Whole Embryo Culture

Tikam Chand Dakal
Mohanlal Sukhadia University

What is primary cell culture

SKIP THIS--- ALREADY COVERED

- Primary Culture

  When cells are surgically removed from an organism and placed into a suitable culture environment, they will attach, divide and grow. This is called a Primary Culture.

- There are two basic methods for doing this:

  Explantation: In this, a small pieces of tissue are attached to a glass or treated plastic culture vessel and bathed in culture medium. After a few days, individual cells will move from the tissue explant out onto the culture vessel surface or substrate where they will begin to divide and grow.

  Enzymatic digestion: In this method, we use digesting (proteolytic) enzymes, such as trypsin or collagenase, to the tissue fragments to dissolve the cement holding the cells together. This creates a suspension of single cells that are then placed into culture vessels containing culture medium and allowed to grow and divide.

Steps in primary cell culture

SKIP THIS--- ALREADY COVERED

- A primary culture is that stage of the culture after isolation of the cells but before the first subculture. There are four stages to consider:

(1) acquisition of the sample,

(2) isolation of the tissue,

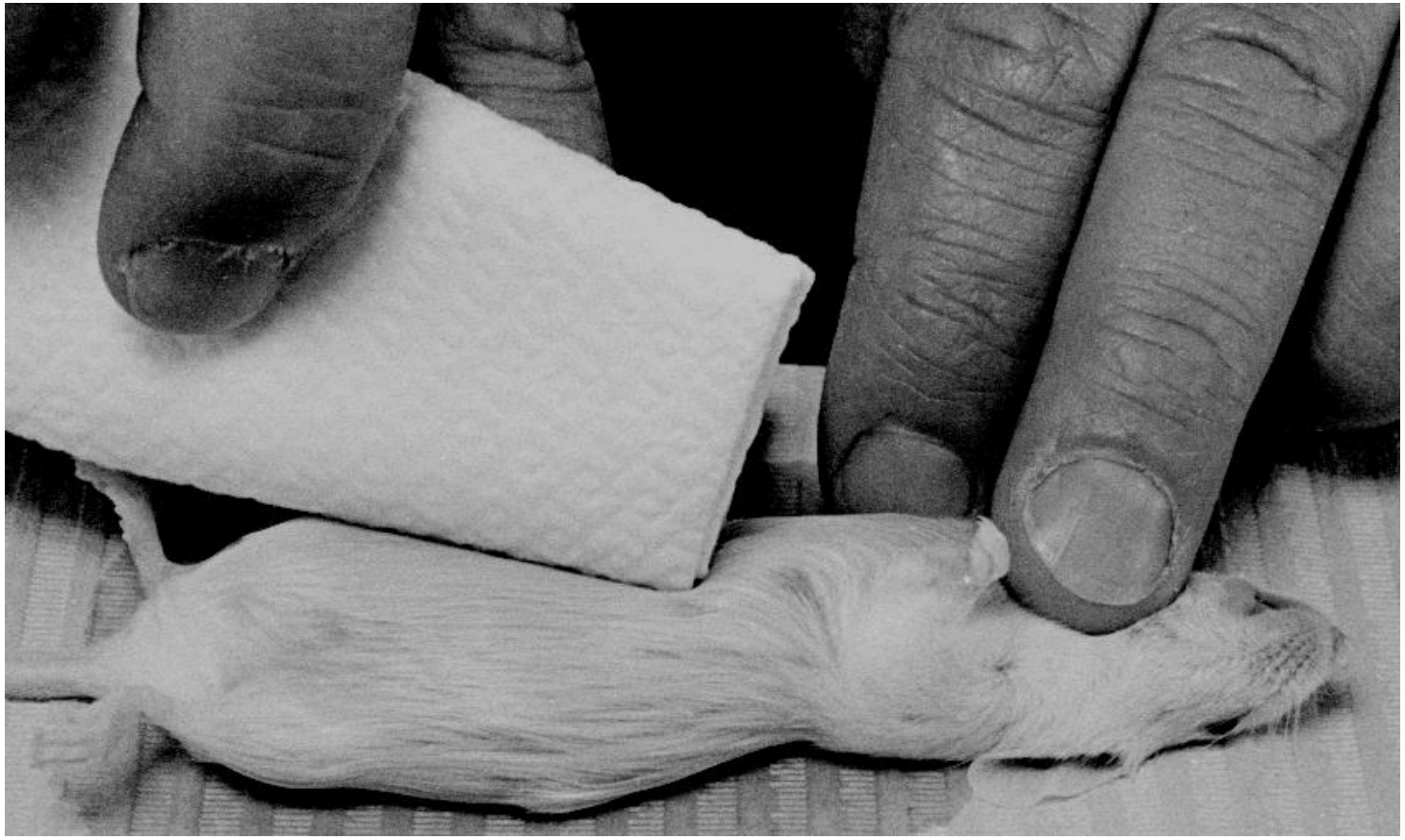
(3) dissection and/or disaggregation, and

(4) culture after seeding into the culture vessel.

