Resumen por el autor, Charles E. Buell.

Notas sobre técnica.

I. Un método para preparar embriones jóvenes montados enteros.

El embrión se fija en formol (10 por ciento) durante doce horas, lavándole en agua y tiñéndole con hematoxilina. Se destiñe en 5 por ciento ó lo por ciento de NaOH hasta que haya desaparecido todo el color azul y la preparación adquiere una tinta parda clara. Se deshidrata y se monta en bálsamo. El resultado se asemeja al obtenido mediante la impregnación argéntica.

II. Disección en parafina.

El embrión inyectado se incluye en parafina del modo ordinario. La disección se lleva á cabo en el bloque de parafina bajo el microscopio binocular, con fuerte aumento. Se disuelve la parafina en xilol, y se monta el embrión en bálsamo.

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NOTES ON TECHNIQUE

- I. A METHOD OF PREPARING WHOLE MOUNTS OF EARLY EMBRYOS
- II. DISSECTION IN PARAFFIN

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I. A METHOD OF PREPARING WHOLE MOUNTS OF EARLY EMBRYOS

In the preparation of total mounts of young embryos, both injected and uninjected, certain well-known difficulties are encountered. If the embryos are mounted unstained, the tissues become too transparent in the clearing reagents. Many stained preparations are so dense that injected vessels and individual structures are obscured. Staining in haematoxylin, carmine, or acid fuchsin gives excellent results in the preparation of very young blastoderms. However, in the stages beyond fifty hours of incubation, owing to the multiplicity of structures and increased density of the tissues, the study of stained embryos is rendered very difficult for the beginner in embryology.

The following technique has certain advantages in that it renders the older stages more transparent and at the same time brings the individual structures into sharp delineation. It offers a variation from the usual laboratory methods and gives a preparation that is impervious to time, acid balsam, and the bleaching effect of sunlight.

The embryo is fixed in formalin (10 per cent) for twelve hours, preferably in a flat-bottomed shallow jar with just enough of the fixative to cover the embryo. This insures a flat preparation—a decided advantage in the case of chick blastoderms. Stain the embryo for two hours in haematoxylin and allow it to decolorize in a 5 or 10 per cent solution of NaOH. The time necessary for the decolorization depends upon the density of the stain, the

strength of the alkali used, and the age of the embryo. In the case of the younger stages, the weak alkali serves best and the time for decolorizing is short, from one to two hours. With the older embryos the stronger alkali and a longer period for decolorization is necessary. When all the blue color has disappeared and the preparation has become a light yellowish-brown tint, the decolorization is complete and the optimum density has been obtained. The blood vessels stand out as brown cords retaining their cell structure. The various organs are stained different shades of brown depending upon the density of their tissues. The whole preparation is translucent and resembles effects obtained by impregnation with silver.

The embryos are washed in several changes of distilled water, dehydrated in graded alcohols, cleared in xylol, and mounted in balsam. Greatest care should be observed, as the embryos are very fragile. Glass supports for the cover-glass must be used to prevent crushing of the specimen. The balsam should be as nearly colorless as possible. Under the binocular microscope with transmitted light, the various structures are readily made out. The mounts are not affected by acid in the balsam, nor are they bleached by sunlight; in fact, they improve with age.

II. DISSECTION IN PARAFFIN

In embryological studies involving injections of the vascular tree, errors in interpretation are prone to occur because of the similarity of arteries to veins and the close interrelation of such structures. It is of great assistance to the illustrator as well as the research worker that the vessels to be demonstrated shall be isolated as far as possible from neighboring structures and that all overlying vessels be removed. In demonstrations for the class-room, it is of a decided advantage that the preparation be as little complicated as possible.

During a recent study on the origin of the pulmonary vessels in the chick, the study of whole mounts of injected embryos was difficult, in that the large cardinal veins and other structures obscured the delicate pulmonary capillaries beneath. In older

embryos this difficulty increased. I have had good results with a simple method of dissection that could be applied to other studies of embryonic circulation.

The injected chick is embedded in paraffin by the usual method and trimmed into a block with its broad surface parallel to the plane of the vessels to be demonstrated. In the case of the capillaries of the lung, this was the sagittal plane of the embryo. With gentle heat fix the block upon a glass slide. In a good light under the high power of a binocular microscope, holding a scalpel lightly in the fingers of the right hand, shave off successive layers of paraffin until all the overlying vessels have been removed. A small window may be formed in this manner, exposing the desired structures and at the same time retaining the form of the embryo for localization and proportion. With a little practice and familiarity with the anatomy of the structures to be demonstrated, the result is in direct ratio to the ingenuity of the dissector. If desired, the block may be reversed upon the slide and the opposite side dissected in the same manner.

After these procedures have been carried out, the block is placed in xylol to dissolve the paraffin and mounted in balsam. In case of injections with dilute ink, the preparation is improved by staining to a light lavender tint with haematoxylin. This method eliminates a fluid medium, gives adequate fixation of the embryo during dissection, and protects the neighboring structures from injury during manipulation.