increases more rapidly than that with the distilled water. Accordingly, it may be considered that the serum contains a viral growth factor.

K. AIZAWA

Virus Laboratory,
Sericultural Experiment Station,
Suginami-ku,
Tokyo.

[1] Aizawa, K., *Bull. Seric. Exp. Sta.*, **14**, 4, 201 (1953).

Electron-Track Autoradiography of Radioactive Frog Embryos

BETA-TRACK autoradiographs offer advantages over other methods of autoradiography, especially when small quantities of weak emitters such as carbon-14 are used[1]. If, in addition, the autograph and tissue can be viewed as a unit, a further advantage is gained[2]. Such preparations permit detection of the characteristic tracks in the presence of heavy background fog; eliminate the problem of spurious interpretation due to the reduction of the silver grains by tissue constituents[3]; require far less radioactivity for detection than other methods of autoradiography; permit viewing at high magnifying power with consequent localization of radioactivity to small areas; and eliminate matching of tissue and autograph. Beta tracks, due to their tortuous path, do not permit determination of a point source.

Radioactive frog (*Rana pipiens*) ova and embryos were produced by injecting sodium formate-^{14}C into the gular lymph sac of females, which were then induced to ovulate by an intraperitoneal injection of pituitaries. Fertilized ova were permitted to develop, and specimens were fixed in absolute alcohol at various developmental stages[4], cleared in oil of wintergreen and embedded in paraffin. Serial sections were cut at 10 μ, floated on absolute alcohol on to slides coated with albumen, and dried.

For preparation of the autographs, the paraffin was removed with xylol and the slides dried at 40° C. on a warm plate. Several drops of Ilford *G*-5 emulsion gel, melted at 45° C., were placed on each warm slide, and the emulsion brushed over the sections with a camel's-hair brush held in a quill. The slides were cooled to room temperature to allow the gel to harden, placed in a lead-shielded dark receptacle for 23 days, and then developed in amidol for 15 min. (amidol, 1·5 gm.; sodium sulphite, 3·5 gm.; sodium bisulphite, 0·5 gm.; distilled water, 500 c.c.). This was followed by 5 min. in a stop-bath of 1 per cent acetic acid and then for 30 min. in hypo solution maintained at 5° C. The hypo was then gradually replaced with cold distilled water over a period of about 45 min. The slides were washed in running, cold tap water for several hours and dried overnight. The emulsion often adheres poorly to plain glass, so 5 per cent sodium sulphate should be added to the stop-bath to act as a hardening agent. However, if subbed-glass (gelatine-coated glass) is used, the hardening agent is not necessary. Subbed-glass can be treated just as plain glass slides are treated.

The slides are best examined with an oil-immersion lens. Emulsion covering non-radioactive areas may show an occasional electron-track which is regarded as 'background', but emulsion covering the radioactive areas exhibits many tracks, and can thus be demarcated from non-radioactive areas.

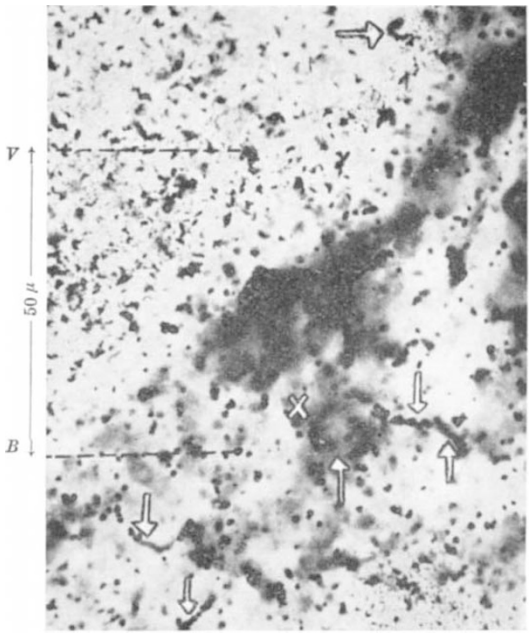

Fig. 1. Hind brain of a stage-21 larva of *Rana pipiens*, sectioned at 10 μ, showing electron tracks, some of which are indicated by arrows. *X* shows a cluster of tracks; the tissue can be seen in the background; *V* indicates the ventricle; *B* indicates the hind brain. (× 1,000)

Fig. 1 shows a portion of the hind brain and ventricle from a radioactive stage-21 larva. The tissue is out of focus, but can be seen in the background. The area in this photograph has at least forty tracks distributed in the emulsion over the tissue; but since the tracks arising from the tissue pass into the emulsion and lie in several planes, only a few can be seen clearly at any one plane of focus. One track can be seen in the ventricle at the periphery of the tissue; but beyond this area there are no electron tracks, and the radioactive area of tissue can be distinguished from the non-radioactive area of the ventricle. It is problematical that the group of tracks seen at 'X' come from a common source such as the same cell, but it can be assumed that they arose from cells in the immediate area of the field viewed.

The method of preparation outlined here loses the fatty substances of the tissue, and thus loses radioactivity. Other methods of preparation, such as freeze-drying, would preserve these and other soluble constituents. Thus, selective fixation and preparation might be used to demonstrate the presence of specified tissue substances.

FREDERICK SPERLING

National Institute of Arthritis and
Metabolic Diseases,
National Institutes of Health,
Public Health Service,
Department of Health, Education and Welfare,
Bethesda 14, Maryland.
July 23.

[1] Yagoda, H., "Radioactive Measurements with Nuclear Emulsions" (John Wiley and Sons, Inc., New York, 1949).
[2] Belanger, L. F., and Leblond, C. P., *Endocrin.*, **39**, 8 (1946).
[3] Everett, W. B., and Simmons, B. S., *Anat. Rec.*, **117**, 25 (1953).
[4] Shumway, W., *Anat. Rec.*, **78**, 139 (1940).