount of rain which falls after June, and especially the amount which falls during August, determine to a large extent the intensity of damage for the season, and the rainfall up to the end of June gives little indication of the severity of attack for the same year.

This generalisation holds good for years in which the total
rainfall is normal or above it, but an exception is provided by the season 1926-29, the driest season of the decade under consideration. Altogether only 22.57 inches of rain fell, and by June 1929, only 12.78 inches were recorded. In addition to the drought there was a period of severe frost during the first two months of the year, the effects of which are discussed below. Despite 4.29 inches of rain in August, 1929, damage that year was negligible. Thus the conclusion stated above must be qualified by the addition that weather conditions in exceptional years may be so adverse during the period November to July that heavy rainfall in August is not enough to enable the population of slugs to recover and cause serious losses six to eight weeks later.

In the warmest part of the year, a month sufficiently dry to curtail the activities of slugs is one in which less than one inch of rain is recorded. The significance of such months in controlling slugs is indicated in Table VII. June, 1925, (0.67 inches) and July, 1935, (0.88 inches) were ineffectual in reducing the number of slugs. In the laboratory young slugs have been kept alive without food for six months, so the short periods of drought which occur in this country cannot be at all effective in the destruction of a slug population already present. But the years 1932 and 1933 illustrate how effective a dry August (0.42 and 0.30 inches respectively) may be in preventing the normal autumnal increase of the pest. In those seasons, although the preceding winter in each case was mild, the rather low rainfall of the first eight months (15.41 and 15.11 inches
respectively), followed by the drought in August, ensured that damage due to slugs was negligible. The effects of a dry August are relative to the rainfall of the months which precede and follow it; if these are very wet the degree of control exercised by the dry month is minimised, but if an average amount of rain falls in July and September, as in the seasons cited, the population of slugs during autumn is materially reduced.

It is a common belief held by farmers and others that a mild winter is conducive to outbreaks of pests during the following season and that a severe winter serves as an effective control. The former is probably true, but so far as Great Britain is concerned there is little proof to substantiate the second part of the statement. In the years 1925 to 1935, 1928-29 and 1930-31 were the most severe winters, and the minimum air temperature recorded was \(-18^\circ\text{C}\). The former was the driest season of the decade and in the latter the summer and autumn were very wet. In 1929 very little damage was done, and in 1931 heavy losses were suffered. In each of these seasons any effects which the winter frosts may have had upon the slugs were obscured by the weather of the following months. A severe winter is no indication that the numbers of slugs will be reduced during the autumn which follows. By itself it accomplishes little, but if it is followed by a dry spring and summer the prolonged period of unfavourable conditions does serve to prevent the increase of the pest.

In the environment of an animal, the factors are of two classes, physico-chemical and biological. The relations of Agriolimax agrestis to environmental factors of the former class have been discussed, and it remains to consider what effects other animal and plant life has upon the slug.

Plant life may be dealt with briefly. The extremely wide range of food plants upon which A. agrestis can subsist has already been noted, and this polyphagous habit ensures that the type of vegetation is never a limiting factor. The abundance of the slugs is affected by the quality of the plant food, for, as shown in Table I, there is a preference for succulent growing tissues, which leads to serious attacks upon seedlings and transplants in spring. In autumn, when active growth of the green aerial parts of plants is much slowed and many die down completely, the underground tuber of the potato is one of the few succulent tissues available. Wherever climatic and soil conditions permit plant growth, the presence of A. agrestis and most related species of slugs can be expected, and in temperate regions the supply of food is usually sufficient to support heavy infestations when other factors permit a large increase of the population of slugs.

The eggs of A. agrestis are subject to parasitism by at least one fungus, which has been identified as Verticillium sp. The fungal spores are present in the soil, they germinate on the surface of the egg, and the hyphae ramify throughout the albumen,
causing the death of the embryo. According to Taylor (1907) Laurent found a minute parasitic fungus present in the egg even before exclusion from the parent slug. The value of Verticillium as a control in nature must be very low, for, although it is prevalent in the laboratory at 20°C and less so at 15°C, it has not often been found to develop upon eggs at outside temperatures. Conditions of temperature in the field during August to November, when most of the eggs are laid, are such as to prohibit successful growth of the fungus.

The animal predators and parasites of slugs form an imposing list but the degree of control which they exercise is a difficult matter to determine. No predator is specifically dependent upon slugs in the way in which the Coleopterus larvae Drillus flavescens and Lamyris noctiluca are supposed to be upon certain Helices. Of domestic stock, pigs, ducks and poultry are useful in keeping down the numbers of slugs. These can only be utilised before the crop is planted, which in the case of the potato crop is five or six months before the attack by slugs develops, hence there is ample time for the field to become repopulated in the interval. Ducks and poultry have been used by farmers in the Lothians in an attempt to clear slugs from the winter stubble, but although many slugs were undoubtedly eaten, it was found that the measure did little to lessen the damage done to the tubers the following autumn.

Slugs form a part of the diet of many wild animals such as the hedgehog, blindworm, toad, rats, voles and mice, but these predators do not particularly frequent plowed land, the molluscs are eaten by chance and are not specifically sought out, and the
usefulness of these natural enemies to the farmer is not significant.

Birds are of much greater service in the destruction of slugs on arable land. The most important are several species of wild duck, the rook, the lapwing, the pheasant and several of the gulls. All except the first-named feed by day and roost at night, which greatly reduces the quantity of slugs they eat; they have been observed to take slugs during wet weather when the latter were very near the surface of the soil, and they also follow the plough and presumably pick up slugs along with the worms and insects which are exposed. A potato field in East Lothian which harboured a dense population of slugs was bordered by a shelter belt of woodland in which there was a rookery. In late winter, before the stubble with its covering of stable manure was ploughed in, the rooks seemed to take a heavy toll of the slugs in this field by turning over the pieces of manure on the underside of which the slugs were to be found unless the weather was too dry or cold. By the autumn there was no apparent difference between this field and others which were less frequented by the birds.

Wild ducks are the most valuable predators of slugs, for their feeding-time in the late evening coincides with the appearance of the slugs above ground. In this connection the experience of a potato-grower whose land lies on the shores of the Firth of Forth is interesting. In pre-war years large numbers of wild duck chose the coastal fields as their evening feeding-grounds and devoured the slugs to such an extent that
damage to the crop was not important. The formation of duck-shooting clubs has reduced tremendously the number of birds and has scared them from the foreshore, so that now duck are seldom seen in the fields. In the opinion of the grower this is a main cause of the greatly increased infestations of slugs which are now to be found on his land.

The interrelations of the soil fauna are as yet imperfectly understood, but it has been established that a number of ground beetles attack slugs and their eggs. A species of Carabus, probably C. violaceus, was induced to prey upon an adult A. agrestis and an egg-mass in the laboratory. It had much trouble in dealing with the copious slime exuded by the former; the eggs were eaten entirely. A small Staphylinid, Aleochara bilineata, attacked and killed a newly-hatched young slug of the same species, but the slime proved too much for it and it did not feed upon its kill. The importance of insect predators in controlling the slug population is difficult to assess. As agents for the destruction of the eggs of slugs they may be of considerable importance, but where slug populations were very intense there seemed to be no corresponding increase on the part of the insect fauna which might prey upon them.

Slugs are known to be the obligatory hosts of the intermediate stages of several trematodes and cestodes, but the control exercised by these parasitic worms is problematical. Recently a protozoan facultative parasite of A. agrestis has been described. Reynolds (1936) has given an account of a change of habit on the part of the ciliate Colpoda steini. It occurred
in large numbers under the mantle of the slug and it is claimed that where the percentage of infection was 20% the number of slugs was reduced by half in two years.

On the whole, natural enemies do not appear to exert a marked influence upon the numbers of slugs. The most important controls are avian predators, and soil-frequenting beetles and their larvae may cause considerable destruction to the egg-masses.

...
Animals are limited both in space and time by climatic conditions, and the relative importance of these as compared with other controlling factors depends upon the habits of particular species. Within their climatic range many species are restricted by the necessity for special forms of food, particular breeding-grounds or nesting-places, associations with other animals, and so on. Further, most animals are subject to the attacks of parasites and predators, increasingly so as they themselves increase in number, and many even suffer from the competition of their own kind when present in unusually large numbers.

But in most groups of animals there exist a few forms which are widespread in their occurrence and diverse in their habits, and which seem to possess no very special requirements that would debar their presence from or limit their numbers within a region. Such species are characterised by a wide distribution and by a marked appearance of success in whatever habitat they penetrate. As a rule they are singularly free from natural enemies, they show a versatility which turns to account new conditions as they are met, and they are prolific breeders. The rat among mammals and the sparrow and starling among birds are examples which illustrate the point. Insects, on the other hand, are a specialised class, leading lives which are confined in a greater or lesser degree by particular needs, although it is probably true that insects which show the most generalised habits are to be
found in the soil. But, of the soil fauna, the majority of the slugs and *Agriolimax* *agrestis* in particular, belong to that class of animals with generalised habits and a lack of special needs, which, when they become pests, always present an exceedingly difficult problem to control. When the natural control of an animal is slender the population remains at a high level and is ever ready to reach epidemic proportions as conditions incline toward the optimum for the species.

Of the non-climatic agencies which affect *A. agrestis* no single one would appear to be of much importance in effecting control. As has been pointed out, this slug subsists upon an almost endless variety of food substances and does not show a marked chemotropism for any of them. Thus the vegetation requirements of the habitat are quite unlimited and suitable food is present wherever climatic conditions are favourable to the slug.

Soil conditions influence the density of the population of slugs, but a very wide range of soils is habitable to them. The factors of greatest importance are the organic content of the soil and the ability of the soil to conserve moisture, the latter being to some extent dependent upon the former. The more these conditions increase the better able is the soil to support a large population of slugs. All agricultural soils are highly organic, and in the cultivation of potatoes especially the soil is enriched by the addition of organic manure.

The natural enemies of slugs appear to be of little account in controlling the pests, and in the study of many severe infestations there has been no evidence of an increase on the part of predators or parasites.
The weather is left as the only agency of real importance in determining the number of slugs present under agricultural conditions at any given time. It has been shown that, of the elements which constitute the weather, rainfall and temperature have the most marked effect upon slugs. An environment of high moisture content is required by the adult and is even more essential to the egg, and in the life-history of the slug there is no resistant stage adapted to withstand dessication. The range of effective temperatures and the optimum temperature for A. agrestis are low in the temperature scale, which fact, along with the high moisture requirement, determines the maximal breeding period as autumn and early winter. But the climatic conditions of this country permit activity and breeding at any time of the year, and only exceptionally, never for prolonged periods, do summer drought or winter frost inhibit these functions. The chief contrasts between the insect and the slug, which make the latter a much more serious pest than most of the former, are three: in the insect the sexes are separate, but the slug is hermaphrodite; the insect gonad undergoes a seasonal cycle, producing ripe products for a very short period only, but the slug is able to reproduce continuously; the range of temperatures and the optimum temperature for the activity of slugs more nearly coincide with weather conditions in Great Britain than do the higher temperatures preferred by most insects.

