TAGGING EMBRYOS OF MULTIPAROUS ANIMALS

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When working with the embryos of multiparous animals it is often necessary to mark embryos subjected to a certain procedure. No reliable methods of marking embryos have yet been devised, and its has been necessary to make a detailed, and often laborious, investigation of the entire litter in order to distinguish the experimental fetuses from the controls.

The method of tagging embryos developed by the authors, being simple and very reliable, may therefore be of interest. It consists essentially of the subcutaneous injection of colored material into the embryo. The best material for this purpose was found to be lanolin, prepared as follows. To 10 g anhydrous lanolin is added 3 g of commercial black ink and 0.5 ml (about 5000 units) of a solution of penicillin or other antibiotic. The ingredients are carefully mixed and any excess fluid is discarded. Hydrous lanolin, colored black, is left in the bottom of the dish. By means of a thin spatula or glass rod, a 2 ml syringe is filled with this material. No air bubbles must be present in the plunger is inserted.

To inject the colored material into the embryo a special needle with a strong stilet is used (Fig. 1). To make it, an injection needle with an external diameter of 0.8 mm is taken, the connector is cut off, and the needle is again soldered to it (1) so that its blunt end is at the level of the base of the connector, as shown in Fig. 1. A portion of an intradermal injection needle (2), about 10 mm long and with an external diameter of 0.5 mm, is inserted into the other end of the needle and soldered to it.

The stilet consists of a steel sewing needle (3) with its end cut plane, which fits snugly inside the needle (1). For convenience in use the steel stilet is fixed with its pointed end inside a shortened injection needle (4).

The needle is attached securely (see Fig. 1, B). The stilet is then introduced into the needle for about

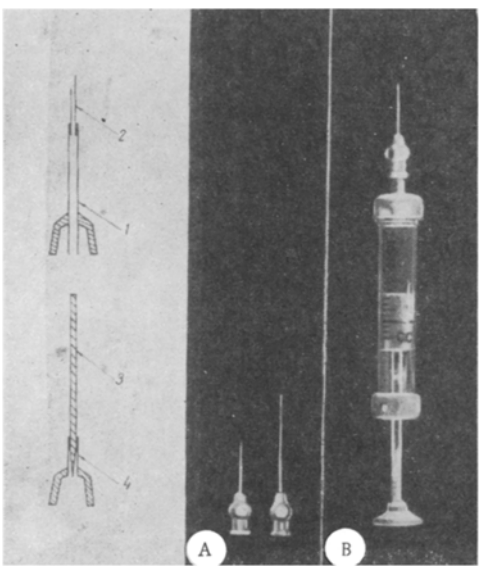

Fig. 1. Syringe for injecting colored material into embryos. A) Needle (scheme and general appearance; explanation in text); B) needles fitted to a Record syringe for filling with colored material.

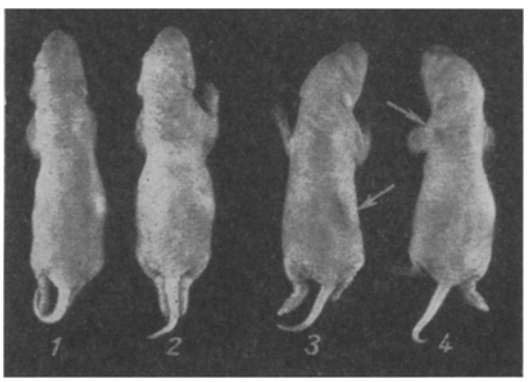

Fig. 2. Newborn rats. 1 and 2) Control; 3) and 4) tagged on the 18th day of intrauterine development. The tags are indicated by arrows.

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one-quarter of its length. The point of the needle is passed through the wall of the uterus and introduced under the embryo's skin near the spine. By pressure of the stilet about 1 ml of colored material is expressed from the needle.

When working with rat embryos the translucency of the fetal membranes makes this procedure extremely simple and it takes only a few seconds. The tag in the newborn rat is subsequently clearly visible (the dark spot or band; Fig. 2). The injected material is not absorbed. When the animal grows its hair the tag can still be seen if a fold of skin is examined in front of a lamp.

By using this method, embryos subjected to a certain procedure could easily be distinguished from the controls, and it proved to be particularly valuable when the fetuses were grown for long periods after birth.