

virus. The brain tissue was ground without abrasive and diluted to make a 10 per cent. suspension in nutrient broth. After centrifugation at 2,500 r.p.m. for 15 minutes, the supernatant fluid was added in 0.3 cc amounts to 2.7 cc of culture fluid media consisting of from 1 to 2 drops of finely minced embryonic tissue suspended in 1 part of fresh mixed normal rabbit serum to 2 parts of Tyrode's solution in a 50 cc Erlenmeyer flask. The inoculated flasks were incubated at 37° C. with successive passages or generations made every fourth day by the direct transfer of 0.3 cc of the preceding passage flask to similarly prepared minced tissue Tyrode-serum mixture in 2.7 cc amounts. The absence of bacteria was established by direct smears from each flask and by subinoculations into Douglas' broth with subsequent aerobic and anaerobic incubation.

During this investigation many experiments were performed, of which two are reported herewith. In both embryonic mouse tissue was used.

*Series A:* Mouse brain tissue virus in the supernatant fluid of a bacteria-free centrifuged 10 per cent. suspension in broth, lethal for mice in a dilution of

10<sup>-4</sup>, was used. This was carried through 19 generations. Undiluted samples of each culture generation were tested for their lethal effect by intracerebral inoculation into mice, under light ether anesthesia. Titration of the sixth generation was lethal in a dilution of 10<sup>-2</sup>; of the twelfth 10<sup>-1</sup>; and of the eighteenth 10<sup>-2</sup>.

*Series B:* An inoculum similar in its preparation to that of Series A, but of a different mouse brain passage, was used to initiate the cultures. This series was carried through 20 generations. Titration of the sixth generation was lethal in a dilution of 10<sup>-2</sup>; of the twelfth 10<sup>-2</sup>; and the eighteenth 10<sup>-2</sup>.

Thus it appears that the virus of St. Louis encephalitis is capable of multiplication *in vitro* by the simple method of cultivation described. Experiments on the serological specificity of the culture virus and its immunological relationships, following repeated cultivation, will soon be reported.

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## SCIENTIFIC APPARATUS AND LABORATORY METHODS

### A GENERAL SOURCE OF RADIATION FOR THE VISIBLE AND INFRARED SPECTRUM

If a portion of Welsbach mantle be introduced into the glow or arc discharge produced by the secondary of a transformer the mantle may be caused to glow with intensities ranging from the barely perceptible to those rivaling a tungsten lamp. High and medium intensities are used in the visible and near infrared spectrum, *i.e.*, up to 15  $\mu$ , while the lower intensities are reserved for the far infrared, where advantage is taken of the unique properties of the Welsbach mantle.<sup>1</sup>

The construction adopted is shown in Fig. 1, where A is a tube of pyrex glass, about 3 cm in diameter, supplied with two side-tubes B<sub>1</sub>, B<sub>2</sub>, over whose ends the water-jackets CC are slipped. Polished plates of rock-salt R and crystalline quartz Q are attached to the open ends of the side tubes with a mixture of beeswax and rosin. The luminous element is supported by two plates of asbestos board DD fitting diametrically into tube A and supplied with lateral openings for the escape of radiation. The plates are given a 2 mm separation by the copper electrodes EE, which are held in place by the small bolts FF. Each copper electrode is supplied with a tongue T, about 1 mm thick, the edge of which is lined with heavy plati-

num wire P. The length of the air-gap between the electrodes is about 15 mm.

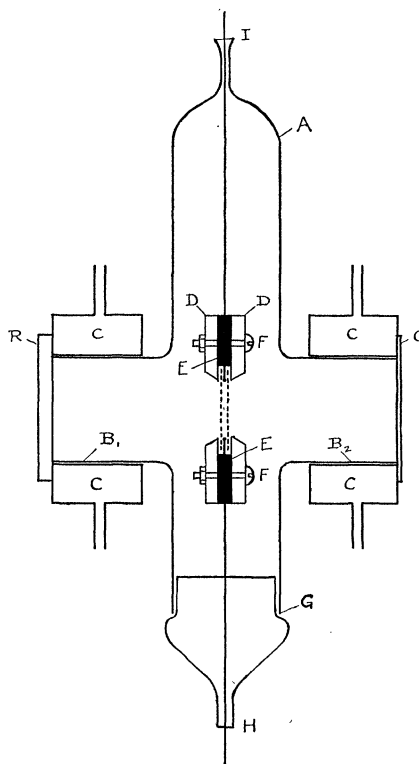


FIG. 1

<sup>1</sup> H. Rubens, *Ann. d. Phys.*, 18, 725, 1905.