It has been shown that the abundance of A. agrestis and other slugs, and especially the severity of attack upon potato tubers during autumn, bear a close relation to weather conditions. The factor of temperature is, in this country, relatively
unimportant as a lethal agent, for the high and low temperatures which are lethal to slugs are very temporary in their occurrence. At the most, some egg-masses placed near the surface of the soil are destroyed by winter frost and summer heat, but the adult slugs can always contrive to evade the unfavourable extreme temperature. Precipitation is the factor of primary importance in limiting or favouring the increase of slugs, and the manner in which the rain is distributed throughout the twelve months prior to the lifting of potatoes closely affects the intensity of the population of slugs and the severity of attack upon that crop.

It is thus possible to forecast to some extent the losses which are likely to be suffered in any season, and the importance of this will be made clear in Part III of this thesis when measures of prevention and control are discussed. The problem of foretelling outbreaks of insect pests on the basis of meteorological observations is one which has received a certain amount of attention; it is the outcome of investigations into the effects of weather upon insects in the field and of laboratory experiments upon the reactions of insects to the individual elements of the weather. The bioclimatic study of Smyntamarus viridis, the Lucerne Flea, by MacLagen (1952) is one of the most complete of these investigations, and deals in detail with the responses of populations of this primitive insect to weather and with the geographical significance of the species in relation to the suitability of the climate. Although an increasing volume of information is being made available and the relations between fluctuations of the numbers of pests according to weather changes are becoming better understood, in few cases have the results been such as to enable better crops to be obtained. A notable
exception is the Pale Western Cutworm, *Porosagrotis orthogonia*, which has been shown by Seamans (1923) and others to be plentiful during the season following a dry May and June and less common if the early summer is wet. The control is indirect, operating through the parasites of the caterpillar, for the latter is brought to the surface of the soil by humid conditions; and until then it cannot be reached by its parasites. It has been shown that when there are less than ten days during May and June on which the rainfall is sufficient to bring the cutworms to the surface, an increased infestation can be expected the following season. On the other hand, if there are more than fifteen such days there will be little trouble the following year.

Such an exact correlation between rainfall and outbreaks of slugs cannot be demonstrated, for in this case control is not dependent upon parasites whose period of flight is confined to several weeks during the summer but is effected directly by weather throughout the season. In the case of *Porosagrotis* the rainfall of a short period determines almost entirely the infestation of the subsequent year; in the case of *Agriolimax* the rainfall of a short period is relatively very important, but the weather during other parts of the year may serve to emphasise or vitiate the effects of this period. As has been noted, the critical time is approximately the month of August.

The correlation between seasonal variation in the abundance of slugs and the weather can best be understood by considering first an average season and thereafter observing the conditions which lead to a deviation in either direction. During the ten years represented in Table VII the seasons 1925-26 and 1927-28
were most nearly normal. Though the rainfall was rather high, which would account for damage being rather above normal, it was evenly distributed throughout the three periods of the year. There was a noticeable lack of droughts and of periods of extreme precipitation, as of very cold or warm weather. These were temperate seasons in all respects. The ideal normal year for the Lothians might be taken as one in which a total rainfall of 27 inches is distributed equally among the four-month periods, but no month receives less than 1 inch or more than 4 inches, and winter frosts are not prolonged or summer heat unusually intense.

Seasons in which damage was slight are characterised by a low total rainfall. In 1929 a lengthy period of severe frosts ended in February and there followed five relatively dry months, so that during July, 1929, it was possible to predict that infestations would be slight during September and October. The damage was far below normal that year despite a relatively wet August. During 1931-32 and 1932-33 weather conditions up to the end of July were much nearer the average than during 1928-29, and at that time the intensity of the autumn outbreak depended upon the weather during the next few weeks. In each case drought during August prevented the development of a dense population of slugs, so that toward the end of that month it could be foretold that the attack would not be important.

By contrast, the month of August in the years 1927, 1930 and 1931 was exceptionally wet and severe outbreaks were recorded later in the year. Until the end of July, 1927 was a normal, indeed a rather dry year, but the high rainfall during August
and again in September turned the tide in favour of a heavy attack. The year 1930 was more persistently wet, and in 1931 any good which was done by the severe frosts of the first three months was amnullied by a wet summer, and finally a very wet August ensured the prolific reproduction of the slugs at the beginning of the breeding season. In each of these seasons it was obvious during August that damage would be unusually severe.

Forecasts of the intensity of the infestation during October may be conveniently summarised:

<table>
<thead>
<tr>
<th>Winter</th>
<th>November to June</th>
<th>August</th>
<th>At end of June</th>
<th>Toward end of August</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Severe (60 days frost)</td>
<td>12 inches</td>
<td>3 inches</td>
<td>Very probably slight</td>
<td>Certainly slight</td>
</tr>
<tr>
<td>2. Mild (about 20 frosty days)</td>
<td>12 inches</td>
<td>3 inches</td>
<td>Probably slight</td>
<td>Slight</td>
</tr>
<tr>
<td>3. Severe or mild</td>
<td>13 inches</td>
<td>Under 1 inch</td>
<td>?</td>
<td>Negligible</td>
</tr>
<tr>
<td>4. Normal (30-40 frosty days)</td>
<td>13 inches</td>
<td>2 inches</td>
<td>?</td>
<td>Normal</td>
</tr>
<tr>
<td>5. Normal</td>
<td>13 inches</td>
<td>6 inches</td>
<td>?</td>
<td>Severe</td>
</tr>
</tbody>
</table>

In example 4 a mild or severe winter would tend to increase or minimise the infestation accordingly. In example 5 a mild winter would intensify the infestation but a severe one would have little effect.

In exceptional years it is possible to predict the extent of damage as early as the end of June, but as a rule the forecast cannot be made with much confidence until August is well-advanced. The practical utility of the forecast lies in the fact that the attack by slugs upon the tubers of potatoes is not fully
developed until the end of September and beginning of October. Some growers follow the practice of lifting the crop early, during September, thus wilfully sacrificing it to some extent in an effort to escape the ravages of the slugs. This is done every season regardless of the probability of attack, but it is only justified when it can be shown that serious damage is imminent. The ideal practice is to leave the tubers in the ground until the haulms have withered, but with a prospective risk of losses by slugs, the best return will be got from the crop when the time of lifting is postponed as long as possible, consistent with the degree of infestation. Hence the importance of making a forecast and effecting the best compromise each season.

It is advisable that the foregoing suggestions should be tested over a series of years. Careful forecasts must be made as outlined here, and they must be compared with records of the degree of damage on successive dates throughout each season.

Settled and throughout England one finds during the years 1910 to 1920 were closely connected with rainfall, especially when the precipitation during late summer.

Biological agencies for the control of slugs are negligible, and are not significant.

The practical application of the knowledge of the effects of weather upon infestations of slugs is best seen by a study of forecasting when severe infestations are expected upon the potato crop.
Summary.

The occurrence and abundance of *Agriolimax agrestis* and other field slugs are discussed with relation to environmental factors.

The reaction, the moisture-holding capacity, and the organic content of the soil have been investigated, and the last two have been found important in determining the slug population.

The effects of temperature upon the activities of *A. agrestis* have been studied. Cool conditions are preferred, for the highest effective temperature for adults is 26°C, the range of temperatures at which eggs develop normally is 0°C+ to 21°C, and the optimum temperature is about 10°C.

The theoretical climatic range of *A. agrestis* is compared with records of its occurrence near the limits of this range and with recorded severe infestations throughout the world.

A correlation has been found between weather conditions and the numbers of slugs. Serious outbreaks in the Lothians of Scotland and throughout England and Wales during the years 1925 to 1935 were closely connected with rainfall, especially with the precipitation during late summer.

Biological agencies for the control of slugs are discussed, and are not significant.

The practical application of the knowledge of the effects of weather upon infestations of slugs is indicated by a method of forecasting when severe attacks can be expected upon the potato crop.
Literature.

   The Hydrogen Ion Concentration of the Soil and of Natural
   Waters in Relation to the Distribution of Snails.
   Scientific Proceedings of the Royal Dublin Society,

   Developpement des Limaces et des Limnées a Differentes
   Temperatures.
   Comptes Rendus de la Societe de Biologie,
   Tome XCI, pp 260-262.

   Influence des Refroidissement Brefs et Repetes sur la
   Croissance des Embryons de Mollusques.
   Comptes Rendus de la Societe de Biologie,
   Tome XCI, pp 1017-1019.

   The terrestrial Air-breathing Mollusks.
   Bulletin of the Museum of Comparative Zoology, Harvard,

   The Habitats of Land Mollusca in Britain.
6. Cameron, A.E., 1913.
General Survey of the Insect Fauna of the Soil within a Limited Area near Manchester; a Consideration of the Relationships between Soil Insects and the Physical Conditions of their Habitat.

Studien über die Respiratim der Landpulmonaten.
Acta Universitatis Lundensis, Bd. XXIII, pp 1-20.

Studies on Soil Reaction II. The Colorimetric Determination of the Hydrogen Ion Concentration of Soils and Aqueous Soil Extracts.

The Mexican Cotton-boll Weevil.
United States Department of Agriculture, Bureau of Entomology, Bulletin 114.

An Ecological Study of the "Lucerne Flea" (Smyrnthus viridis, Linn.) - I, II.

The Ecological Significance of Soil Reaction (pH Value) in Relation to Terrestrial Animals.

12. MacLeod, J., 1934.

Ixodes ricinus in Relation to its Physical Environment: the Influence of Climate on Development.
Parasitology, Vol. XXVI, pp 283-305.


On the Ecology and Control of Slugs.


The Insect and Other Invertebrate Fauna of Arable Land at Rothamstead. Part II.


Studies on the Temperature of Individual Insects, with Special Reference to the Honey Bee.

*Colpoda steini*, a Facultative Parasite of the Land Slug, *Agriolimax agrestis*.


Soil Conditions and Plant Growth.

London.


How to Foretell Outbreaks of the Pale Western Cutworm in the Prairie Provinces.

