great extent. If, however, the cavity is treated with sodium fluoride only, then similar penetration is observed in both cavities (Fig. 2), as Amler (*1*) has shown. This preliminary work indicates the possible use of the "direct section" autoradiograph and also the effect of a layer of calcium fluoride in decreasing the permeability of the dentine.

References

1. AMLER, M. H. *J. Dental Research*, **27**, 635 (1948).
2. WAINWRIGHT, W. W., and LEMOINE, F. A. *J. Am. Dental Assoc.*, **41**, 135 (1950).
3. EVANS, T. C. *Proc.. Soc. Exptl. Biol. Med.*, **64**, 313 (1947).

Temporary Immobilization of Salamander Larvae by Means of Electric Shock[1]

Norman E. Kemp and Alonso Gamero-Reyes[2]

Department of Zoology,
University of Michigan, Ann Arbor

The use of electricity for killing or stunning fish has become common practice among fisheries biologists (*1–3*). While witnessing a demonstration of the technique of temporarily paralyzing fish in a pond by means of an electric shocker, it occurred to the senior author that the same principle might be employed in the laboratory as a substitute for chemical anesthesia to immobilize amphibian larvae during operations or for short periods of microscopic examination. Alternating (tetanizing) current from the secondary of an ordinary Harvard inductorium powered by two 1½-v dry-cell batteries has proved to be entirely satisfactory for this purpose. The nature of electronarcosis and how it compares with chemical anesthesia have been investigated only slightly (*4, 5*).

As the apparatus is most commonly employed, larvae are immobilized by maneuvering them between 2 platinum electrodes dipped into the operating dish, then closing a hand-operated switch and allowing the current to flow until the animals are completely paralyzed. Regulation of voltage is easily accomplished merely by moving the position of the secondary coil relative to the primary. At low voltages the larvae usually escape from the electric field after first feeling the shock; consequently it is often necessary to replace them several times before they become motionless. Excessively high voltages, on the other hand, frequently paralyze the animals almost immediately and sometimes kill. In practice an intermediate voltage in sublethal dosages which stuns fairly rapidly is employed. The range of voltages attainable with one pair of batteries used intermittently during an entire academic year was 0 to *ca* 40 v at first. The upper limit had declined to 20 v by the end of the year; but this was well beyond the voltage required for electronarcosis. Amperage in the circuit was barely measurable with a Simpson a-c milliammeter; it was of the order of 5–10 mv.

In order to render animals motionless before introduction into the operating dish, an electropipette (Fig. 1) was devised. This was made by inserting two short lengths of 30-gauge platinum wire through holes blown in opposite walls of a glass pipette, then sealing the openings. The ends of the wires inside the tube thus served as electrodes in contact with the fluid introduced into the pipette. The advantage of this pipette is that larvae may be exposed to current in a relatively small volume of fluid from which they cannot escape. Lower voltages and shorter times of exposure are thus feasible.

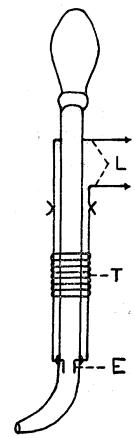

FIG. 1. Electropipette. *E*, electrode of platinum wire; *T*, thread wound around platinum leads; *L*, leads of copper wire to inductorium.

Because of the observed lethality of excessive shocking of swimming larvae, a series of experiments was performed to test the tolerance of various stages of embryos of *Amblystoma punctatum* to various dosages of electricity. In one group of experiments, animals at the blastula, yolk-plug gastrula, closing neural tube, or tailbud stages were exposed to voltages ranging from 2.5 to 20 v for 5–15 sec. The number of animals alive 3 days after shocking was used to determine percentage of survival. A given dosage (5 v for 5 sec) applied to each of the 4 stages resulted in 2% survival for the blastula, 79.4% for the gastrula, 97.3% for the neurula, and 100% for the tailbud embryos. Possibly the thickened epidermal surface coat in the older embryos protected them from injurious effects of the shock. Increased voltage over a given period of time (5 sec) at the gastrula stage caused decreased survival. The percentage was 90 for gastrulae exposed to 2.5 v, 64 after exposure to 10 v, and only 6 after 20 v. Holding the voltage constant at 5 v and increasing the time to 15 sec likewise led to decreasing survival of gastrulae.

Another group of experiments was performed on swimming larvae of *A. punctatum* at stages 45–46+ to test the time of recovery after various dosages of electric shock. The results show an obvious correla-

[1] Aided by a grant from the Horace H. Rackham School of Graduate Studies, University of Michigan.

