the fat prior to beginning the treatment with KOH. Of the several common fat solvents tried, acetone was found most satisfactory. It acts quickly and does not injure the tissue or affect its clearing and staining qualities.

The material is first thoroughly fixed in 95 per cent. alcohol and then transferred directly to acetone, being left there for several days, depending on the bulk of the object being treated. Following this treatment, the specimen is transferred directly back to 95 per cent. alcohol for twenty-four hours or longer. After washing in alcohol, the clearing and staining with alizarin may be carried out routinely. In our work we are using a modification of the alizarin method, which results in a progressively selective staining of the bone (Dawson, 1926).[2]

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MACERATION OF GREEN HYDRA

IN working with *Hydra viridis* I hit upon a method of maceration that gave results which were better than those obtained by other recorded methods.

For the benefit of those who may be interested, the method is given below.

This involved a fixing fluid composed of equal parts of 40 per cent. formalin, 95 per cent. alcohol and glacial acetic acid. This fluid has been devised and used by Mr. J. B. Looper in the fixation of certain protozoa here in this laboratory. This mixture is placed in a vial that has a mouth of from one half to three fourths inches in diameter. Place the Hydra on a microscopic slide in a very small drop of water. Then draw off as much of the water as possible with a fine pipette, leaving only a film of water surrounding the specimen. Invert the slide over the vial containing the above macerating mixture, in such a manner as to completely cover the mouth of the vial, for eight to ten minutes. At the end of about ten minutes—some specimens requiring a shorter time than others—remove the slide from the vial and add one or two drops of water and draw off. Add water the second time and draw off. Then add a drop of 40 per cent. glycerine. Tease or break up the Hydra with fine needles. Apply the coverglass and examine. If the cells are not separated sufficiently, gently press the coverglass with a needle; however, care should be taken not to crush the delicate cells. At this point, if pressed too hard, the cells are easily smashed and the preparation ruined.

[2] Dawson, A. B., 1926, "A Note on the Staining of the Skeleton of Cleared Specimens with Alizarin Red (sodium alizarin monosulphonate)." *Stain Technology* (to appear in the October number).

Excellent preparations have been made from freshly collected specimens and specimens which have remained living in the laboratory aquaria for as long as two or three months. Many of these preparations show fine cell structure.

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SPECIAL ARTICLES

GROWTH AND TRANSFORMATION OF PARASITIC GLOCHIDIA IN PHYSIOLOGICAL NUTRIENT SOLUTIONS

IN experiments completed at the U. S. Bureau of Fisheries Biological Station at Fairport, Iowa, by us this summer, artificial nutrient solutions were prepared in which the glochidia of the freshwater mussel, *Lampsilis fallaciosa* Smith (known as the Creeper or Slough Sand-shell), were carried through their various developmental stages from glochidium to the free-living juvenile mussel. The glochidia of this species of freshwater mussel are parasitic on the gills of the short-nosed gar, *Lepisosteus platostomus* Rafinesque, for a period varying from two to several weeks, during which time the glochidia undergo marked internal changes and differentiations and emerge from their cysts at the end of this sojourn on the fish as free-living juvenile mussels. The nutrient fluid was perfected so that this period of parasitic life on the fish could be replaced by a period *in vitro,* during which the growth and differentiations ordinarily made by the glochidium in the cyst could be studied and controlled.

The glochidia used in these first series of experiments were dissected out of their cysts on the gills of artificially infected gar, eighteen and ninety-six hours after encystment was begun. The freed glochidia were transferred at once to the solutions in which their development was to be followed. Glochidia removed from the cyst eighteen hours after attachment to the fish gill differed little if at all in appearance from ripe glochidia in the maternal marsupium. Glochidia removed at the end of ninety-six hours showed considerable development of the organ anlagen, although the glochidia were still in a very embryonic stage, as was evidenced by the presence of a large portion of the larval mantle cell mass. In the most favorable solution tested the glochidia were carried through the twelfth day in the solution, at which time their development equalled that of control glochidia which had been carried on the fish and were just ready to emerge from their cysts. When this stage was reached by the glochidia *in vitro* they were transferred from the nutrient solution to river water in

which they made their final transformation in less than a half hour. In actual time the transformation stage was reached by the glochidia *in vitro* more quickly than by the glochidia on the fish, but there may be several factors involved in this comparison. Certainly, however, the growth and development of the glochidia *in vitro* was not delayed.