Department of Agriculture, Canada, Entomological Branch, Circular No. 12.


*Bulletin* No. 66.


*Bulletin* No. 99.

Ministry of Agriculture and Fisheries, London.


Air Ministry, Meteorological Office, London.
PART III

THE CONTROL OF SLUGS ATTACKING POTATOES.

1. Introduction.
3. The Slugs Concerned, and Their Habits.
   a) The Two Important Species.
   b) Feeding Habits.
4. The Causes of Severe Infestations.
5. Control.
   a) Review of Previous Methods.
   b) The Present Problem.
      (i) Cultural Methods.
      (ii) Farm Hygiene.
      (iii) Chemical Methods.

Literature.
In the previous parts of this thesis which dealt with the life-history and ecology of *Agriolimax agrestis*, the Grey Field Slug, some idea has been given of the mode of life of this species, and its economic importance will have to be realised. No other agricultural pest is so universally distributed or causes damage to so wide a range of crops as does *A. agrestis*. And when, under very favourable conditions, this or other slugs are present in epidemic proportions, the losses for which they are responsible are equal to those caused by any other type of pest. They are continually at work, but their nocturnal and unobtrusive habits ensure that the tremendous toll of produce which they take passes to a large extent unnoticed, or is not assigned to the proper cause.

Slugs are less easily studied under field conditions than are, for example, insect or acarine pests which are active by day, feeding openly and causing characteristic types of damage; which occur in the destructive form throughout a short period of the year; and which have several clearly-defined stages in the life-cycle, offering attractive life-histories for investigation. Hence slugs have not received the attention which they merit from the practical biologist, whose attention has been directed to many insect pests of doubtful economic significance, to the neglect of this important group of land molluscs.

Apart from the persistent damage done by slugs, there are two types of attack which cause more serious losses. The first of these occurs in spring upon young shoots and transplants.
Destruction of the growing tips and succulent leaves is a form of damage which seriously impairs the growth of the plant, and it has been encountered upon potatoes in the Lothians. The other type of damage which is very important economically occurs to potato tubers underground during autumn, and this prompted the present investigation.

During a number of seasons prior to 1934, potato farmers in the Lothians had made complaints that very serious losses were being incurred as the result of the attacks of slugs, which produced "worm-holes" in the tubers and rendered them unfit for sale. The few attempts which were made to control the outbreaks were not successful for there was no information available with regard to infestations of this nature. The existing knowledge referred to attacks under garden and market-garden conditions, and damage was being done to the green parts of plants and not to underground tubers, so that the control measures recommended were quite inapplicable in the present instance. Therefore it was felt that a fuller knowledge of the biology of slugs should be obtained, and particularly that the factors which lead to an abnormal increase of the numbers of slugs in potato fields should be clearly understood. The information contained in Parts I and II of this thesis, upon the breeding and other habits of *A. agrestis* and upon the relations between the slug and its environment, is used in the interpretation of the cause of severe infestations, and the most opportune methods of dealing with these are indicated.

The problem is not one of recent origin. Although damage seems to have been most severe and widespread during the last twelve or fifteen years, the pest was in evidence as far back as thirty years ago. The damages effected by adult and young slugs burrowing into and feeding upon the substance of the tubers before they are lifted. When *Agriolimax agrestis* is the species of slug concerned, the entrance hole, which is usually very small, is often covered with soil and easily overlooked, and as apart from this the skin is not eaten, badly tunnelled potatoes may show very little outward sign of damage. This has serious implications, for it makes the separation of damaged tubers during dressing a difficult matter, and it may only be after peeling that the tuber is found to be useless. When *Arion circumscriptus* is the causative agent, the holes are more obvious and large portions of the surface of the potato are removed.

From information received from growers, while slug damage has on occasion been so severe as to result in the loss of the entire crop, and in frequent instances four tons per acre have been removed during the dressing of potatoes for the ware market, a loss equivalent to one-quarter of this amount may be regarded as a bad attack, and this is a common occurrence. In the course of the present investigation it was found that in the 1935 season the worst attack observed affected two-thirds of the tubers being pitted, while in other cases one-quarter of the crop was unfit for sale. To this loss must be added the additional labour involved
SLUG INJURY TO POTATOES.

Kerr's Pink damaged by *Agriolimax asrestis*. The entrance-holes are small and the tunnels penetrate deep into the tuber.
in removing the slug-holed tubers and, what is more serious, the loss of goodwill on the part of merchants and Southern growers through the sale of damaged tubers which have, inadvertently, escaped attention.

Late varieties only are attacked and these only toward the end of the season. Damage is negligible up to the end of August, but crops at that period showing no trace of damage have been found to be badly slug-holed four to six weeks later. The varieties which are stated to suffer most are Golden Wonder, King Edward, Kerr's Pink and Great Scot in that order, but there is little evidence to show that slugs have a preference for any one of these more than another, and on occasion all of them have been subject to heavy infestation. In the case of one grower, a succession of losses has caused him to plant mainly the last-named of these varieties, and to lift the crop early while the haulms are still green and before the slugs have begun to attack the tubers.

Complaints of damage have come mainly from coastal and lowland farms, upland districts above 250 ft. not being affected. As shown on the map, part II, Fig. 3, the trouble is worst in West Lothian to the west and south of South Queensferry, and in East Lothian east and south of North Berwick, and South of Dunbar, being present to a less extent in the vicinity of East Linton, Drem and Longniddry. It is by no means uniform and during the lifting season of 1935 it was noticed repeatedly that a farm where damage was severe might be adjacent to areas where it was negligible
3. The Slugs Concerned and Their Habits.

a) The Two Important Species.

Five species of slugs have been found in potato fields. Agriolimax agrestis (The Gray Field Slug), Arion circumscriptus, Arion subfuscus (The Bushy Slug), Arion hortensis (The Garden Slug) and Arion intermedius. The last two are comparatively uncommon and have never been found attacking potatoes in the field, although a very severe attack by the former slug has been observed in a badly-kept garden. Arion subfuscus is more plentiful and is evenly distributed, not being more abundant in areas showing severe damage than elsewhere. It is more readily found in grassy banks at the sides of fields, and in the few instances in which it has been found feeding upon tubers it has been associated with a heavy infestation of A. agrestis and A. circumscriptus. In the laboratory it is not usually a primary cause of damage to the tuber but appears to be a secondary pest, finding its way into potatoes already badly attacked by other species. It is of minor economic importance.

The two first-named species are the cause of all serious attacks and differ materially in their distribution and habits. Agriolimax agrestis. This slug is ubiquitous, occurring in every part of the area and in almost any situation. It is particularly prevalent in Mid and East Lothian, the heaviest population being east of North Berwick, where over 100 per square yard were killed on rye-grass by copper sulphate dusting at night, while dunged stubble in winter yielded 16 slugs and 30 eggs per square yard. It is an abundant species in potato fields around Dunbar, East
Linton, Drem, Inveresk and Dalkeith, and is also numerous to the south and east of South Queensferry.

**Arion circumscriptus.** This slug does not have the same widespread distribution as *A. agrestis*, and apart from a few districts where it occurs in great numbers is not readily found. The most important of these lie just south of North Berwick and east and south of South Queensferry.

The breeding habits of *A. agrestis* have already been described and the relations between this slug and its environment have been discussed. It has been found to tolerate and flourish under a wide range of soil reaction, and it has been shown that soils with a high moisture-holding capacity are associated with the most intense infestations. This agrees with the distribution of these infestations, for lowland and coastal farms with heavy clay soils suffer most, and the lighter soils of upland farms and of fields bordering the coastal sand-dunes of East Lothian support a comparatively low population of slugs. In the laboratory, slugs given the choice of such soils showed a marked preference for the heavier type.

While at rest during the day, *A. agrestis* is usually found singly whereas *A. circumscriptus* shows a tendency to be gregarious, collecting in close groups up to a dozen or so under a stone, dead leaves, or at the roots of a potato plant.

**b) Feeding Habits.**

The methods by which a slug finds its food are important in considering control measures. Repeated observations in the field, corroborated by laboratory tests, have shown that slugs are
not attracted to food materials from a long distance, and their powers of vision and smell seem to be very poorly developed. Slugs which have been starved for several days will pass in the search for food within less than an inch of suitable material and fail to detect it, whereas when it is placed directly in their path they feed at once with avidity.

Slugs are active by night and during dull, wet weather, and it is at these times that most of the feeding is done, except in the case of young *A. agrestis*, *A. circumspectus* and *A. subfuscus*, which can be found feeding on grass, weeds, and the lower leaves of potato plants both day and night in late summer and early autumn.

It is improbable that individual slugs feed every consecutive night, even where conditions are suitable, or that members of a slug population are active simultaneously. This is a question which can scarcely be investigated in nature, but under laboratory conditions all the slugs in a container are seldom found on the soil surface at once. Different slugs may come up at different times during the night or, alternatively, they may not feed every night. In either event, when contact poisoning is being carried out, it becomes necessary to select a night following upon a spell of weather unsuited to slug activity or to repeat the operation more than once.

Slugs exhibit a wide choice of food, as might be expected from the poor development of their food-detecting senses. *A. agrestis* has been found feeding upon rye-grass, hay, oats, weeds, and potatoes, both haulms and tubers. It does show one food preference which has an important bearing on the present problem, namely that
fresh green food above ground is taken before the underground tubers. The comparative suddenness with which slugs turn to the tubers for food during September has been remarked upon. This is not the result of a sudden invasion of the field by slugs from outside, since the heavy infestation is present during August and early September and can be seen feeding above ground on such grass and weeds as are present, and also upon the potato leaves, although these seem to suffer little damage. It is when the haulms begin to dry and other green food presumably becomes less attractive with the slowing of transpiration, and when nights with suitable weather conditions are becoming less frequent, that underground feeding on tubers becomes serious. This preference of slugs has been tested by keeping a number in a container in soil in which potatoes were buried with rye-grass or lettuce placed on top. So long as the latter was kept fresh, the potatoes were left untouched in as far as _A. agrestis_ was concerned, but _A. circumscriptus_ was found to feed upon both food materials impartially. Another important difference in the feeding habits of the two species is found in the fact that _A. agrestis_ seldom remains inside the tunnel it has made in the tuber, while _A. circumscriptus_ is often found inside an attacked potato, remaining there for days until an enormous cavity is formed. Thus, in the case of an infestation of the latter slug, damage to individual tubers is always more extensive, and while _A. circumscriptus_ enters the pits in large numbers inside affected tubers, _A. agrestis_ seldom does.
4. The Causes of Severe Infestations.

