or less complete maceration of the musculature of the animal. The nervous system, at least the brain, the spinal cord, the spinal nerves and the limb plexuses, remains intact. The action of nitric acid seems to be sequential in effect, acting first on the bone, then on the connective tissue. The result is the dissolution of the skeletal and muscular organization. The epithelium all over the body, both externally and internally, is soon disintegrated and the viscera do not long retain their organization.

The sympathetic nervous system does not seem to endure the maceration process. On the other hand, the central nervous system, together with the spinal nerves, resists the action of nitric acid for some time, with the result that by simple and careful teasing of the macerated tissue by pointed glass rods the whole nervous system is obtained complete or nearly so, depending on the care used in teasing. Most of the spinal nerves and the central nervous system are composed of relatively heavy myelinated fibers, while the fibers of the sympathetic nervous system are not or only very thinly so. It may well be that the presence of myelin sheaths slows up the action of nitric acid on the spinal nerves and the central nervous system for a long enough time to allow complete maceration of the rest of the body.

In the case of mammals, such as rats and guinea pigs, somewhat more care and apparatus are required for the best results. As the first action of nitric acid is the decalcification of bone, some care in extracting of the brain must be exercised. If the whole animal is simply immersed, as in the case of the frog, the head, consisting chiefly of the bony skull, is soon disintegrated. Thus the brain is ready long before sufficient maceration of the rest of the body has taken place. The precaution is necessary because the action on the nervous system appears to be one of hardening, the result being that if the immersion of some areas, particularly the brain, is too long, say 24 hours or longer, such tissue becomes exceedingly brittle, making easy handling of it practically impossible.

Maceration of complete small laboratory animals, such as rats or guinea pigs, should be carried out somewhat as follows: (1) Skin the animal completely, including the tail, the appendages and the head. This is to make the action of the acid on the bones and muscles more uniform and rapid. (2) Form a glass hook from glass rodding or tubing large enough to pass under the neck of the animal. Suspend the head by this hook from some suitable level above the immersion bath. (3) Immerse the rest of the mammal in the bath of 30 per cent. nitric acid for 36 hours. A large crystallizing dish or large Petri dish is perhaps the most satisfactory container for the bath. These dishes should not exceed 10 to 15 cm in depth. (4) After the 36-hour period allow the head to be immersed in the bath, leaving the head and body for another 8 hours. (5) Carefully tease the macerated muscle away from the nervous system. Not all the muscle tissue can be removed in this way. After all is removed that can be with safety, transfer the nervous system from the acid bath to an empty Petri dish of the same size as used for the bath. This transference can be easily done by simply inserting one of the glass rods under the brain and lifting the tissue out of the liquid. (6) Place the tissue in the empty Petri dish under a water faucet and let a stream of water gently drop on the tissue from some height. The force of the water will generally remove the remaining muscle tissue clinging to the nervous system.

The intact nervous system can be preserved in glycerine jelly, this method making a very satisfactory means of demonstrating to members of biology classes.

In such preparations by maceration, it will be found that the nervous system at the tips of the extremities, together with some of the spinal nerves, are occasionally lost. Skinning of the animals (unnecessary with frogs) aids in preventing this. In addition any loss can be considerably reduced if the most careful teasing is employed. The whole process seems to depend finally on the length of time of immersion. Inasmuch as the nervous system is quite resistant to the action of the acid, a period of 24 hours in the case of frogs and 48 hours with small laboratory mammals is not too long, excepting for the head region of mammals, immersion of which should not exceed 8 to 10 hours.

WILLIAM S. CORNWELL
THE UNIVERSITY OF ROCHESTER
SCHOOL OF MEDICINE AND DENTISTRY

THE ABSORPTION OF METHYLENE BLUE BY THE NEPHRIDIUM OF THE EARTHWORM

THAT the method for demonstrating the nephrostomes of the earthworm, published several years ago,[1] has met with some lack of success in the hands of certain workers has recently come to my attention. This difficulty is apparently due to failure to inject the dye in quantity sufficient to render the treated segments turgid. Under conditions of semi-flaccidity in the worm, the dye, when introduced dorsally, may fail to reach those parts of the nephridium lying near the mid-ventral axis.