Juvenile mussels which transformed in these artificial nutrient solutions were kept in river water for three weeks after transformation without the loss of a single individual, the juvenile mussels making excellent growth of both shell and soft parts during that time and seeming in every way to be very vigorous. This was a bit surprising, as there is known to be a rather high mortality, on the contrary, among mussels during the first few days after leaving the fish following the natural parasitic cycle.

The several series of glochidia carried in the various nutrient solutions *in vitro* showed that parasitic life on the fish is not essential to development and transformation if the proper food substances be supplied in the proper environment; that the glochidium receives by its encystment a much-needed protection against certain bacterial and protozoan enemies; and that the glochidium is a true parasite while on the fish, receiving essential food substances from the host fish. This last statement was repeatedly tested in a variety of experiments and the so-called protective physiological solutions containing only inorganic salts were neither adequate to produce growth and differentiation nor to maintain glochidia already well started on their way to transformation.

The successful solutions contained sodium chlorid, potassium chlorid, calcium chlorid, sodium bicarbonate, dextrose and a mixture of amino-acids, together with small quantities of phosphates and traces of magnesium salts. Detailed data of these experiments as well as experiments on glochidia taken directly from the maternal marsupium are to be published.

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CRITICAL POTENTIAL MEASUREMENTS: A CORRECTION FOR HIGH-EMISSION CURRENTS

IN experiments on critical potentials the voltage E_1 (Fig. 1) applied to a filament and grid is usually measured by means of the potentiometer R_1R_2.

When the current I_3 is zero, then

$$E_1 = \frac{R_1}{R_1 + R_2} E \qquad (1)$$

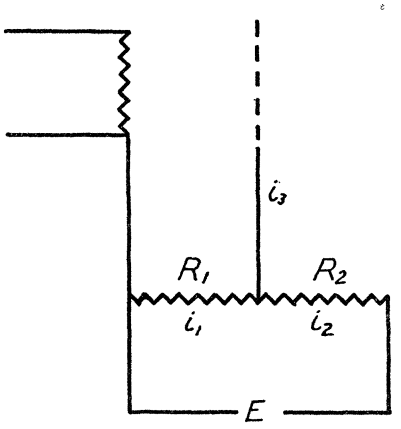

FIG. 1

However, if the current I_3 is large, then

$$E_1 = \frac{R_1}{R_1 + R_2}[E - I_3R_2] \qquad (2)$$

This equation is derived from Kirchoff's first and second laws

$$E = E_1 + E_2 \text{ and } I_2 = I_1 + I_3 \qquad (3)$$

and Ohm's law

$$E_1 = I_1R_1 \text{ and } E_2 = I_2R_2 \qquad (4)$$

Combining equations (3) and (4) and simplifying gives equation (2). In this derivation the laws for networks have been applied to conductors only, and no special assumption has been necessary regarding the resistance of the filament-grid space.

Equation (2) has been tested with a four-electrode tube containing helium. The following table shows clearly that the correction is a necessary one, when I_3 becomes large.

CRITICAL POTENTIAL OF HELIUM AT 0.3 MM PRESSURE

I_3 microamperes	Experimental Critical Potential in Volts uncorrected	corrected
2.4	21.01	21.01
160	21.63	21.24
800	23.50	21.39
	Average	21.21

A further correction for initial electron velocity and contact potential has to be applied to this value.

In another test a portion of the voltage E_1 has been measured with a standard cell, and it was found that equation (2) is correct.

I wish to thank Messrs. R. H. Dalton and W. P. Baxter for making the experimental tests described above.

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