running into the top bowl and trickling down to those below, has proved successful and economical of space. As maturing females lay eggs, the clusters can be seen through the glass. The females may be detached, the eggs removed, and the animals replaced to lay again. (Conklin in Galtsoff *et al.* [*2*]).

Growth and sexual activity are reduced to a minimum at temperatures below about 15° C. It is possible that reproduction might be induced during the winter if running sea water were warmed as described by Loosanoff (*3*).

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Egg Capsules of River Limpet Snails: Material for Experimental Biology

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In the literature of experimental embryology and sexual physiology there are only a few reports of studies made on representatives of pulmonate gastropods. During the last few years, Raven and his co-workers at the University of Utrecht, Holland, have made a comprehensive investigation of the development of the pond snail, *Lymnaea stagnalis* L. (*8*), to get a better insight into the determination processes in animals with so-called mosaic development. In order to make a detailed analysis, the Dutch scientists at first studied oviposition and normal development. Further, some preliminary experiments were carried out on the earliest stages of development of the egg.

In 1941, I began to study the transparent egg capsules of fresh-water pulmonate gastropods. By 1942 the capsule of the patelloid *Ancylus fluviatilis* Müller had proved particularly useful in studying embryonic development.

The egg capsules of *Ancylus fluviatilis* Müller and *Ancylus fuscus* C. B. Adams are almost unknown. As it is impossible to remove the capsules without injury from the substratum, only a few superficial descriptions and exceedingly primitive sketches are to be found in the literature (*2–6*). By following a special technique, it is now possible to describe in detail the capsules of pulmonate gastropods in fresh water.

The breeding season of *Ancylus fluviatilis* Müller extends from April to the middle of August. The capsules are laid on the undersides of stones mostly in running streams, but sometimes in lakes with flowing water. The form of the capsule is very well suited for such a habitat, being slightly domed and sloping a little more toward one side (Fig. 1). Like all other capsules laid by pulmonate gastropods in fresh water, the *Ancylus* capsule is transparent, and all phases in the development of the embryo can be followed easily. During the laying of the capsule the animal rotates so that the capsule becomes a spiral, in which the terminal point overlaps the initial one (Fig. 1), in a way similar to that of *Planorbis*.

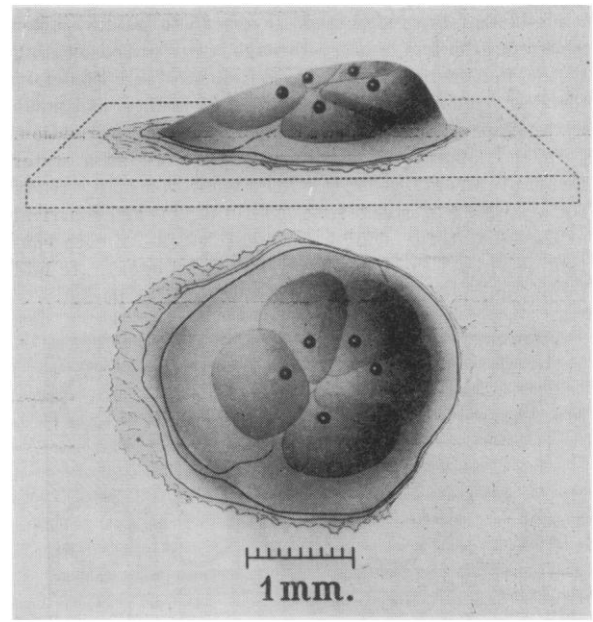

Fig. 1. Egg capsule of *Ancylus fluviatilis* Müller spawned on a glass slide in a wire-netting container. Upper: side-view. Lower: top view. Egg No. 1 is situated farthest to the left just above the initial point. The outermost irregular outline of the capsule limits the secretion from the foot gland, the quaternary envelope. The lid opens to the right along the farthest of the two side-running lines. The hinge of the lid is where the terminal part covers the initial point of the capsule (to the left on the figure).

As shown by the author in a more detailed paper (*1*), it is possible to classify nearly all pulmonate-gastropod capsules in the following way: Snails with the genital aperture on the right side will lay capsules turning to the left (counterclockwise) as in *Lymnaeidae*. Those with the genital aperture on the left side will turn in the opposite direction (clockwise) as in *Physidae*, *Planorbidae*, and *Ancylus*. The terminal point of a capsule, as a rule, is elongated into a thin tail (*exitus terminalis*), and it is accordingly possible to orient a capsule in the correct way and define the beginning and the end of the egg mass. With the pulmonate gastropods, however, it is very difficult in most cases to number the eggs in the order of their appearance.

Ancylus fluviatilis Müller and related forms, e.g., *Ancylus fuscus* C. B. Adams, are the only species in which it can be precisely determined which egg is laid first, second, etc., since the eggs are always in one row. It is useful, when determining the kind of abnormality of an egg or its content, to designate the ordinal number for each egg in the series.