In further work on the staining effect of methylene blue on the annelid nephridium, a better technique has been devised. An earthworm is anesthetized by immersion in a 0.2 per cent. aqueous solution of

[1] Elbert C. Cole, "The Demonstration of Nephrostomes in the Earthworm." SCIENCE, 62: 50–51, 1925.

chloretone, a mid-dorsal incision is made in the post-clitellar portion of the body and the septa carefully cut away so that the specimen may be pinned out flat on the wax pad of a dissecting dish. The digestive tract is then removed, care being taken to cut the septa close to the under-surface of the intestine, in order that the nephridia may remain undisturbed. The preparation is then wet with several drops of methylene-blue saline solution. Methylene blue, lot NA3, certified by the Commission for the Standardization of Biological Stains, has proved entirely satisfactory for this purpose. For anatomical study one part of dye in 2,000 parts of 0.6 per cent. NaCl solution is a suitable concentration; for the study of cilia in motion a concentration of 1: 20,000 is preferable. After the stain has acted for ten minutes it should be washed off with saline solution (0.6 per cent. NaCl in distilled water), and the preparation covered with this solution.

Two regions of the nephridium stain heavily with the dye—the nephrostome and the ampulla. Within wide limits the more dilute the stain the greater the contrast between the amount of dye absorbed by these regions and that absorbed by the remainder of the nephridium. Since the stained nephrostome is clearly visible, it is not difficult to remove the entire nephridium for study under the microscope. In the more dilute solutions the cytoplasm of the central cell, as well as the cytoplasm of the marginal ciliated cells, is strongly stained. The nature of the ciliary action and the direction of the effective beat can be clearly made out. In more concentrated solutions ciliary action is likely to cease, concurrent with the staining of nuclei of the cells of the nephrostome.

The ampulla is stained distinctly in solutions of the concentrations mentioned above, due to the accumulation of the dye within the cells.[2] The outer portion of the ampulla does not betray its cellular nature in dilute solutions, but in strong concentrations cell nuclei are clearly delineated. The inner portion of the ampulla stains strongly in any case. This area, said by Maziarski[3] to consist of rod-shaped bacteria packed closely together, is so strongly stained that when examined macroscopically it may be mistaken for the nephrostome. Its position at the distal part of the long loop of the nephridium serves to distinguish it from the nephrostome, which lies much nearer the median ventral axis.

Complete nephridia, strongly stained, may readily be removed, dehydrated rapidly in absolute alcohol, cleared in xylene, and mounted in balsam. If desired, to retain the dye fully, such preparations may be fixed in ammonium molybdate and washed before dehydration. Such mounts usually show fine detail, together with unusual translucency, and are therefore well adapted for careful study.

ELBERT C. COLE

WILLIAMS COLLEGE

SPECIAL ARTICLES

ALTERNATING CURRENT CONDUCTANCE AND DIRECT CURRENT EXCITATION OF NERVE

THE Fourier integral has proved to be a powerful and useful tool in many branches of science. In the Heaviside operational calculus form it has been particularly valuable in studying the transient behavior of electric circuits. When certain simplifying assumptions and approximations are made, this type of analysis points out a relation between the alternating current conductance and the direct current excitation of irritable biological tissues.

Alternating current resistance and capacity measurements over a wide frequency range show that biological materials may be considered electrically equivalent to a circuit containing two fixed resistances and a polarization element having an infinite impedance at zero frequency and a zero impedance at infinite frequency. This element may be considered as a resistance and a capacity in series, both of which decrease with increasing frequency, n. When $r(\omega)$ is the resistance and $x(\omega)$ is the reactance $(1/C\omega)$ of the capacity, it is often found that $r(\omega) = r_1\omega^{-\alpha}$, $x(\omega) = x_1\omega^{-\alpha}$, where $\omega = 2\pi n$, r_1 and x_1 are the resistance and reactance for $\omega = 1$, and α is a constant between zero and one. The impedance of the element $z(\omega) = z_1 p^{-\alpha}$, where $z_1 = \sqrt{r_1^2 + x_1^2}$, $p = j\omega$, and $j = \sqrt{-1}$, and the phase angle is constant, $\phi = \tan^{-1} x_1/r_1 = \alpha\pi/2$,[1] (1).

When a constant current i is started through this element at time $t = 0$, the potential difference across the element may be found by either the Fourier integral[2] or the operational[3] method to be $e(t) = z_1 i t^{\alpha}/\Gamma(1+\alpha)$, where $\Gamma(1+\alpha)$ is the gamma function. This means that when the equivalent polarization element of a biological tissue has a constant phase angle and an impedance which is a power func-

[2] R. Chambers, "Some Changes in Dyeing Cells," *Proc. Soc. Exper. Biol. and Med.*, 20: 367–368, 1923.

[3] S. Maziarski, "Sur la structure des néphridies des Vers de terre," *C. R. Soc. Biol.*, Paris, Vol. 53, 1901.

[1] H. Fricke, *Phil. Mag.* (7) 14: 310, 1932.

[2] G. A. Campbell and R. M. Foster, "Fourier Integrals for Practical Applications." Bell Telephone System Monograph B–584, New York, 1931. Pair No. 516.

[3] V. Bush, "Operational Circuit Analysis," p. 197, New York, 1929.