Slugs are animals so ubiquitous in their habits, so unrestricted in their choice of food, so prolific in breeding and so resistant to natural controls that they can never be completely eradicated and will always claim a percentage of produce. But the abnormally heavy infestations which constitute the present problem are the result of a combination of factors which, once understood, may be modified with a view to effecting some measure of control. A severe attack is caused by a definite sequence of events and there are a number of contributory factors which serve to aggravate the case.

In the warmest and driest months of the year slugs are not much in evidence in potato fields, and eggs are scarce. But during August cooler and more humid nights cause a renewal of feeding and breeding. In badly infested fields in August and early September both adult and young slugs can be seen on suitable evenings feeding above ground upon grass and weeds, and to some extent upon the haulms. *A. circumscriptus* is less often encountered above ground, but has been seen feeding upon the lower leaves of potato plants. During late September and October a radical change of feeding occurs, and while a certain number of slugs are to be found above ground, practically all the feeding takes place in the tubers. The green parts of the potato plants are drying and possibly other vegetation is becoming less attractive to the slug, which requires succulent tissue to feed upon. Also, an increasing number of nights are wet or windy, conditions which slugs avoid.

The effect on incidence of attack of clean cultivation or the reverse is worthy of remark. The idea is prevalent that
particularly clean fields with no wrack and only a few scattered blades of grass suffer more damage than fields with much weedy growth in the furrows. As a result of comparisons made, it does seem probable that in the latter case slugs are maintained above ground until later in the year, so that if the crop were lifted fairly early, damage would be moderate. But the field in which most woods were present, lifted on 28th October 1935, suffered very heavy damage.

Following the harvesting of the potato crop, the slugs are suddenly deprived of their food supply. In a heavy infestation the immense number of slugs present now becomes apparent, for on suitable nights they appear on the bare soil surface. They feed upon such little vegetation and few potatoes as are present, and shelter in large numbers beneath the heaps of dead haulms until these are removed. At this time, the slugs are more in the open and more easily seen and collected than at any other time of year.

In neighbouring fields oat or hay stubble is being prepared for the following season's crop of potatoes. A varying amount of dung, twelve to fifteen tons per acre, is laid down in small lumps evenly spread over the stubble and the field is left in this condition until ploughed about March. The oats or hay supported a very small population of slugs, and few can be found when the dung is first applied. There now takes place a gradual populating of the dung-strewn stubble by slugs emanating from the potato fields. With the removal of the crop, these areas have become many times over-populated in relation to the available food supply and the slugs disperse from these centres. There have been no complaints
of damage by slugs on crops following potatoes, even where the latter crop suffered a severe slug attack. Slugs can cover considerable distances in a single night, and in this case the fields are often only a few yards apart. There is no evidence that the dung exerts any attractant influence over a distance, but rather it is a case of those slugs which reach the stubble remaining there on finding suitable shelter and feeding in the moist lumps of dung, and an escape from the most intense cold. In addition, a catchcrop of clover or rye may have been sown with the grain crop, thus providing a further food supply. The gradual accumulation of adult slugs in dung-strewed stubble has been followed during the three months after lifting of the potatoes, and in all likelihood this is the chief explanation of the dense population of slugs found in the field the following autumn. Instead of reproductive activity being curtailed during the coldest months, it is continued under these conditions, although eggs are scarce in other situations. In the severe weather of January–February 1956, when the ground was frozen hard for long periods, slugs found shelter and eggs were developing in the moist centres of pieces of dung, which provide suitable feeding for the newly-hatched young slugs. In the previous winter, the slug population of a stubble field was estimated at almost 80,000 adults and 150,000 eggs per acre. Thus the number of slugs effecting damage in the autumn is maintained and increased throughout the following winter.

In addition to this effect, there is the fact that soil-inhabiting animals are most numerous in soils containing a high percentage of organic matter. Miles, Wood and Thomas (1931),
investigating the relation between organic manures and slug prevalence, state:

"The presence of organic matter has a dual importance in relation to slug development. Variation in the water content of similar soils follows closely the differences in the amount of organic matter present. Because of its water-holding capacity, the presence of organic matter encourages slug development, and since it also serves as food for slugs at various points in the life-cycle, its influence is increased. Newly hatched slugs feed at first on undecomposed organic matter and broken, damaged plant tissue. In the early spring, before crops are available, the organic matter in the soil appears to be one of the main sources of food for young slugs, and during the winter when conditions are unfavourable for surface feeding and metabolic processes are reduced, the organic matter in the soil also provides food for the adults. The importance of organic matter in the soil was demonstrated in the laboratory when newly hatched slugs were kept alive for over six months under conditions in which moisture and organic matter only were present.

"Examination of slug infested soils in East Lancashire suggests that there is a fairly close relationship between the amount of organic matter present in the soil and the extent of the slug population. The application of organic manure to the soil increases its water-holding capacity, thus rendering it more favourable to slug development. It also provides undecomposed humic material on which slugs feed. This favourable influence of
organic matter has already been noted by Morris, who has shown that at Rothamsted the application of farmyard manure to land previously unmanured increased the number of Pulmonates present from 13,500 per acre to 33,700 in the course of a year, an increase of about 150 per cent.

Other factors which favour the presence of slugs are grassy banks and untidy fields, adjacent woods, ditches and hedge-bottoms with a bed of moist dead leaves and grasses, and wet sacking, large stones, etc., so often used as a humid shelter for eggs.

There is a fundamental difference between the slug pest and the insect one. The slug multiplies whenever conditions permit, regardless of the time of year, and does not conform to an annual cycle with a comparatively short breeding season. Under normal circumstances, the numbers of a pest are kept within reasonable proportions because the rate of multiplication is balanced by a high natural mortality. In the present instance, breeding conditions are so favourable and so extend beyond the usual limits that the numbers of slugs have been allowed to increase with food supply as the only limiting factor of any account. And food supply includes potatoes.

Few populations of animals which are crop pests ever reach saturation point with regard to the available food supply. Other factors intervene and in the seasonal cycle the periods of active feeding and multiplication are comparatively short. But when these other factors are prevented from exerting their full effect and the pest is one which can avail itself of suitable breeding
conditions at any time, and is moreover endowed with prolific powers of increase and a wide variety of food plants, the result is a dense population which suffers no seasonal reduction in numbers.

Lertz and Blum (1950) studied field, garden and meadow species in Germany and, after carrying out a series of tests where the slugs were attacking crops grown, advocated a combination of repellent and poison bait. The former was a 1:1:1:1 mixture of 2-4-5-B sprays upon the affected plants, and the latter was chopped lettuce (16 parts) mixed with calcium arsenate (1 part) and scattered in small heaps.

Hodson (1959 and 1963) dealing with A. nemoralis and A. hortensis at agricultural parks found a good contact poison to be paratone sodium sulphate plus twice its weight of lime applied at the rate of 1 oz. to the area. An effective standard poison consisted of 1 lb. bran (of which slugs are fond) mixed with 1 lb. Paris green, blended with water to which a little sugar had been added, the mixture being spread by hand at the rate of 30-40 lb. to the acre. The time of application, however, was all important. The poisons had not to be too dry, so the slugs did not crawl and after heavy rain the poison was diluted. The ideal time was after a downpour, following a shower, or a warm evening. The operation required to be repeated a week later. The experiments of Armstrong and Taylor (1950) carried out in Yorkshire during June 1955 on A. nemoralis and A. hortensis which were attacking cabb
5. Control.

a) Review of Previous Methods.

Slugs have frequently been the subject of control experiments which have differed according to the nature of the attack. The more recent of these are considered here, and their application to the present problem is discussed.

Lovett and Black (1920) studied field, garden and greenhouse species in Oregon and, after carrying out a series of tests where the slugs were attacking crops above ground, advocated a combination of repellent and poison bait. The former was Bordeaux Mixture 4-4-50 sprayed upon the affected plants, and the latter was chopped lettuce (16 parts) dusted with calcium arsenate (1 part) and scattered in small heaps.

Hodson (1924 and 1925) dealing with A. agrestis and A. hortensis as horticultural pests found a good contact poison to be powdered aluminium sulphate plus twice its weight of lime, applied at the rate of ½ cwt. to the acre. An effective stomach poison consisted of 20 lb. bran (of which slugs are fond) mixed dry with 1 lb. Paris green, moistened with water to which a little sugar had been added, the mixture being spread by hand at the rate of 20–40 lb. to the acre. The time of application, however, was all important. The ground had not to be too dry or the slugs did not emerge and after heavy rain the poison was diluted. The ideal time was after sundown, following a shower, on a warm evening. The operation required to be repeated a week later. The experiments of Anderson and Taylor (1926) carried out in Yorkshire during June 1925 on A. agrestis and A. hortensis which were attacking oats,
sugar beet and peas, resulted in the important discovery of a contact insecticide which, provided it reached the upper surface of the slug, was at once lethal. This was copper sulphate sprayed as a 3% solution or mixed dry with 20 parts commercial kainit (6 lb. copper sulphate to 1 cwt. kainit) and distributed by hand or manure distributor at the rate of 3 cwt. to the acre.

Contact poisons and poison baits which have given success in New South Wales (1927) were (1) boiled potatoes sprinkled with dry white arsenic or Paris green and (2) arsenate of lead as a spray (1½ to 3 lb. in 40 gall. water) or as a dust (1 lb. to 4 lb. lime).

The ecology and control of slugs (Agriolimax agrestis, Milax sowerbii, and Arion subfuscus) under market gardening conditions in Lancashire and Cheshire were studied by Miles, Wood and Thomas (1931). The causes resulting in the increase of slugs were explained and the results of several methods of control were compared by means of field trials carried out with cabbage plants. Copper Sulphate, washing soda and creosote were found to be efficient contact poisons, while a bait of bran and Paris green was of doubtful value as a stomach poison. Repellents were most satisfactory in the form either of a spray consisting of 1:1000 corrosive sublimate or of a dust of 1:100 creosote and precipitated chalk. The final conclusion reads:

"Since the slug problem in the north-west of England is closely bound up with conditions of soil and climate, the control measures which have been described can only be regarded as giving temporary relief. Even where such measures are carried out persistently there seems little likelihood that any marked influence on the
slug population will accrue. Any considerable relief can only 
follow alteration or modification in the character of the soil 
so that conditions are no longer suitable for the maintenance of 
a large slug population. It seems likely that in the north-west 
of England this change in soil conditions may be produced by 
alteration in the system of manuring, for allotment holders who 
have substituted artificial manures for organic dressings have 
observed a remarkable reduction in loss from slug attack, although 
on adjoining allotments receiving the usual quantities of organic 
manure without artificials losses from slug attack were very severe. 

