ually at an earlier age than they do in the more northern waters. This faster rate of living in warmer waters has been reported often by investigators of other poikilothermous animals.

References

1. GUNTER, G. *Am. Midland Naturalist*, **43**, (2), 438 (1950).
2. COE, W. R. *Biol. Bull.*, **71**, (2), 353 (1936).
3. *Ibid.*, **74**, (1), 64 (1938).
4. BURKENROAD, M. D. *Science*, **74**, 71 (1931).
5. NEEDLER, A. B. *Contribs. Can. Biol. and Fisheries*, **7**, 284 (1932).
6. PAUL, M. D. *Proc. Indian Acad. Sci.*, Sect. B, **15**, (1), 1 (1942).
7. INGLE, R. M. *Science*, **112**, 338 (1950).

Direct Tissue Radioautography Technique Applied to Teeth

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Radioactive isotopes have been used in the study of the permeability of the enamel and the dentine, and the pathways by which these isotopes penetrate the tooth structure have been recorded by the radioautographic method in which the radiograph of the tooth section after the penetration of the isotope is determined by comparison with the tooth section itself. In 1948 Amler (*1*) using this method studied the penetration of radiophosphorus in the dentine following the use of various medicaments, and more recently, Wainwright and Lemoine (*2*) illustrated the penetration of the enamel and dentine by urea containing C^{14}, by using the comparison of the radiograph to the ground tooth section.

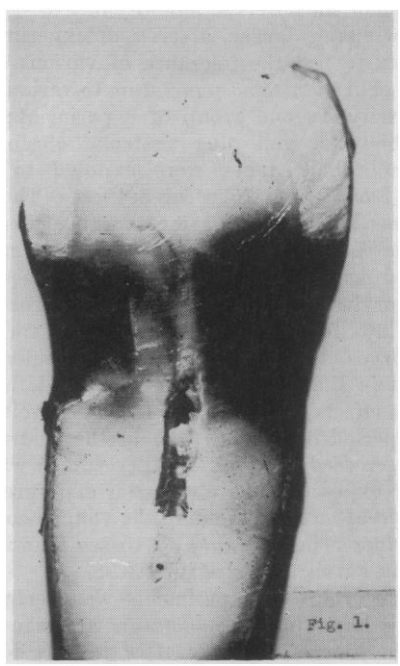

FIG. 1.

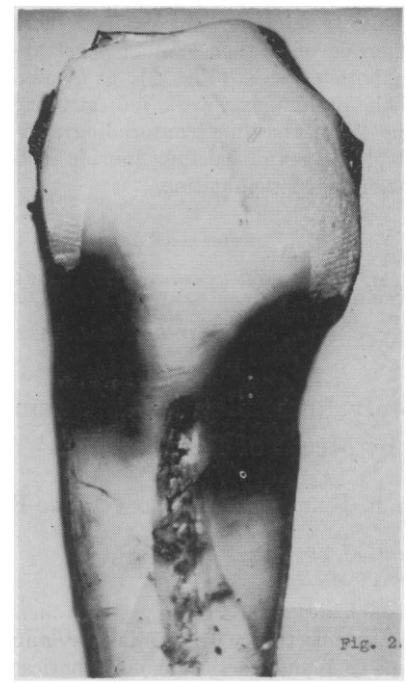

FIG. 2.

In an investigation of the permeability of the dentine and enamel to P_{32} labeled NaH_2PO_4 following the treatment of the teeth by fluorides and other compounds, the authors thought that the application of the method of Evans (*3*) might provide a technique that would permit a direct viewing of the isotope penetration and, furthermore, microscopic examination of the sections that would give additional information as to the mode of penetration.

This technique consists essentially of placing a very fine grain stripping-film emulsion[1] on the surface of the ground tooth section and exposing to the radiation of the absorbed isotope. This emulsion is developed still in contact with the tooth section, and thus a direct view of the penetration is possible (Fig. 1).

In the specimen shown in Fig. 1, similar cavities were prepared on the mesial and distal surfaces of an anterior tooth and with the right cavity serving as a control the left was treated with 2% NaF and then 2% $CaCl_2$, a precipitate of CaF_2 forming in the cavity. Into each cavity 0.02 ml of radiophosphorus in the form of NaH_2PO_4, with a specific activity of 30 μc, was placed, and the cavities were sealed. After 24 hr a ground section of the tooth was prepared, and the film emulsion laid in contact with it. It was exposed for a length of time proportional to the radioactivity of the section and then developed.

The resultant composite radioautograph and section show a diffuse penetration of P_{32} in the control cavity, while the precipitation of calcium fluoride in the treated cavity has decreased the diffusion to a very

[1] Kodak–London Autoradiographic plates.

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]

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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.

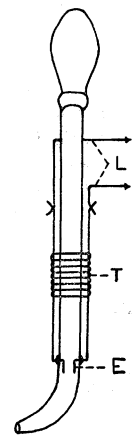

FIG. 1. Electropipette. *E*, electrode of platinum wire; *T*, thread wound around platinum leads; *L*, leads of copper wire to inductorium.

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-

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