[1] My thanks are due to Mr. Percy G. Bird, Aughton, Lancs., England, and Prof. A. G. Huntsman, Department of Zoology, University of Toronto, Canada, for their suggestions.

The capsule of *Ancylus fluviatilis* Müller is typical of a sessile gastropod inhabiting a locality exposed to strong water currents (cf. egg mass of *Acmaea testudinalis* Müller [7]). In such forms the eggs are placed in one layer fastened to the substratum by a special membrane. This quaternary envelope (in addition to the three formed by the egg cell, by the follicular epithelium, and by the efferent duct) within the mollusc is observed for the first time in these patelloid snails and proves to be a product of the foot gland. From 25 to 30 days after spawning, the larvae hatch through a preformed opening in the top of the capsule, a special structure found in the *Planorbidae* and *Ancylus fluviatilis* Müller, and made by an impression of the foot during capsule formation.

In the laboratory, spawning takes place at a water temperature of 10°–12° C. The snail is usually ready for spawning a short time (8–10 days) after arrival at the laboratory. Only a few capsules (4–7), each containing from 1 to 10 eggs (averaging 2.6 eggs), are laid by an individual snail during a reproductive period.

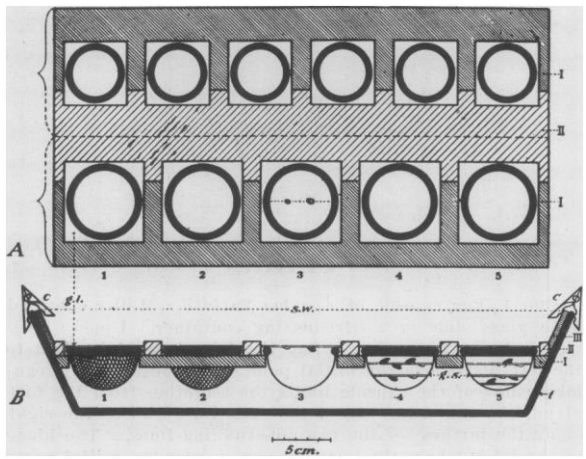

FIG. 2. Apparatus for observing oviposition of *Ancylus fluviatilis* Müller. *A*: Top view. I—Teak plates carrying wire-netting containers. II—Teak bar for fastening the glass lids, *g.l.* *B*: Longitudinal section. 1 and 2—The wire-netting containers hanging in the teak plate. 3—Median section with a specimen and an egg capsule on the glass lid. 4 and 5—Tangential section showing snails and capsules on glass slides resting on the walls of the strainers. III: Diagonal list which, by means of a clothespin, *c*, fastens the apparatus in the tray, *t*; *s.w.*—surface of current of running water.

The best results are obtained when specimens are placed in a special apparatus, shown in Fig. 2. The principle of this apparatus is to force the snails to oviposit on glass in order to get living preparations for microscopic examination. The apparatus is so constructed that the specimens will be able to spawn only on the thin glass plates, which can be easily cut into suitable pieces. In rectangular teak plates, 11 × 43 cm, a series of regular circular holes is cut, into which wire-netting containers (strainers) in two sizes (diam 6.5 cm and 5.3 cm) are fitted. In these containers pieces of glass slides are placed in two or three tiers resting horizontally on the rounded, sloping slides of the strainers.

TABLE 1

Number of abnormal eggs and egg cells	1943	1944	1945
	47	97	117
	%	%	%
Dwarf eggs, normal developed egg cell ...	32	19	25
Dwarf eggs, undeveloped egg cell	6	5	1
Dwarf eggs, without egg cell	28	37	5
Normal egg, undeveloped egg cell	6	13	24
Normal egg, without egg cell	13	11	23
Crushing of egg cell	5	5	8
Egg with two egg cells	2	6	3
No egg but egg cell	2	2	8
Neither egg nor egg cell (empty capsule) .	6	0	2
Gigantic egg	0	2	1

Finally, the teak plates with the strainers hang down into a deep tray (a dissecting tray), *t*, in a current of running water. In order to prevent the wooden apparatus from floating, it has to be fixed to the end walls of the tray.

By standing the apparatus in the laboratory sink, it is possible to move a long-armed binocular microscope over the horizontal glass lids and so observe the spawning and other biological habits of the animals. For studying egg capsules under the microscope, the small glass slides with capsules on them, identified by numbers scratched on by means of a glass cutter or a diamond, are put into a Petri dish half-filled with water.

For experiments with capsules under different conditions, the glass slides can be put into special small Petri dishes. Instead of running water, air is conveyed through tubes to each Petri dish, where it is released in the form of bubbles to freshen the water and provide a steady circulation.