These instances of slug attack differ in some respects from 
the present case and this must be borne in mind when control 
measures are considered. In the first place, they are all 
concerned with damage to the green parts of plants during spring 
and summer, in which case the slugs are feeding above ground. 
When the feeding is done in autumn below ground, a different 
problem is presented, and at the time of attack the slug is not 
accessible. Also, methods which have met with success under 
horticultural and market-gardening conditions are not always 
practicable on a field scale.

b) The Present Problem.

From the foregoing account of the habits of field slugs 
and the nature of the problem presented by their attacks upon 
potato tubers, and from the consideration of such data as are 
available regarding the control of slugs under other conditions, 
it is possible to indicate which methods of control can be applied 
here with most hope of success. The available methods which might
be adopted are either preventive, e.g. cultural methods, farm hygiene, or curative, e.g. the use of insecticides. These various lines of action may be considered in the light of the information which has been obtained upon the biology of the slugs.

(i) Cultural Methods.

The whole aim of ecological work upon animals of economic importance is the attainment of relatively simple methods of control, based upon a sound knowledge of the pest itself and the relations of the pest to its environment, in particular to the crop which is attacked. It has been realised that chemical methods are more costly and less effective in the long run, and as a wider knowledge of practical animal ecology is obtained these methods are being superseded by the more fundamental and lasting measures which involve alterations of farming practice. Imms (1931, Recent Advances in Entomology, Chap. X) states:

"There are many insect pests affecting agriculture which do not lend themselves to direct control measures with any real prospects of success. Or, the crop may be of such an extent or character which renders such measures impossible from the practical standpoint. Under such conditions control methods largely resolve themselves into devising modifications of cultural practice as will place the pest at some disadvantage and, at the same time, ensure a better crop yield. In formulating such measures an acquaintance with the chief ecological features in the life of a given pest in relation to its plant-host is called for. Knowledge so gained, when translated into practice, resolves itself into what may be termed cultural methods of control."
"Cultural methods of control have definite practical advantages as contrasted with physico-chemical methods. In the first place they are, as a general rule, preventive, and serve to restrain losses that would otherwise supervene. Further, in the case of most crops, they usually incur little or no extra financial expenditure upon the practical grower, they are generally easy to apply, and frequently involve no drastic disturbance of accepted routine. It has also to be remembered that the present trend of agricultural conditions in many countries is such as leaves a narrowing margin of expenditure available for insecticidal treatment, or other more or less costly measures. There is, in consequence, an increasing tendency to study the relations between insect attack and the conditions under which the crops are grown. This in its turn involves the application of ecological methods, and in the present chapter endeavour will be made to show that success, in this direction, is dependent upon adequate knowledge of the biology of the insect in relation to the growth phases of the crop which it attacks."

The practice of spreading large quantities of stable manure at the beginning of winter upon stubble designed for the following season's potato crop is the crux of the present problem. Ordinarily the slug would be a sufficiently serious pest in this area, particularly in wet seasons. But the manorial practice which provides for the slugs a safe retreat, a supply of food and a suitable breeding-ground during the coldest weather, and which raises the organic content and moisture-holding capacity of the soil to a high level, permits the development of an excessively large population of slugs.
A striking example of the increased temperature which obtains inside masses of manure is given by Austin (1936) who investigated the effects of temperature upon the house fly (*Musca domestica*). The larvae of the fly die at temperatures of 40.5°C and over, and if piles of horse manure in which fly larvae are living are closely compressed, the heat generated by decay inside the heap is sufficient to kill the larvae except at the edges, and to cause a migration of the larvae away from the hotter centre toward the periphery.

The grower desires nothing less than to reduce the amount of stable manure used in the cultivation of potatoes. The potato is the profitable crop, and a large amount of organic manure ensures a high yield, especially in dry seasons. The manure represents a valuable bye-product of the cycle of agricultural operations in the Lothians, and it must be utilised else it becomes a dead loss and the inorganic salts used to replace it become an additional expenditure. The cattle which provide the manure are brought in to feed upon the turnips, which along with hay and oats, are needed to complete the rotation of crops. But, with a view to obtaining an increased quantity of manure and so raising the yield of potatoes, growers have resorted to the practice of buying in both cattle and turnips; in other words, they are trying to get more return from the land than it would normally give if each farm were self-supporting. And it so happens that the method adopted, namely the spreading of large quantities of stable manure upon stubble, encourages the most important animal pest of potatoes in the area. Economically the necessity of utilising the manure is
too pressing and, it must be admitted, traditionally the practice
too deeply rooted, for a radical change of practice to be welcomed.
It can merely be urged that as little organic manure be applied as
consistent with a satisfactory yield, that it be not spread
immediately after potatoes are lifted from adjacent fields, and
that it be ploughed in as soon as possible. No matter how high
the yield, the crop is useless if the tubers are damaged by slugs
and therefore unsaleable. The grower must try to strike the most
profitable balance for himself.

The second cultural practice which will assist in preventing
the attacks of slugs upon potatoes concerns the time of lifting
the crop. This has already been referred to in the discussion in
Part II of this thesis on the relation between weather and the
infestation of slugs, to which the reader is referred. The
grower who follows with an intelligent interest the effects of
changing conditions of weather upon the populations of slugs in
his own fields will find himself at the end of August in the best
position to decide upon the most suitable date on which to lift
the crop un each season. Sampling the crop at weekly intervals
from the beginning of September onwards will enable the attack to
be discovered at the very onset. It develops rapidly.

A further means whereby losses due to slugs can be reduced
refers to the method of lifting and pitting the tubers. It can
best be illustrated by an example. On 24 October, 1935, a crop
of Kerr's Pink was examined as it was being lifted and pitted.
The field was not level, but contained a hill which drained into
a very damp hollow. There was a very heavy infestation of
Arlon circumscriptus, and damage was extensive but not uniform throughout the field. In the drier ground on top of the hill one tuber in ten was affected, whereas in the hollow every other tuber was damaged. Yet the potatoes were mixed indiscriminately as they went into the pit, with two results. As has been noted, this species, *A. circumscriptus*, often remains inside the tuber, and many slugs were being introduced into the pit there to contaminate sound potatoes. Also, the merchant who would examine the crop at a later date would be likely to get a very much worse impression of damage than otherwise. In circumstances such as these it would well repay the farmer to sample the crop just before lifting, and, if necessary, to demarcate areas where damage was worst and keep the tubers from such areas separate from the others.

(ii) Farm Hygiene.

The cleaner the farming, the fewer the situations which provide food and shelter for slugs. The presence of weeds in potato fields has been remarked upon and has been the subject of experiment. In July 1935, a clean field with a dense population, where damage had been severe in the past, was sparsely sown with rye-grass, one-quarter bushel to the acre, with the object of providing sufficient surface feeding for the slugs and so enabling the crop to escape severe damage. Damage was probably lessened, but such a practice does nothing to reduce the numbers of slugs in future years.

(iii) Chemical Methods.

Chemical control aims at reducing an already high infestation by (1) providing poisoned food, (2) applying an external poison or (3) keeping the pest away from the vicinity of the crop. Stomach poisons have yet to be proved successful against slugs on
the field scale. Of repellents, corrosive sublimate and creosote have been claimed to be useful. The former, however, is costly, while a potato field in East Lothian heavily dosed with creosote showed no improvement. A contact insecticide, however, suitable in every respect, is available. Copper sulphate is cheap, easy to apply dry or wet, and is specifically lethal to slugs on a first application, the requisite dosage causing no harm to foliage. The principal problem in its use is one of application, for to be effective it must be brought into direct contact with the upper surface of the pest. The difficulty of control does not arise because of the lack of an efficient insecticide, but because the slug, nocturnal and subterranean in habit, seldom exposes itself to the risks of direct attack.

This last method of control has been successfully employed in this area. In 1928 an attack during April on Brussels Sprouts and Savoys in Midlothian was controlled by dusting at night with copper sulphate - kainit mixture, and a field of Epicure, the shoots of which were being eaten above ground during the same month, was successfully treated with copper sulphate. In a case where seedling sweet peas had been eaten as soon as they appeared above ground, on replanting, rings of copper sulphate powder round the growing shoots prevented further attack. These relatively simple methods, however, hardly apply to the case of autumn attacks on potato tubers. Control measures must be undertaken long before the slugs have commenced feeding underground. It has been pointed out that they are most accessible just after the potato crop is lifted and that it is necessary to anticipate the following year's
attack by dealing with those slugs which will find their way to the dung-stream stubble and over-winter there. Trials are necessary for effecting a comparison of copper sulphate dusting or spraying with the poison baits referred to above. Collecting too, is a method not to be ignored, the heaps of withered haulms acting as natural traps.

The preference of slugs for grasses such as Italian rye-grass has been noted, and a 'kill' of 112 slugs to the square yard on thick grass 6" high has been made by copper sulphate dusting. Provision of belts of this grass as a trap crop, with dusting at regular intervals under the correct conditions, will result in a reduction of numbers, and similar treatment of grassy banks near fields, especially in spring and early autumn, is necessary.

The migration of slugs into the stubble fields in early winter has been found to be the starting-point of the following year's infestation. Prevention of this is difficult and it is desirable to carry out field trials of poison baits and traps at the borders of the infested potato field, and of a copper sulphate belt, renewed if necessary, in a shallow ditch along the side of the stubble field, especially where it adjoins an infested field of the previous year. Moist bran or damaged tubers which have just been lifted can be used as traps or baits, and wet sacking laid on the ground will provide a situation attractive to slugs, especially in dry weather. Systematic collecting of slugs and renewal of baits is necessary. Such control measures entail much trouble and some expense, but from what has been written of the
habits of the slugs and of how agricultural practice favours their increase, it will be appreciated that there is not likely to be available any very simple or very speedy method of eradication.

   The House Fly.  
   British Museum (Natural History), Tenth Series Vol. 14, London.

   Preliminary Experiments in the Control of Slugs.  

   Some Further Notes on the Control of Slugs.  

   The Grey Garden Slug, with Notes on Allied Forms.  
   Oregon Agricultural College, Station Bull. 179.
Literature.

   The Slug Pest.
   Department of Agriculture, University of Leeds,
   Bulletin No. 143.

   The House Fly.
   British Museum, (Natural History), Economic Series No. 1a.
   London.

