of rubber tubing which could be opened or closed with steel spring clips. Before the ampoules were inoculated, the manifold with the arms closed was connected to a 'Speedivac' pump with a phosphorus pentoxide trap. By means of a small leak in the manifold, the pressure was adjusted to *c.* 10 mm. mercury.

The suspensions used for the inoculation of the plugs were prepared by diluting nutrient broth cultures of the organisms 1 : 250 with 5 per cent glucose. Cultures at or near the end of the logarithmic phase were used. By means of a capillary pipette tipped with paraffin wax to which a syringe was attached, a single drop (*c.* 0·02 ml.) of the suspension was discharged on to the surface of each plug. As soon as each ampoule had been inoculated it was connected to a manifold arm which was then opened. Usually, 12–18 ampoules were inoculated at a time. Approximately 5 min. after the last ampoule had been connected, the leak was closed and high vacuum (*c.* 0·01 mm.) was established. The ampoules were taken off one at a time, constricted, replaced on the manifold and finally sealed off *in vacuo* after 1 hr. The ampoules were stored at room temperature in the dark.

The immediate effect of the inoculation of the freeze-dried plug is a localized viscous solution of the solids. Wetting of the entire plug is prevented by subsequent rapid removal of water by the pump. The exposure of the plugs to the higher pressure (10 mm.) induces a mild foam which may rise 1–2 mm. above the surfaces of the plugs; at the lower pressure (0·01 mm.) dehydration of this viscous foam proceeds very rapidly.

Some preliminary experiments have shown that the method may be simplified where percentage estimates of survival are not required. Smaller plugs (0·25 ml.) in tubes of ¼ in. diameter have been 'stab' inoculated by means of a straight wire charged with bacterial suspension. With such modifications no visible foaming of the peptone has occurred under reduced pressure, and drying *in vacuo* has been achieved by exposure of the plugs to high vacuum as soon as they have been inoculated.

At present a number of factors are being investigated in relation to the method. Meanwhile, it is of interest that the survival-levels shown in these early experiments compare favourably with those obtained by previous drying methods.

I am indebted to the Medical Research Council for a personal research grant.

D. I. Annear

Department of Pathology,
University of Cambridge.
May 4.

[1] Fry, R. M., and Greaves R. I. N., *J. Hyg. Camb.*, **49**, 220 (1951).

Egg-laying in Ctenophora

While occupied with field-work on Siphonophora, I placed six small *Pleurobrachia* sp. measuring about 2·5 mm. in length in a watch-glass at 1100 hr. on April 29. An hour later I noticed that eggs were being extruded through the ectoderm over the gastrovascular canals. Since the fertilization membrane was present as the eggs were being extruded, it would appear that fertilization took place inside the meridional canals. By 1430 hr. the first and second cleavages of the majority of the eggs were taking place as in *Beroe*. By 1830 hr. the numerous micromeres were covering the macromeres by epiboly and many of the larvæ had reached the stage of gastrulation. Within twenty-four hours of extrusion the larval ctene-plates could be seen beating within the egg envelope. The same phenomenon was witnessed with another batch of *Pleurobrachia* on May 7, when less than half the eggs segmented.

A recent text-book states that ripe sex cells in Ctenophora are discharged through the mouth except in *Coeloplana* and *Ctenoplana*, in which the testes open on the aboral surface by ducts.

I am informed by Miss M. A. Harvey, who confirmed my observations of April 29, that she has observed eggs being extruded through the ectoderm in a similar way in *Beroe ovata* and *Lampea pancerina*; but in the former, development of the eggs did not take place, while the eggs of the latter were not kept.

A. K. Totton

Station Zoologique,
Villefranche-sur-Mer,
Alpes Maritimes,
France.
May 7.

Permeability to Water of the Lamprey Integument

The relationship between the anadromous lamprey, *L. fluveatilis*, and the purely fresh-water form, *L. planeri*, has been the subject of much discussion, and doubt has sometimes been expressed as to whether they should be regarded as distinct species. As Weissenberg[1] showed, although there are profound differences in the biology of the two forms, there are no important morphological criteria (other than size) by which they may be differentiated. It is therefore of especial interest to find that significant physiological differences exist between these species in regard to the permeability to water of the integument.

The rate of osmotic uptake in fresh water has been determined by weighing at intervals animals in which the urino-genital papilla has been ligated. In the sexually mature animal, the possibility of water absorption in the gut can be excluded, since the foregut is no longer patent. Expressed as percentage increases of weight, the mean rate for *fluveatilis* was found to be 1·1 per cent per hr. in tap water at 13·5° C., compared with 5·1 per cent per hr. for *planeri* in tap water at 12° C. As measured by the thermoelectric method, the osmolar concentration of the blood of *fluveatilis* at the time of the experiments varied from 135 to 150 mM with a mean value for six animals of 143 mM; the corresponding mean value for a large number of determinations on *planeri* was 110 mM. The osmotic gradient in tap water is therefore rather greater for *fluveatilis*, and hence the difference in permeability between the two forms would appear to be even greater than is indicated by the rates of uptake of water.

The rate of flow of urine implied in these figures is, for *planeri* at least, far in excess of that recorded for any other aquatic vertebrate in fresh water, and even the reduced rate in *fluveatilis* is about twice that of the eel[2], the skin of which bears a resemblance to that of the lamprey.

These results are in general agreement with those obtained for the rate of loss by osmosis in hypertonic solutions. In Fig. 1, the mean percentage hourly