[2] Present address: Instituto Pedagójico, Caracas, Venezuela.

tion between strength of dosage and rate of recovery. In one series, for example, where the time of exposure was always 5 sec, animals became motile in less than 1 min after 0.5 v, in 1–2 min after 1.0 v, in 2–5 min after 2.5 v, in 5–10 min after 5.0 v, and after 20–30 min shocking at 10 v. These data suggest a roughly straight-line relationship between dosage and time necessary for recovery. Significantly, all these swimming larvae survived, even at the higher dosages.

If used judiciously, electronarcosis of amphibian larvae and certain other small aquatic larvae is a valuable tool for the laboratory experimentalist. It is a quick, convenient substitute for chemical anesthesia. Stages of *Amblystoma* prior to completion of gastrulation are particularly susceptible to injury by electric shock. Subsequent to this time, however, animals become increasingly resistant and are able to tolerate a fairly wide range of dosages. It is possible to select dosages that will immobilize swimming larvae for roughly predictable periods without apparent permanent injury.

References

1. HASKELL, D. C. *Trans. Am. Fisheries Soc.*, **69**, 210 (1940).
2. ———. *Progressive Fish Culturist*, 33 (March–April 1940).
3. HOLMES, H. B. *Spec. Sci. Rept. No. 53, U. S. Fish Wildlife Service* (1948).
4. SCHEMINZKY, F. *Pfluger's Arch. ges. Physiol.*, **202**, 200 (1924).
5. VAN HARREVELD, A., PLESSET, M. S., and WIERSMA, C. A. G. *Am. J. Physiol.*, **137**, 39 (1942).

Spectrophotometric Determination of Hemoglobin and Oxyhemoglobin in Whole Hemolyzed Blood[1]

Gabriel G. Nahas

Mayo Foundation, Graduate School, University of Minnesota, Rochester

The difficulty of performing spectrophotometric analysis anaerobically has prevented the wide application of this method to determination of oxyhemoglobin (*1*). In view of this fact a lucite cuvette,[2] allowing for anaerobic analysis, was built to fit the Beckman D. U. spectrophotometer. It consists (Figs. 1, 2) of two lucite plates, a metal gasket 0.01 cm thick, and supporting plates of nickel-plated brass. The metal gasket and plates contain 4 apertures (so that when they are clamped in place 4 chambers are formed between the lucite plates, which are 0.01 cm thick and conform in shape and area to the standard Beckman cuvette holder). For filling and cleaning purposes, 8 L-shaped holes, one to serve as an inlet and one as an outlet channel for each of the 4 chambers, are drilled through one of the lucite plates so as to end on each side of a lateral wall of each chamber. These L-shaped holes, which are hidden in a frontal view by the metal plates of the cuvette, may be seen in a side view (Fig. 2). Filling of the chambers with blood is performed by inserting into the inlet channel a 20-gauge rubber-tipped needle attached to a syringe containing the blood sample. Coagulation is prevented by filling the dead space of the syringe with an anticoagulant solution (heparin, 10 mg/ml) before withdrawal of the blood sample. Hemolysis of the blood, which is necessary in order to obtain a clear solution (*1–3*), is produced anaerobically by adding to the blood sample 1/100 of its volume of saturated solution of saponin Merck. This procedure is performed by inserting into the sampling syringe a rubber-tipped needle fitted to the tuberculin syringe containing the saponin.

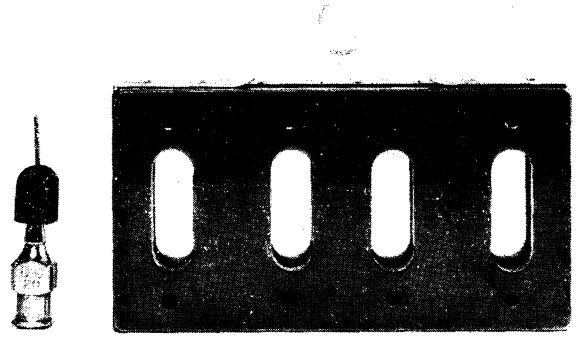

FIG. 1. Front view of cuvette with needle used for filling chambers.

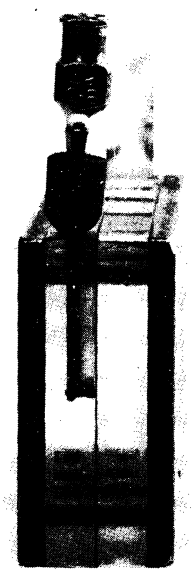

FIG. 2. Side view of cuvette with filling needle in place.

[1] The author wishes to acknowledge the cooperation of E. H. Wood, under whom this study was made, of M. H. Power, and of the Section on Engineering of the Mayo Clinic.

[2] Manufactured by the Waters Conley Company, Rochester, Minn.