   Preliminary Experiments in the Control of Slugs.
   Journal of the Royal Horticultural Society,

   Some Further Notes on the Control of Slugs.
   Journal of the Royal Horticultural Society,

   The Gray Garden Slug, with Notes on Allied Forms.
   Oregon Agricultural College, Station Bu 111etin 179.
   The Gray Field Slug (Agriolimax agrestis, Linn.).
   Journal of the Ministry of Agriculture,
   Vol. XXVIII, pp 451-455.

   On the Ecology and Control of Slugs.

   Common Pests of Field and Garden Crops.
   Agricultural Experiment Station, Michigan, Bulletin No. 183.

9. --- 1927.
   Cutworms, Slugs, Snails and Slaters.
   Department of Agriculture, New South Wales.
   Insect Pest Leaflets, No. 1.

10. --- 1935.
    Slugs and Snails.
    Ministry of Agriculture and Fisheries, London.
    Advisory Leaflet, No. 115.
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R.C.
THE SPERMATOGENESIS OF THE AXOLOTL
(AMBLYSTOMA TIGRINUM)

BY

ROBERT CARRICK, B.Sc.

[WITH THREE PLATES.]
III.—The Spermatogenesis of the Axolotl (Amblystoma tigrinum). By Robert Carrick, B.Sc., Carnegie Research Student, Zoology Department, University of Glasgow. Communicated by Professor J. Graham Kerr, F.R.S. (With Three Plates.)

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I. INTRODUCTION.

The material for this work was obtained from the stock of Axolotls which breed in the Zoology Department of Glasgow University. The objection to using captive specimens for cytological work is overridden by the fact that the Axolotl, unlike some other urodeles, breeds readily in captivity, so that the germ-cell cycle can be taken as normal.

The reasons for wishing to add yet another account of urodele spermatogenesis to the considerable number already existing are twofold. First, widely different interpretations of a similar process in different species have been put forward, and one of the most recent accounts (Stieve, 1920, on Proteus) is entirely at variance with the usual course of spermatogenesis in urodeles. Second, there has been great discrepancy in the number of chromosomes assigned to this species.

It was hoped that the male cells in the Axolotl would provide suitable material for these investigations, due to their large size and comparatively small number of distinctive chromosomes, varying greatly in size and form. Somatic mitosis and oogenesis have been thoroughly studied, but one writer alone (Champy, 1913) has made scanty reference to spermatogenesis in a comprehensive paper on batrachians in general.

I wish to thank Professor J. Graham Kerr and Mr C. W. Parsons for help and advice in the course of the work, and the former also for providing the material.

II. METHODS.

Testes were fixed during the breeding season in May 1932 and in March and October 1933. The fixatives used were Flemming (strong formula), Bouin, and Carnoy. The first two gave excellent results, but Carnoy’s fluid was unsatisfactory, tending to clog the finer leptotene and zygotene threads during prophase. All the material was imbedded in paraffin.

The standard stain was Heidenhain’s iron-alum haematoxylin, with eosin or congo red as counter-stain. Delafield’s and Ehrlich’s haematoxylin, gentian violet and eosin, and safranin and gentian violet were tried but found less useful. Feulgen’s technique was also employed for purposes of comparison with the Heidenhain preparations.

The optimum thickness for sections varied from 8 µ to 12 µ, dependent on the stage being studied, and some sections were cut as thin as 6 µ and 4 µ.

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All sections were mounted between thin cover-slips. The value of being able to examine and draw cells from both sides is that accurate counting of chromosomes is ensured and unravelling of clumps of chromosomes or crossing threads is possible. It is only the exceptional cell in which every chromosome stands out distinctly from its neighbours.

The optical apparatus consisted of the usual one-sixth and one-twelfth oil-immersion objectives, used in conjunction with a Zeiss 12.5 x binocular eyepiece. Binocular vision confers great advantages, especially for chromosome counts and in studying the twisting of fine leptotene threads.

III. Structure of the Testis.

The testis of the Axolotl is a compact, lobed structure, no more than one inch long even in the breeding season, and unlike that of several other urodèles the surface is not pigmented. The primary germ-cells are each surrounded by connective-tissue cells, and by repeated division they give rise to tubules, subdivided into cysts, and replace the previous tubules, which degenerate after being emptied of mature sperms. There is little evidence of seriation of stages, for the tubules convolute at random throughout the substance of the testis. A cross-section will usually show all stages in the cycle—degenerating tubules filled with débris, mature sperms, spermatocytes, spermatogonia, and, in the connective, primary spermatogonia. There is thus no advantage in cutting sections in one plane rather than another.

Testes fixed at the three seasons previously mentioned show in a general way the annual changes which take place. Mature sperms are always present in large quantities. In early summer, which is the breeding season, the testis is slightly larger than at other times, and every stage in the cycle is represented. Meiotic divisions are plentiful. In autumn there seems to be complete inactivity, and no maturation divisions were found. In spring, apart from large numbers of sperms and spermatogonia, there were many primary spermatocytes in prophase and very few in metaphase.

The sequence of stages was not so difficult a matter to determine as at first appeared. Neighbouring cells divide synchronously in little groups, and contiguous groups within a cyst are at approximately the same period. Different cysts within the same tubule may show a greater variation, and two neighbouring tubules may contain stages at the opposite ends of the cycle. And, in general, the stages resemble those of closely related species in which long narrow tests show seriation along their length.

IV. The Chromosome Number.

Previous estimations of the diploid number of chromosomes range from sixteen to thirty. Oguma and Kakino (1932) give the following list. Fick (1893 and 1899) found eight chromosomes (haploid) in polar divisions of the ovum, and Kölliker (1899) gives twenty-four as the number in dividing nuclei of blastomeres. Jenkinson (1904) estimates fifteen chromosomes in each of the oogenetic divisions, though the polar bodies may have fourteen or sixteen. The fertilisation spindle has about thirty. Muckermann (1913) gives twenty-four as the somatic number in Siredon, and Mack (1914) finds a similar number in Amblystoma. Parmenter (1919), in a detailed study of the chromosomes of Amblystoma tigrinum, states that there are twenty-eight elements in somatic cells, forming fourteen homologous pairs.

In the testis of the Axolotl the equatorial plates of primary spermatogonia and first meiotic division lend themselves admirably to accurate counting of chromosomes.

In the former the cell is extremely large and the chromosomes long. Five cases were
counted, and in three the number was clearly twenty-eight, and in the remaining two probably the same. Plate I, fig. 7, shows the same cell occurring throughout three consecutive sections. Parmenter (1919) concluded that “there is also a suggestion that there is a sex chromosome attached to a euchromosome.” Plate I, fig. 7b (x), shows the condition which he also finds in somatic cells.

In the metaphase of the first meiotic division fourteen pairs of chromosomes are arranged on a flat equatorial plate, usually complete in one 10 µ section. When the spindle is seen in polar view the chromosomes stand out very distinctly, the larger V-shaped ones forming a ring with apices pointing inwards, and the two smallest occupying the centre of the plate (Pl. III, fig. 23). The constancy of this arrangement is evident in the four cells figured. Indeed, there is a suggestion that each chromosome is brought into a constant position relative to its neighbours on the spindle, and chromosomes may even divide in the same order and follow the same path as the division progresses. Fifteen pairs is the normal number at this stage, although a number of cells showed only fourteen. Since the somatic number is twenty-eight the additional pair would be explained by the presence of an accessory chromosome usually free from the autosomes during this division.

This conclusion is borne out when the metaphase of the second maturation division is examined. Here the cell is smaller, the chromosomes are less distinct from each other, and the metaphase is of very short duration, so that fewer cells are suitable for counts. Approximately equal numbers were found to show fifteen bivalents and fourteen bivalents plus one univalent. A few even showed only fourteen bivalents. Plate III, fig. 28, is an advanced metaphase with fifteen bivalent chromosomes, one small element having been added from the next section.

The possibility is raised that in some cases the sex chromosome divides in the first division and remains whole in the second, and in other cases remains attached to an autosome in the first and divides in the second. A precise account of the sex-determining mechanism could be given and the identity of the sex chromosome established only after a comparison of male and female chromosome constitution during maturation divisions.

A sex chromosome has been recorded by King (1912) in the case of one urodele, Necturus maculosus, but Iriki (1931) finds no cytological evidence for such a chromosome in Diemycetilus or Megalobatrachus, and assumes that urodeles must be of the XX-type with the chromosomes “in a very primordial, morphologically undifferentiated condition.”

I have found the haploid chromosome number to be fourteen, with the addition of an X-chromosome which normally divides in the first maturation division and remains whole in the second.

V. The Spermatogonial Divisions.

The primary spermatagonia are easily recognised by their large size, often 50 µ in diameter, spherical form and nuclei ranging from an extreme polymorphic type to regular spheres. Each is set in a capsule of connective cells. The more irregular the nucleus the more faintly does it take the chromatin stain, and the spherical nuclei show deep-staining chromatin masses. One large, and often several small, nucleoli are present. The cytoplasm stains well, and in the more regular type of nucleus a depression contains the sphere with minute centrosomes (Pl. I, fig. 1).

As the chromatin blocks become more pronounced they are seen to occupy a peripheral position, and lines of chromatin radiate from them (Pl. I, figs. 4 and 5). This is the incipient spireme, which when fully formed has used up all the chromatin masses and consists of an
apparently continuous band winding about inside the nucleus just underneath the membrane (Pl. I, fig. 6).

The nuclear membrane breaks down and long chromosomes appear and are arranged on the equatorial plate. There is a marked tendency for chromosomes of the same size and form to group themselves together—a phenomenon first noted by Montgomery (1904) in several Amphibia and mentioned by Agar (1911) in *Lepidosiren*.

The centrosomes at this stage frequently have the appearance of a group of granules, and the astral rays are long and pronounced, reaching the cell boundary. The chromosomes split longitudinally and pass more or less together to the two poles, where reconstitution of the daughter nuclei occurs. The division between the two cells seems to constrict the spindle fibres in the middle (Pl. II, fig. 10), and many writers have described the mid-bodies which result.

Mitosis of secondary spermatogonia is essentially the same, except that the cells become progressively smaller in size and more closely packed together as divisions succeed one another; chromosomes are smaller and astral rays are less pronounced.

The characteristic polymorphic or reniform nuclei of resting primary spermatogonia have given rise to the idea that amitosis is taking place. Meves (1891 and 1894) describes and figures these nuclei in *Salamandra* as being halved "by the constricting power of a ring-shaped centrosphere," and vom Rath also finds amitosis in the same material. McGregor's figure of binary division by simple constriction in *Amphiuma* is strongly suggestive of Plate I, fig. 2, in which the nucleus has apparently divided into two parts. The condition is quite common in Axolotl, but in every case a careful examination of neighbouring sections showed that the apparent division was due to the removal of two lobes from an irregular nucleus. In no case was there any indication of cytoplasmic division.

