In practice, cats have been used as test animals, confined in cat boxes. One electrode is a stout wire bit placed in the animal's mouth, the other a metal plate between the ears, upon an area of fur which has been thoroughly moistened with soap solution. The two electrodes are kept in place by a simple bridle of string. Experiment shows that convulsions are produced at a slightly lower amperage if the speed of the commutator is increased up to 80 contacts per second (the limit available with the apparatus employed) than at lower speeds. The current is turned on for 10 seconds for each test; if the stimulus is above threshold, a convulsion will often begin in less than half this time. As there are no means of predicting accurately what the resistance of the circuit will be, the slider is adjusted to the desired milliamperage after the switch is closed.

The animals appear to be unconscious while the current is flowing, though naturally rigid. The method appears to involve no undue cruelty, and indeed is similar to that used for executing stray animals by some animal protective societies. A convulsion is marked by the persistence of tonic and clonic movements for a variable interval after the current is turned off, dilation of the pupils and subsequent stupor. Each animal has a characteristic threshold—usually between 6 and 15 milliamperes under the conditions stated—which seldom varies spontaneously more than 10 per cent. on any one day, and usually less than 25 per cent. from one day to another. If 5 minutes is permitted between shocks when no convulsion is produced, and an hour after each convulsion before the next shock, several determinations of threshold may be made daily.

The apparatus would probably be almost as efficient and somewhat simpler if a transformer of the "Variac" type were used on an alternating current line through a milliammeter. The difference in effectiveness between a 60-cycle current and the 80 shocks per second used at present can not be great.

The anticonvulsant effect of most of the common drugs has been studied by this apparatus, continuing the work of Spiegel.[6] Under the conditions of the experiment, it is easy to demonstrate that a dose of sodium bromide sufficient to prevent a cat from walking (about 2 gm) will raise the convulsive threshold only about 50 per cent., while a dose of phenobarbital producing similar symptoms (about 0.1 gm) may treble or quadruple it. Cats so protected may survive shocks of an intensity which proves fatal to untreated animals. Comparable doses of other familiar barbiturates have little anticonvulsant activity. This, and the fact that Harrison, Mason and Resnik[7] have produced evidence that the conjugated phenols are responsible for the motor depression of uremia suggested a search among phenyl derivatives as well as among standard hypnotics.

Accordingly, a large number of the less toxic phenol compounds was studied. They included phenyl, cresyl and tolyl sulfonates, benzoates, ketones and esters of such radicals as carbamic, malic, barbituric acids and hydantoin. The compounds which appear to have the greatest anticonvulsant activity combined with the least relative hypnotic effect of those tested so far are diphenylhydantoin, acetophenone and benzophenone.[8]

Whether the drugs found most effective under the conditions of the test will also prove of value in clinical practice remains to be seen. The experimental method appears, however, to constitute at least a provisional index of their activity.

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[6] R. T. Harrison, M. F. Mason and H. Resnik, *Jour. Clin. Invest.*, 15: 463, 1936.

[8] H. H. Merritt, T. J. Putnam and D. M. Schwab. Material in preparation.

EXTRACTION OF THE NITROGENOUS MATERIALS FROM DRIED GRASS[1]

As a part of a quantitative study of the nutritionally essential amino acids in the more important forage plants, it was considered highly desirable if not absolutely necessary to find a method for removing all the protein from such materials.

It is the object of this paper to report that extraction of the air-dry grass in a Soxhlet apparatus with 90 per cent. formic acid brings most of the sample into a solution which includes *all* the nitrogen. This procedure was developed as a result of some experiments in which several reagents were tested by agitating samples of grass at room temperature with successive small portions of each reagent. Ninety per cent. formic acid was found most effective, removing about 88 per cent. of the total nitrogen. Since repetition of this treatment removed further small quantities of nitrogen, it was decided to try the Soxhlet method of extraction. When the charge was mixed with a suitable material to facilitate percolation, such as 50-mesh ground glass, the Soxhlet extraction was completed in three to eight hours. Aliquots of two such extracts, representing 0.2 g of grass, contained 5.21 and 5.07 mg of nitrogen or an average of 100.8 per cent. of that present in the grass. Such extracts are being examined to determine their usefulness in a study of the nutritionally essential amino acids.

It is interesting to note that in a material containing

[1] Contribution from the Bureau of Plant Industry in cooperation with the Bureau of Animal Industry. The possibility of using formic acid as a protein solvent was suggested by Dr. H. W. Titus, of the latter bureau.

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 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 Richardson[2] that 400,000 to 500,000 eggs have been taken from a four- to five-pound 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:

[2] R. H. Carr, SCIENCE, 69: 407–8, 1929.
[1] F. J. Brinley and C. W. Creaser, SCIENCE, 74: 295–296, 1931.
[3] R. H. Carr, SCIENCE, 83: 355–6, 1936.
[2] S. A. Forbes and R. E. Richardson, Natural History Survey, 1908.