crystals was confirmed by means of the radioautograph technique.

The yield of crystalline material was sufficient to permit a countercurrent analysis for vitamin B_{12} content based both on color (5500-A band intensity) and on radioactivity distribution. The fraction of total color distributed in the fourth and fifth tubes, where vitamin B_{12} concentrates, was equal within experimental error to the fraction of total radioactivity found in these tubes. This correspondence between color and radioactivity definitely establishes the identity of the radioactive vitamin B_{12} with the normal vitamin. The intensity of absorption at 5500 A in the fourth and fifth tubes amounted to 22% and 19% of total, and the corresponding radioactive cobalt content was 23% and 18.2%, respectively, as compared to theoretical values of 29% and 24.7% for the pure vitamin. Color distribution results thus indicate a purity of 76% for both tubes, while the activity measurements show that 79% and 73% (average 76%) of the radioactive cobalt is present as vitamin B_{12}. As judged by the color and activity distributions and the absorption spectrum of the crystals, the second component (also radioactive) appears to be largely vitamin B_{12a} (*4*) or vitamin B_{12b} (*5*).

TABLE 1

COUNTERCURRENT DISTRIBUTION OF CRYSTALLINE RADIOACTIVE VITAMIN B_{12}

Tube	Water phase % of total		Benzyl alcohol phase % of total	
	Color	Activity	Color	Activity
1	1.9	2.5	1.1	1.0
2	5.9	6.2	4.6	4.2
3	11.7	11.4	9.8	10.0
4	14.6	15.3	12.9	12.5
5	11.9	12.1	10.6	10.2
6	6.4	5.6	5.4	5.2
7	1.4	1.5	1.6	1.7
8	(0.1)	0.2	(0.1)	0.4

In view of this preliminary investigation, larger scale fermentations were conducted and about 100 mg of high purity, crystalline, radioactive vitamin B_{12} was isolated. The absorption spectrum of the product was characteristic of the normal vitamin in all its detail (*1, 2*) and indicated a vitamin B_{12} content of 97%. The results of an 8-stage countercurrent distribution analysis are reported in Table 1, which lists the percentage of total color and of radioactivity found at each stage and in both phases.

This distribution of radioactivity is identical within experimental error with the color distribution and corresponds closely with that expected for vitamin B_{12}, thus definitely establishing that the preparation is truly radioactive vitamin B_{12}. The activity of the present crystalline product is $\simeq 0.25$ μc/mg. By starting with cobalt sulfate of higher specific activity than employed in this work, radioactive vitamin B_{12} of much higher specific activity than 0.25 μc/mg can be produced.

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Culturing *Crepidula plana* in Running Sea Water

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The simple method of culturing *Crepidula plana* described here is designed to prevent waste of material and lessen the expense of collection at seaside laboratories, where the eggs of this small gastropod are in demand for embryological work.[1]

The shells of hermit crabs may contain attached communities of *C. plana* numbering several dozens of individuals of all sizes and sexual phases from tiny spat to old females. Immature specimens and males are more numerous and are usually discarded and lost, although they are all potential egg-producers. If all the individuals are detached and transferred to 4-in. finger bowls under running sea water they will attach themselves to the glass, and the young will grow to maturity and provide eggs, requiring no more attention than an occasional dumping of debris from the bowls.

At Woods Hole, males removed from the communities in June and cultured may reach the adult female phase by the end of August. Small spat, 2–5 mm in length, may not produce eggs until the next summer season, but these animals survive the winter with very low mortality. The natural tendency to community sex adjustment in *C. plana* insures that any artificial community, unless it consists exclusively of adult females, will eventually contain both sexes. It is immaterial whether it is started with sexless young, immature males, adult males, regressive males, or any mixture of these phases. Adult females should be accompanied by small individuals which will develop the male phase and fertilize the eggs.

The number of individuals which may be cultured in one dish is limited only by the area available for attachment and growth. Dense crowding limits size and increases the proportion of males. A continual movement of unfiltered sea water over the animals is important, since it provides the microscopic food organisms necessary. A stack of finger bowls separated by squares of coarse galvanized iron wire netting, with the sea water

[1] Other workers have conducted laboratory experiments on a related species.

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.*

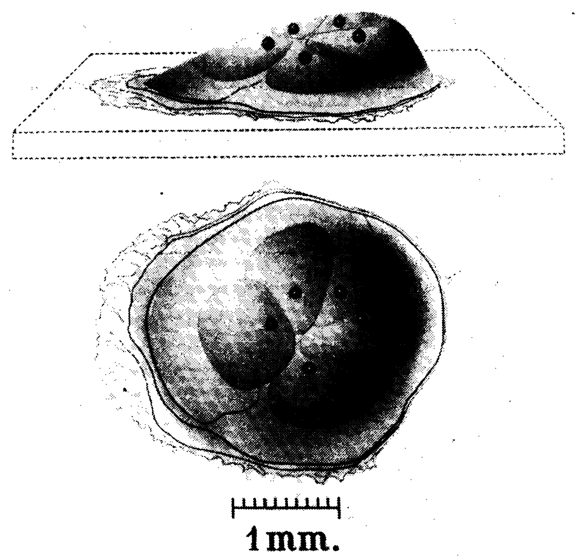

Fig. 1. Egg capsule of *Ancylus fluviatilis* Müller spawned on a glass slide in a wire-netting container. Upper: side-view. Lower: top view. Egg No. 1 is situated farthest to the left just above the initial point. The outermost irregular outline of the capsule limits the secretion from the foot gland, 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.

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