There is no evidence in Axolotl that amitosis occurs. The insertion of such inexact divisions just previous to the precise maturation meioses seems very doubtful, and much more convincing proof is necessary before McGregor's statement that "amitosis occurs among primary spermatogonia, and is, apparently, a normal process" can be accepted.

VI. The Maturation Divisions.

Repeated spermatogonial divisions result in the small secondary spermatogonia, which are closely packed together and completely occlude the lumen of the tubule. They are dark-staining, with coarse masses of chromatin and one large spherical nucleolus. The growth period ensues, during which the resting primary spermatocyte is formed, about twice the diameter of its preceding secondary spermatogonium.

At the commencement of prophase fine chromatin granules scattered evenly throughout the nucleus give it a speckled appearance, and there is a conspicuous nucleolus (Pl. II, fig. 12). The whole substance of the nucleus is faintly basophilic, and gradually clears as the prophase proceeds. The successive stages of the prophase merge gradually into each other, and, judging by the large number of every stage present, the prophase occupies a large proportion of the time taken to complete the cycle. This is important when it is remembered that it is during this period, if at all, that synkinesis and an exchange of hereditary substance occur between maternal and paternal chromosomes.

(In order to facilitate description the conclusion is anticipated that side-by-side union of maternal and paternal chromosomes takes place during leptonema, and the terms homologue, dyad, bivalent, etc., are used.)
A certain amount of seriation is evident here, for some tubules cut in cross-section showed resting spermatocytes towards the outside gradually giving way to the metaphase and telophase interiorly. A dense network of fine threads with no discernible arrangement is gradually built up out of the chromatin granules. The cell in prophase is pear-shaped, the nucleus being pushed over to one side, and the sphere with centrosomes is embedded in the cytoplasm at the other side. The leptotene threads which form seem at first to be more distinct towards the proximal pole of the nucleus, i.e. next the sphere (Pl. II, fig. 13).

When the leptotene threads are fully formed polarisation becomes evident. The ends of the threads straighten out and point towards the proximal pole, where they unite at their tips to form V-figures. The distal half of the nucleus is still occupied by a dense tangle of threads. Few cells show V-figures alone, the majority having also Y-figures in which fusion of two thin leptotene threads has formed the thicker stem of the Y. This is the zygotene stage, or amphitene of Janssens, and the free arms of the Y can often be seen running parallel for a considerable distance into the distal part of the nucleus, where the tangled leptotene gradually unravels. The longer the Y-stems the smaller is the mass of unpaired threads.

Each leptotene thread consists of deep-staining granules of varying size strung out in linear series along the chromosome, and it can be seen that syndesis consists in the apposition of corresponding chromomeres in homologous chromosomes (Pl. II, figs. 15b and 16). Pairing threads often seem to be spirally twisted about each other, but a consideration of this is left until later.

The pachytene stage follows, similar to Janssens' bouquet, in which complete fusion has resulted in thick horse-shoe-shaped dyads, with ends still orientated towards the sphere. Occasionally a suggestion of bivalence persists till this stage, but as a rule fusion is apparently complete. The thick irregular pachytene chromosomes are often joined by fine cross-connections, as shown in Plate II, fig. 17, and Plate III, fig. 18b. It is difficult to count the number of free ends which concentrate at the proximal pole during zygonema and pachynema. Several counts indicate that the number is approximately thirty, and this, bearing in mind that both ends of the bent dyad have been counted, supports the interpretation of the process as that of fourteen fused pairs of homologous chromosomes.

Plate II, fig. 15b, represents one of a small group of zygotene and early pachytene nuclei, which are much larger than usual, and which seem to have arrested development at this stage, for all other cells in the tubule have completed the divisions. A similar case is figured by Agar (1911, fig. 15). The double nature of the pachytene is here clearly shown, and there is a suggestion of spiral twisting of the components.

The nucleolus in earliest prophase is smooth and spherical. At the commencement of syndesis it has lost shape (Pl. II, fig. 14b), and soon breaks up into a number of deep-staining masses lying just inside the nuclear membrane (Pl. II, fig. 15b). By the end of prophase it has disappeared entirely.

The gradual contraction and thickening of threads which has been proceeding from the beginning of prophase now seems to accelerate, and simultaneously with the formation of smoother deep-staining bands a longitudinal split appears (Pl. III, fig. 20). This is the diplotene or so-called dispireme stage. As it progresses the contracting chromosomes become more peripheral in their distribution inside the nucleus. This stage has been interpreted by several writers as a continuous, longitudinally-split spireme, but sections containing entire nuclei show with perfect clearness the numerous free ends of paired chromosomes. Homologous chromosomes are again separate.
It is soon followed by the well-known strepsinema (Pl. III, fig. 21) with twisted homologues often seen joined at the ends, lying just beneath the nuclear membrane. Untwisting of the chromosomes and breakdown of the nuclear membrane occur simultaneously, and closely apposed pairs of chromosomes are arranged on the equatorial plate. No metaphase was found in which fusion was so close as to mask the double nature.

Plate III, figs. 25 and 26, illustrate anaphase and telophase, which differ little from previous descriptions. Conspicuous centrosomes and spindle fibres are seen, and usually one or two pairs of chromosomes separate in advance of the others. As the chromosomes near opposite poles in anaphase, anaschistic V are formed (Pl. III, fig. 25), showing the precocious split of the equation division which is to follow.

A detailed description of the second division is unnecessary and has no bearing on the reduction question. From a short but complete resting period there emerge fourteen split chromosomes, presumably identical with the anaschistic chromosomes of the previous division. The distinguishing features of the homotypical division are the smaller size of the cell, smaller and more slender chromosomes, lack of polarisation in prophase, and no split in anaphase.

VII. SYNDESIS AND REDUCTION.

The number of urodeles in which spermatogenesis has been studied is considerable, and a survey of these accounts shows clearly that a very similar process is taking place in each case. Yet interpretations of pairing and reduction are extremely varied, and cannot be reconciled with each other.

There are, mainly, four:

1. Syndesis does not occur and the individuality of the chromosomes is denied.
2. Syndesis does not occur and both divisions are regarded as simply equational.
3. Qualitative reduction takes place by metasyndesis.
4. Qualitative reduction takes place by parasyndesis.

1. Earlier papers by Fick, Della Valle, and Meves, and more recent accounts by Meves (1911) and Champy (1913), find no support for pairing of chromosomes in any manner whatsoever, and go so far as to deny the individuality of the chromosomes. Meves (1911) states his belief that germ-cells entering the growth period simply inherit the special property of bringing forth half the number of chromosomes, and Champy (1913) agrees with Flemming and Meves that the paired elements of the first metaphase represent single split chromosomes.

Such a facile explanation seems a mere avoidance of the problem, and though these authors figure some of the significant stages of the first prophase—particularly polarised zygotene and pachytene—they fail to give them their real importance.

2. McGregor (1899, Amphiuma) reaches the conclusion that "since the distribution of chromatin is accomplished by two longitudinal (equation) divisions, there is no 'reduction' in the Weismannian sense, and of the four spermatids derived from one primary spermatocyte all are exactly equivalent as regards their chromatin." Eisen (1900, Batrachoseps) and Kingsbury (1901, Desmognathus) agree with this, but it now seems clear that these writers were led astray by the supposition that a transverse division was necessary to true qualitative reduction.

3. The metasyndetic view is based on one of two ideas. Either the polarised, pairing, leptotene threads represent, not whole chromosomes, but a kind of precocious fission, the spireme being formed as a double structure from the beginning (this theory of Meves is as yet unsupported by other observers), or a continuous, longitudinally-split spireme, in which
the chromosomes are in linear series, is first formed and segments, so that pairs of chromosomes are left attached end to end. Loop formation and the strepsitene stage may follow. This has been supported by Montgomery (1908, Desmognathus), Farmer and Moore (1908, 1905, Triton), and Moore and Embleton (1906).

One of the latest accounts of urodele spermatogenesis is that of Stieve (1920). In two detailed papers on Proteus all the essential results of the supporters of parasyndesis have been contradicted. The spireme is described as unsegmented right to the end of pachynema, and no Y-figures are seen forming the amphitene stage. When the spireme does segment it is into the diploid number of univalents, which later come together end to end in metastylitic union.

4. Parasyndesis as the mode of pairing has been upheld by Janssens (1903–1908, Batrachoseps, etc.), the Schreiners (1908, Salamandra), Wilson (1912), Snook and Long (1914), and others. The respects in which the course of spermatogenesis in Axolotl agrees with this conception and invalidates metasyndesis are:

(a) As the succession of stages during prophase is traced serially along a tubule it is seen that the polarised V’s gradually give place to Y’s, the stems of the Y’s gradually lengthen at the expense of the arms, and the pachytene loops appear next. A series of cells from the same tubule is shown in Plate II, figs. 12, 13, 14a, 15a, 17, and Plate III, fig. 18a.

(b) The pairing leptotene threads, examined in detail, show a beaded structure, and chromomeres of one are comparable in size and position with those of its neighbour (Pl. II, fig. 16).

(c) The number of Y-stems during zygonema and of free ends during pachynema shows that the chromosome number at this period is exactly haploid (Pl. II, fig. 17).

(d) There is no evidence of a continuous spireme in the pachytene stage, or of the subsequent appearance of univalents which unite end to end. Cells about the stage of Plate III, fig. 20, with comparatively smooth, paired chromosomes lying peripherally in the nucleus, may give the impression that a continuous spireme is splitting longitudinally. But when the complete cell is seen in one section numerous free ends are always visible. No indication of end-to-end syndesis can be found.

VIII. THE SIGNIFICANCE OF TWISTING DURING LEPTONEMA.

It has been noted that the pairing threads of the first prophase often show spiral twisting about each other as they become apposed side by side in pairs. Homologous chromosomes are united first at their ends, and contact takes place from each polarised end towards the middle. The cells of Plate II, fig. 16, are mainly the distal parts of nuclei in zygonema, and twisting is obvious. In some instances (Pl. II, fig. 16, e, g, h) the thick pachytene stem which results from the union of two leptotene threads is shown, and an appearance of complete fusion is presented.