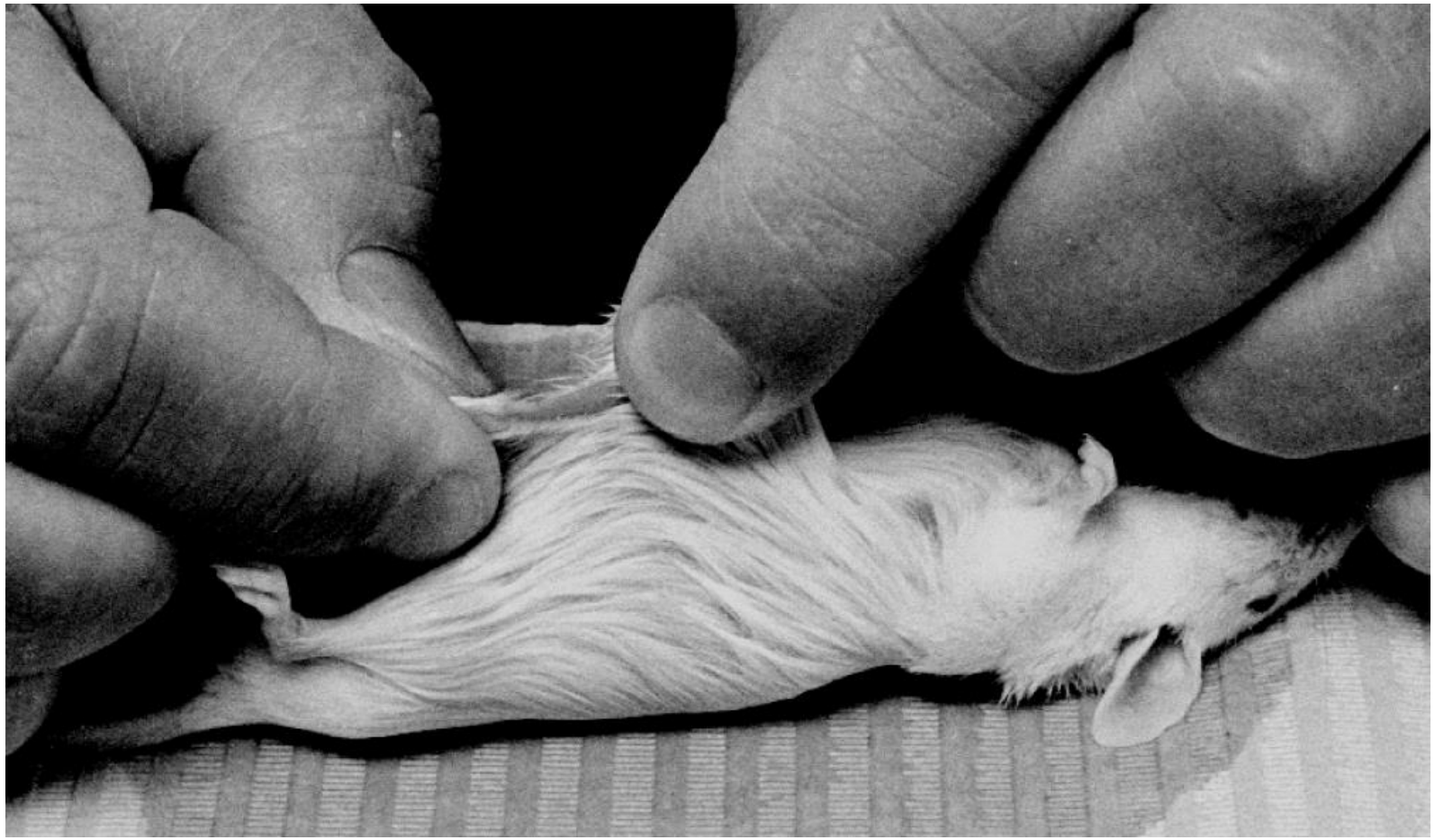
Isolation of mouse embryo

- Kill the mouse by cervical dislocation, and swab the ventral surface liberally with 70% alcohol.
- Tear the ventral skin transversely at the median line just over the diaphragm (Fig. 12.3b), and, grasping the skin on both sides of the tear, pull in opposite directions to expose the untouched ventral surface of the abdominal wall.
- Cut longitudinally along the median line of the exposed abdomen with sterile scissors, revealing the viscera. At this stage, the uterus, filled with embryos, is obvious in the posterior abdominal cavity (Fig. 12.3e).
- Dissect out the uteri into a petri plate and then transfer them into 25-mL or 50-mL screw-capped vial containing 10 or 20 mL DBSS.

