SCIENCE

22.2 per cent. of crude fiber, the residue from this extraction averages 21.9 per cent., but *it must not be inferred* that the two substances are identical.

In a limited search of the literature two pertinent papers by Dr. R. H. Carr were found. In the first, "Structure of Plant Compounds and Solubility,"² it is reported that not much protein dissolves in cold 75 per cent. formic acid. In the second, "Preparation of Transparent Specimens of Leaves, Worms, Bees, Butterflies, etc."³ it is reported that cold 90 per cent. formic acid dissolves most of the proteins of both plant and animal tissues. There are no analytical data in either of these papers.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

EGGS OF FRESH-WATER FISHES SUITABLE FOR LABORATORY STUDIES

BRINLEY and Creaser, in 1931, published a short article on eggs of fresh-water fishes suitable for physiological research.¹ Since that time, one of us (F. J. B.) has continued the study with the hope of extending the list and adding new species that would produce eggs at other times, so that there would be a continuous supply of eggs during the entire year.

Trout and Salmon: Eggs or embryos of the brown (Salmo fario) and rainbow (S. irideus) trout, and the Atlantic salmon (S. solar) have been used by a number of investigators and may be readily obtained from the various state hatcheries during the late fall and early winter months. The eggs in the "eyed" state can be shipped long distances in cool damp moss packed in thermos jugs.

If running, cool spring water is not obtainable in the laboratory, the embryo may be kept until after the yolk is absorbed (March or April) in shallow dishes, such as finger bowls, in a refrigerator, maintained at a temperature of 8 to 10° C. A supply of pond water should be kept in the refrigerator, and water in the dishes should be changed daily. The dishes must be kept clean and all dead embryos removed. The eggs vary in size from 5-8 mm in diameter, and not more than 50 eggs should be kept in a finger bowl. The eggs are covered with an opaque shell which must be removed in order to see clearly the enclosed embryo. It has been found that the embryos develop faster, with less mortality, and that the dishes are more easily kept clean from fungus and scum if the shells are removed as soon as they are received from the hatchery. As the fish develop and the gills begin to function, the water should be filtered to remove any scum or fungus, which, when taken into the mouth of the fish during respiration, will clog the gills and result in suffocation. If this accumulation on the gills does occur, the stringy scum can be removed by gently pulling and working it loose from

² R. H. Carr, Science, 69: 407-8, 1929.

¹F. J. Brinley and C. W. Creaser, SCIENCE, 74: 295-296, 1931.

the gills with dissecting needles and fine pointed forceps. This condition has been erroneously called a fungus disease by several authors.

Great northern or common pike (*Esax lucius*) and the wall-eyed pike (*Stizostedion vitreum*) spawn in the early spring, and their eggs may be obtained from hatcheries during March and April in this latitude and kept under the same conditions as stated above. These eggs are much smaller, averaging about 3 mm in diameter. The wall-eyed pike embryos become very active as soon as the shells are removed, therefore they are difficult to study and their use for physiological investigation is rather limited. The egg of the common pike is well adapted for class and research use.

Eggs of the European carp (*Cyprinus carpio*) may be taken in large numbers by collecting those that are naturally spawned in the shallow water along the shore of lakes or ponds. Spawning takes place in early to mid-morning and the males can be observed chasing the females in the shallow water. It has been reported by Forbes and Richardson² that 400,000 to 500,000 eggs have been taken from a four- to fivepound female. By observing the fish at spawning time, fertilized eggs may be collected shortly after being laid. The adults may also be seined at the time of spawning and the eggs stripped from the female and fertilized by sperm from the male.

The eggs are considerably smaller than the pike, and there is a transparent jelly-like substance between the egg shell and the embryo, which interferes with the removal of the shell. The jelly does not adhere to the embryos and can be picked off after removal of the shell. The long narrow yolk sac is attached almost to the anal opening. The embryos are very active and undergo numerous winding and bending movements of the tail which make them difficult to observe under the microscope.

Dates at which fresh-water fish eggs may be obtained and kept in the laboratory:

³ R. H. Carr, SCIENCE, 83: 355-6, 1936.

²S. A. Forbes and R. E. Richardson, Natural History Survey, 1908.

March and April: Yellow perch (1); wall-eyed pike; common pike. May and June: European carp.

June and July: Log-perch (1); straw-colored minnow (1); common shiner (1). October to April: Trout and salmon.

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ACETO-CARMIN MOUNTING MEDIA

BELLING's aceto-carmin technique,^{1, 2} which has been so valuable for a quick examination of meiotic divisions, has proven to be especially useful in the investigation of the giant chromosomes in the salivary glands of the Diptera. The chief disadvantage of the method lies in the fact that the preparations are temporary and the specimens can not be preserved without further treatment. Often specimens so prepared are too valuable to be discarded and a number of methods have been devised for transforming these temporary preparations into permanent slides. (McClintock,³ Steere,⁴ Buck,⁵ Marshak,⁶ etc.). These methods serve the purpose for which they were designed, but they lose the chief advantage of Belling's original technique, its extreme simplicity and speed.

The aceto-carmin technique can be greatly improved and the preparations made permanent by adding to the fixing solution certain inert substances which do not alter the fixation image but which serve as mounting media when the acetic acid and water evaporate. A number of such substances are available, *i.e.*, dextrose, gelatin, glycerine, gum-arabic, pectin, etc. A detailed investigation of these water-soluble mounting media is now in progress. Two mixtures, however, have already shown their usefulness.

(1) The specimen is macerated on a slide in a drop of Belling's aceto-carmin (a saturated solution of carmin in 45 per cent. acetic acid plus a trace of iron). Then several drops of the following solution are added:

Belling's aceto-carmin	80 cc
Karo Corn Syrup (Dextrose)	10.cc
Certo (Pectin)	10 cc

(The commercial products in the mixture can be purchased at any grocery store.) The preparation is then heated, as in the Belling technique. The coverglass can be pressed down until the specimen has reached the desired thinness. If an excess of the solution is squeezed out around the edges of the coverglass it need not be removed, as it forms an excellent sizing and dries as hard as balsam.

(2) The following mixture may be used alone and

- ² Biol. Bul., 50: 160, 1926.
- ³ Stain Tech., 4: 53, 1929. ⁴ Stain Tech., 6: 107, 1931.
- ⁵ SCIENCE, 81: 75, 1935.
- 6 Amer. Nat., 70: 406, 1936.

thus it reduces the fixing, staining and mounting to a single operation. It may be used undiluted or it may be diluted with Belling's aceto-carmin in various proportions (3:1, 1:1, 1:3, etc.), depending upon the nature of the specimen to be examined. With greater dilutions more fluid should be used to allow for the greater evaporation of water and acetic acid.

Acetic acid (glacial)	50 cc
Water	50 cc
Glycerine	1 ee
Gelatin (powdered)	10 grams
Dextrose	4 grams
FeCl ₃	$0.05 \mathrm{~grams}$
Carmin	To saturation

The gelatin should be dissolved in the water and the other components added. The mixture should be boiled and filtered, just as is done with Belling's solution. As the acid and water evaporate, the medium becomes as firm as balsam and, unlike the familiar glycerine-jelly, it will not liquefy, even when heated to 80° C.

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¹ Amer. Nat., 55: 533, 1921.