Upon having unbolted this system, pieces of commercial Welsbach mantle about one inch square are placed on both sides of the tongues T and are ashed with a small, pointed gas flame. Care must be taken that these pieces of mantle be quite free, *i.e.*, not clamped, since otherwise the mantle will tear upon warping when heated. Having reassembled the system it is introduced into the tube at G—the opening being closed subsequently with a waxed-in plug. Connections (not shown) to vacuum pump, manometer, etc., are made at H. The opening I at the upper end of the tube is closed with de Khotinsky cement.

At a potential of about 10,000 volts the discharge strikes if the pressure be 30 cm of mercury or less. The glow discharge raises the temperature of the mantle to incandescence. The current is not carried by the mantle, since the radiant emission is not affected measurably when the mantle has a crack running squarely across the line of discharge. Other things being equal, the discharge current is virtually independent of the gas pressure, *i.e.*, between 25 cm and 1 cm of mercury. However, the luminosity, which is very feeble at low pressures, becomes intense at higher pressures. While the discharge, once initiated, will proceed even at atmospheric pressure, the light emission is somewhat unsteady. Probably the most satisfactory operation at moderate intensities is realized at a pressure of 20 cm and a current of 40 m.a. Under these conditions the intensity of radiation is remarkably constant—being limited only by the voltage constancy of the alternating current supply.

As previously stated, for the visible and near infrared the high-intensity mantle and rock-salt window are used, while, for the far infrared, the low-intensity mantle and quartz window are employed. The usefulness of this new lamp in vacuum spectrographs is evident.

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### LOCALIZED VITAL STAINING OF TELEOSTEAN EMBRYOS

SLIGHT modifications of the technique devised by Vogt<sup>1</sup> for local vital staining of amphibian embryos have been most practicable in the study of teleostean development. Pasteels<sup>2,3</sup> has used Vogt's method in studying the blastula of the trout egg. In the present investigation the cleavage blastomeres as well as later stages have been locally stained by applying stain-impregnated Cellophane. This is employed as the carrier of the stain in place of the agar used in Vogt's

technique. It is, however, prepared in the same manner, colorless Cellophane being treated with aqueous solution (1 to 5 per cent.) of the dye. The embryos take up the stain just as readily from Cellophane as from agar. In addition, Cellophane is not a bacterial medium, it does not swell, it is more easily cut to a desired shape which it maintains, and it is more rigid and more easily manipulated than agar.

Nile blue sulfate and neutral red are both satisfactory for staining the teleostean blastoderm. Fundulus eggs take up the vital dye far more readily when handled in a solution of double-strength Ringer's solution than when in normal Ringer's solution or seawater. Normal Ringer's solution is favorable for use when staining perch eggs. It has been found that Nile blue stain remains definitely localized for a longer period of time when used in combination with neutral red than when used alone. In work on Fundulus, the greatest degree of success was obtained by staining the blastoderms with Cellophane that was treated in a mixture of equal parts of 1 per cent. neutral red and 1 per cent. Nile blue.

When the early blastomeres of Fundulus are stained, the dye is retained in cell inclusions throughout the blastomere to which the Cellophane is applied. The inclusions are first purple in color, but become blue in a few hours; the blue remains localized throughout gastrulation and is visible even when the stained cells form yolk-sac epithelium. Experiments performed by this method show not only that the embryonic axis does not necessarily coincide with the axis of the first cleavage plane, but that it frequently follows the second plane of cleavage or an intermediate axis.

When stain is applied to the blastula, the dye remains localized until after the differentiation of somites and brain-vesicles, and even after its diffusion the white chromatophores of the stained region contain pale blue dye. This method is applicable to the study of the extensive migrations which the blastular cells undergo in attaining their definitive locations in the embryonic shield. It has also afforded proof by direct observation that during gastrulation cellular materials pass from extra-embryonic regions into the shield at its anterior part as well as at the blastoporic lip.

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<sup>1</sup> W. Vogt, *Arch. f. Entwmech.*, 106: 542-610, 1925.

<sup>2</sup> J. Pasteels, *C. R. Soc. Biol.*, 113: 425-428, 1933.

<sup>3</sup> J. Pasteels, *C. R. Assoc. des Anat.*, 21ième Réunion, Bruxelles, 451-458, 1934.