Whether actual fusion takes place during pachynema is a doubtful point. Plate III, fig. 18b, showing no signs of duplicity, is typical of most cells, but this may be the result of imperfect technique, for in a proportion of cells the dual nature of the chromosomes is evident even in an advanced zygotene or pachytene stage (Pl. II, figs. 15a, 15b; Pl. III, fig. 18a). It has been suggested by Wilson (1925, p. 555) that apparent fusion may be due to a close twisting together of the leptotene threads, but he adds that “on the whole there is little to support the hypothesis of a synaptic twisting.” While the results of most investigators are negative, such twisting has been recorded by Grégoire (1907), Agar (1911, Lepidosiren),
Bolles Lee (1911, *Helix*), and Gelei (1921). Janssens (1906) clearly figures the condition in *Batrachoseps* but does not describe it.

After pachynema the homologues separate while still relatively rough and uncontracted (Pl. III, fig. 19), and twisting is at once evident. Further contraction leads to the smoother strepsitene figures being formed (Pl. III, fig. 21).

In Axolotl, therefore, the evidence is that syndetic twisting takes place from the onset of the apposition, and the true strepsitene stage is to be regarded as merely the last and most obvious phase of the process.

The importance of twisting of the homologues while still in early prophase lies in the support which this gives to the chiasmatype theory as a possible explanation of the mechanism for the segregation and recombination of mendelian factors. The relatively long time which parasyndesis occupies in the cycle, and the intimate nature of the union, suggest that here is an opportunity for the interchange of chromatic substance concerned with hereditary transmission.

IX. GENERAL CONCLUSIONS CONCERNING MEIOSIS.

The main purpose of this paper is to emphasise that the mode of syndesis is a side-by-side pairing, this probably being true for all urodeles despite the recent account upholding metasyndesis. The latter mode is now largely discredited, particularly for animals, and when an attempt is made to harmonise cytological facts on the one hand with the truths of genetics and evolution on the other, parasyndesis alone permits of a reasonable explanation. This discussion of the intimate nature of meiosis excludes considerations which would arise if metasyndesis were admitted.

The actual causes, of which the obvious results are syndesis and meiotic division, are quite unknown. Much guesswork has been indulged in, and hypotheses depending on chemical variations or physical differences of sign or potential between homologues have been produced. It is not intended to enter into these theoretical arguments, but it seems that a useful purpose would be served by a concise restatement of such general conclusions as can be drawn from the known facts of meiosis.

The whole process can be stated in terms of attractive forces, successive stages being the result of increasing or decreasing intensity of these attractions.

Firstly, chromatin particles of like nature are mutually attractive. This is evident in two ways:

1. Minute pieces of chromatin concentrate during prophase first of all into chromomeres, which then condense to form chromosomes.

2. Homologous chromomeres are drawn together in parasyndesis, the result being that homologous chromosomes lie side by side with the same orientation.

In very early prophase, when chromomeres are first becoming distinct in the nucleus, each is joined indiscriminately to those around it by very faintly-staining threads. As prophase proceeds and the attractive force between like pieces of chromatin intensifies so does the thread of "linin" on which the chromatin of each chromosome is suspended become more visible. In addition, there are visible lines connecting homologous chromomeres, and the original network between unlike chromomeres remains very faint. This seems to indicate that the attractive force between chromatin and chromatin produces a definite staining chemical substance, the amount produced varying with the degree of similarity of the chromatin particles.

A third evidence of this type of attraction is seen in the ordinary mitosis of many animals,
notably the Diptera, where maternal and paternal chromosomes lie close together on the metaphase plate.

Chromatic attraction reaches its peak in metaphase, when chromosomes are condensed to their utmost, and thereafter diminishes. This diminution is made evident by the separation of homologous chromosomes. Somatic nuclei of early blastomeres of *Ascaris megalococephala* provide further evidence, for fragmentation of the chromosomes begins in anaphase; and the second spermatocyte division may occur in the first anaphase, as in Axolotl.

Unusual and abnormal cases provide interesting evidence concerning the attraction which exists between homologues. In triploid, tetraploid, and polyploid cells of *Drosophila* the chromosomes come together in groups of three, four, or more; and in the hemipter *Metapodius formatus* Wilson (1910) has shown that the *m*-chromosome alone is trivalent, and that the three elements unite in synecosynthesis. This evidence seems to rule out the possibility, which has been suggested, that there is in the homologues a difference of electric charge or chemical constitution which requires to be satisfied by their union in pairs.

Frequent references are found to cells undergoing meiosis which seem to turn "sick," lose their power of chromatic attraction, and arrest their progress before division is complete. Such a cell is figured in Plate II, fig. 15b, which has remained in prophase and enlarged while its neighbours in the cyst continued to divide. Variations in the intensity of attraction have been accomplished experimentally by subjecting gonad nuclei of *Ascaris* to radium treatment.

Secondly, there is attraction between centrosomes and chromatin, also evident in two ways:

1. The pairing leptotene threads of early prophase, at first a meaningless tangle, gradually become orientated with respect to the centrosome. There exists an attractive force which causes polarisation of the ends of the chromosomes towards the centrosome, and synecosynthesis begins at these ends and progresses towards the middle. This attraction is a means which materially aids syndesis and disentanglement of the leptotene threads. There is no probability of any direct organic connection between centrosome and chromosomes, and the nuclear membrane intervenes.

2. As the intensity of the attraction between homologous chromosomes is diminishing, the attractive power of the centrosomes relatively increases, and the haploid groups pass to the poles.

In polarisation it is the free ends of the chromosomes which are attracted towards the centrosome; in anaphase the apex of the V-shaped chromosome, which is the point of attachment to the spindle, leads the way and the ends separate last. This seeming change in intensity of attraction between the parts of the chromosome and the centrosome may be explained by the fact that the ends of the chromosomes, having remained longest in synecosynthesis, establish a more intimate and lasting attraction for each other, and so the chromosomes separate near the middle first and pass to the poles as V-shapes.

The centrosomes also exercise control over the cytoplasm, and astral rays and spindle fibres are formed. The old comparison with a magnetic field in which steel particles take up their position with their long axes along the lines of magnetic force would appear to be perfectly valid, although of course the attractive force within the cell cannot be called magnetic.

The meiotic division, as it is understood from the foregoing account, accomplishes four things. The first two of these tend towards exact reproduction from generation to generation, and consequent stability of the species; the second two lead to those slight variations in
the offspring, the raw materials which, sorted in the sieve of natural selection, produce evolutionary change.

1. Accurate pairing of homologous chromatin particles due to mutual attraction.
2. Separation of the diploid chromosome group into two homologous haploid ones.
3. Possibility of crossing-over of elements within the linkage groups.
4. Exchange of some whole maternal and paternal homologues so that daughter nuclei have a mixed parentage.

X. Summary.

1. The haploid chromosome number in the Axolotl is fourteen plus an accessory chromosome, which usually divides in the first maturation division.
2. Amitotic division does not occur among primary spermatogonia.
3. The first maturation division is meiotic and is preceded by parasyndesis.
4. The pairing homologues twist about each other from the very onset of syndesis, and equivalent chromomeres along their length are brought together during the process.

The author is greatly indebted to the Carnegie Trustees for the Universities of Scotland for a post-graduate scholarship during session 1933–1934.
REFERENCES TO LITERATURE.

(Only essential references are listed here. All others can be found in The Cell in Development and Heredity, by E. B. Wilson, New York, 1925, pp. 1145–1203.)


DESCRIPTION OF PLATES.

(All figures were drawn with the Abbé camera lucida, using a Winkel 1/12 oil-immersion objective and No. 4 eyepiece. Different shades of chromatin indicate different planes in the nucleus. Drawings are all from Heidenhain preparations with the exception of 23c and 23d, which are from Feulgen preparations.)

PLATE I.

Figs. 1–10. Spermatogonial Division.
Fig. 1. Primary spermatogonium with polymorphic nucleus, surrounded by nuclei of connective tissue capsule. The sphere is visible in the cytoplasm.
Fig. 2. Polymorphic spermatogonium showing apparent amitosis.
Fig. 3. Spherical resting nucleus, with masses of chromatin and conspicuous nucleoli.
Fig. 4. Early prophase; chromosomes forming from masses.
Fig. 5. Later prophase; spireme beginning to form.
Figs. 6a and 6b. Late prophase with smooth peripheral spireme. The same cell is drawn in two consecutive sections. Fig. 6b shows the aster.
Figs. 7a, 7b, and 7c. Metaphase; the same cell in three consecutive sections, showing twenty-eight chromosomes arranged on the equatorial plate. Most chromosomes show a longitudinal split, and one pair in fig. 7a has separated.
Fig. 8. Side view of metaphase. The centrosomes appear granular.
Fig. 9. Anaphase.

PLATE II.

Fig. 10. Telophase, showing intercellular bridges and mid-bodies.
Figs. 11–27. First Maturation Division. Fig. 28. Second Maturation Division.
Fig. 11. Resting stage, before growth period.
Figs. 12, 13, 14a, 15a, 17, and 18a are successive in the same section.
Fig. 12. Prophase; scattered chromatin with faint indication of lines and a prominent nucleolus.
Fig. 13. Prophase; early leptotene, with first indication of polarisation.
Figs. 14a and 14b. Prophase; leptotene showing V-figures.
Figs. 15a and 15b. Prophase; zygonema showing Y-figures.
Fig. 16. a–h. Prophase; pairing and twisting leptotene threads.
Fig. 17. Prophase; the cell is cut at right angles to previous figures so that a polar view of twenty-eight cut ends of pachytene chromosomes is obtained.

PLATE III.

Figs. 18a and 18b. Prophase; pachynema, fig. 18b showing the cross-connections clearly.
Fig. 19. Prophase; early diplonema.
Fig. 20. Prophase; late diplonema, with smoother chromosomes.
Figs. 21a and 21b. Prophase; strepsinema.
Fig. 22. Very early metaphase with nuclear wall breaking down.
Fig. 23, a–d. Metaphase; equatorial plates in pole view.
Figs. 23a–23d. Show fifteen pairs of chromosomes (the clear pair in 23b has been added from a neighbouring section).
Fig. 24. Metaphase in side view, showing two well-marked centrosomes and spindle with stronger fibres passing to points of attachment of chromosomes.
Fig. 25. Anaphase, with anaschistic chromosomes.
Figs. 26a and 26b. Telophase; intercellular bridges.
Fig. 27. Resting stage with chromatin arranged in rough masses.
Fig. 28. Metaphase of homotypical division, with fifteen split chromosomes. (The clear one is added from the next section.)
R. Carrick: "Spermatogenesis of Axolotl."—Plate I.
R. Carrick: "Spermatogenesis of Axolotl."—Plate II.
R. Carrick: "Spermatogenesis of Axolotl."—Plate III.
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