11160 P

Vital Staining of Virus Lesions on Chorio-Allantoic Membranes by Trypan Blue.

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In the study of the early focal lesions of viruses on chick chorio-allantoic membranes, some difficulty is often experienced in recognizing the earlier focal lesions on direct examination of the membrane even with moderate magnification. The recognition of the foci and the preservation of such membranes for more leisurely study under the microscope has been greatly facilitated by staining the lesions with trypan blue, and, after formalin fixation, mounting them in glycerin gelatin.

In this method, 1 cc of a 0.5% aqueous suspension of trypan blue is employed. It is placed directly upon the membrane when ready for examination through the window in the shell. Gentle rotation of the egg facilitates the general distribution of the stain, after which the shell is closed and the egg placed in the incubator for from 10 to 30 minutes depending on the age and extent of the lesions. The more extensive lesions require a shorter time to take up the stain and may become too intensely colored for satisfactory examination if the stain is left on too long.

The membrane is then removed in the usual manner, washed gently in physiological saline to remove the excess of trypan blue, and fixed flat in 10% formalin for a few minutes. After draining away the excess liquid the membrane is flattened on a 2 x 2.5 inch glass slide and mounted in a glycerin gelatin containing 50% glycerin, 5% gelatin, and 1% phenol. The gelatin should be soaked in water about 2 hours before adding the glycerin and phenol, then heated gently for a few minutes while stirring. For mounting the membrane, the glycerine jelly is melted and while still hot (about 70°C) dropped on the membrane until it is well covered, flaming the slide gently but not enough to produce bubbles. It is then mounted with a warmed cover slip and gentle pressure applied for a minute or two until the jelly has begun to set. When fully hardened the excess jelly is removed and the edges sealed with Canada balsam or asphalt cement.

Under the low power of the microscope uninoculated membranes or those to which simple broth or sterile serum has been added 24 to 48 hours previously, show that very little trypan blue is taken up by the cells, although sometimes certain of the ectodermal cells and

sometimes sheets of them have absorbed some of the stain. Cells around traumatized areas also are often stained. The foci of proliferation in virus lesions, however, stand out prominently as more or less isolated clumps of well stained cells which are easily recognized from their distinct focal arrangement.

Most of our observations have been made with the lesions of the St. Louis type of encephalitis virus in which it is usually possible to recognize the virus foci in the vitally stained membrane in 18 hours and always in those 24 hours old. The focal lesions from vaccinia and variola virus also are well stained by trypan blue and easily recognized. The finer cell structure of the virus lesion is not easily made out in the thickened membrane, but the cells show well in paraffin imbedded sections.

11161 P

A Consideration of Extra-Valvular Elements in the First Heart Sound.

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The relation between auricular systole and the first sound, the rôle of the atrio-ventricular valves, and myocardial contraction have been considered, together and separately, as factors contributing to the production of the first heart sound.[1-7] Some observers have felt that closure and tensing of the atrio-ventricular valves at the onset of systole are the sole elements in causing the first sound, while others have adhered to the theory that the initial heart sound is partly muscular and partly valvular in origin. We investigated the problem, applying a method designed to prevent atrio-ventricular valve movement.

The instrument here used to record the heart sounds was a cathode-ray stethograph, furnished by the Burdick Corporation. The instrument is aperiodic to most vibrations produced by the heart, and the

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