TABLE 2

POSITION OF DWARF EGGS COMPARED WITH OTHER EGGS WITHIN THE CAPSULES OF *Ancylus fluviatilis* MÜLLER

Position in the capsule	Frequency			Avg
	1943	1944	1945	
	%	%	%	%
No others	16	14	18	16
Beginning	19	20	34	24
Middle	0	0	0	0
End	65	66	48	60

Finally, for studying egg cells and embryos in capsules, the glass slides are placed in such a way that the capsule hangs inverted in a small cell or chamber with water. Hanging-drop slides can be used for this purpose, but it is better to use a glass ring mounted on a glass slide, forming a chamber filled with water.

The egg (egg cell + perivitelline fluid and *membrana interna*) of *Ancylus fluviatilis* Müller has been shown to be the biggest, in comparison with the size of the adult, among the eggs of fresh-water pulmonate gastropods. Only the eggs of the much bigger *Planorbis corneus* L. and *Myxas glutinosa* Müller are slightly larger in absolute size, and those of *Lymnaea stagnalis* L. are about the same size. My experiments have shown that a

larger amount of albumen at the disposal of the embryo is of special value in giving it a better start in life outside the capsule.

Three years of laboratory culture of *Ancylus fluviatilis* Müller have shown that this species has a higher percentage of abnormalities (20%) in the eggs than any other species examined. The frequency of the different kinds of abnormal eggs and egg cells is shown in Table 1.

Most common in all three years are dwarf eggs, smaller than ordinary size (1.42 × 1.20 mm). Dwarf eggs may be a phenomenon definitely connected with the starting or stopping of oviposition. In both series of eggs, those throughout the egg-laying period and those in a single capsule, dwarf eggs are found mainly at the end of each series, but partly at the beginning. The percentages in which they occur in different positions within the capsules are given in Table 2.

The considerable number of dwarf eggs and other irregularities given in Table 1 also make *Ancylus fluviatilis* Müller an interesting subject for studies of the earliest phases of embryonic development. Even when still *in ovo* the embryos may meet with various influences that interfere with their normal development—e.g., variations in the quantity of albumen, crowding because of abnormal development of a neighboring embryo or accidental formation of "nurse eggs" (competition), or abnormal development of the egg cell itself, determined by intrinsic factors (e.g., lack of fertilization) or extrinsic factors (e.g., crushing of the egg cell during the formation of the capsule).

The advantages of the egg capsules of *Ancylus fluviatilis* Müller as experimental material can be summarized as follows. The limpet is a suitable animal for studying oviposition and embryogenesis under laboratory conditions. The apparatus described makes it possible to obtain living eggs, laid in transparent capsules, for microscopic investigation of living preparations. The capsule is easily turned, and the eggs can be studied in series exactly in the order in which they were laid. The frequency of abnormalities of several sorts in the eggs make the capsules particularly well suited for studies of cell physiology and other aspects of experimental biology.

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Presence of Iodinated Amino Acids in Unhydrolyzed Thyroid and Plasma[1]

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De Robertis (*1*) demonstrated the presence of a proteolytic enzyme in the colloid of the thyroid follicle. He postulated that this enzyme causes the breakdown of thyroglobulin with the release of biologically active fragments which are of low enough molecular weight to diffuse out of the follicle, and which presumably constitute the thyroid hormone. The only compound of small molecular weight found so far in unhydrolyzed thyroid is thyroxine (*4*). In the present work, the technique of paper chromatography has been used to confirm this result, and to study the production of other iodinated amino acids which might be formed during the breakdown of thyroglobulin. The appearance of these substances in the circulation has been studied by observing their presence in *n*-butanol extracts of plasma. In addition, chromatographic analysis of whole plasma has supplied evidence as to the nature of the circulating thyroid hormone.

Methods. Female albino rats weighing 150–200 g and maintained on Remington's low iodine diet No. 342 (*5*), to which 10% brewers' yeast had been added, were injected subcutaneously with about 100 μc of carrier-free radioactive iodide (NaI^{131}). After 48 hours the animals were anesthetized with ether and exsanguinated with a heparinized syringe via the inferior vena cava.

Chromatography of n-butanol extracts of thyroid and plasma. The thyroids were removed immediately, ground in 1 ml of ice-cold saline in a chilled mortar, and extracted three times with an equal volume of *n*-butanol. Similarly, the plasma was extracted three times, first with a double volume and then twice with an equal volume of butanol. In both cases, the combined butanol extracts were reduced to dryness *in vacuo* at room temperature. The dry residue was taken up in distilled water, 0.1 ml and 0.2 ml being used for the thyroid and plasma residues, respectively.

Aliquots of 0.02 ml of this solution were then chromatographed by the capillary ascent method of Williams and Kirby (*10*), with or without the addition of 20 μg of each of the following carriers: DL-thyroxine, DL-diiodothyronine, DL-diiodotyrosine, and DL-monoiodotyrosine. The aliquots were placed in the lower left-hand corner of 10 in. × 12 in. Whatman No. 1 filter paper sheets and

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