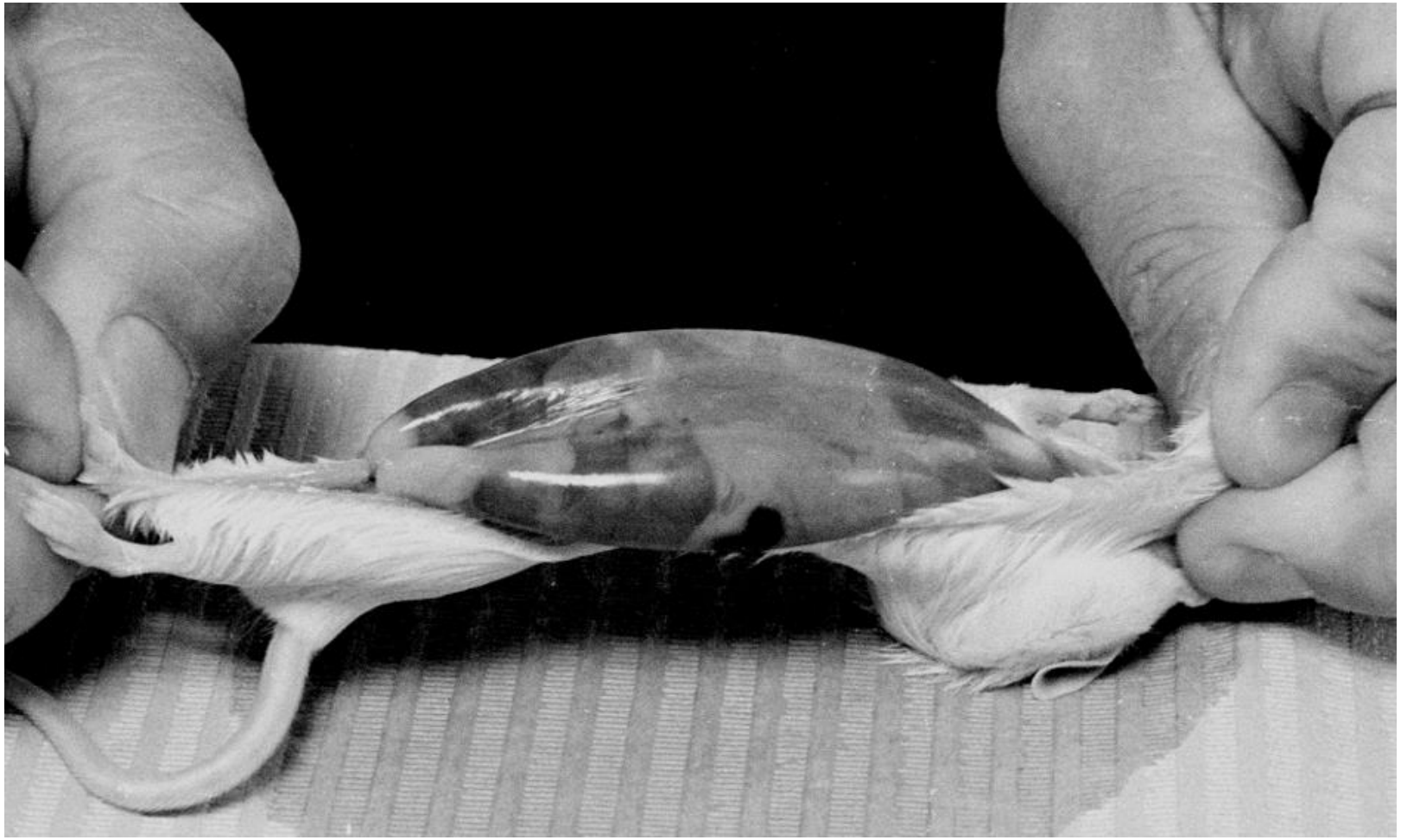
Swabbing the abdomen



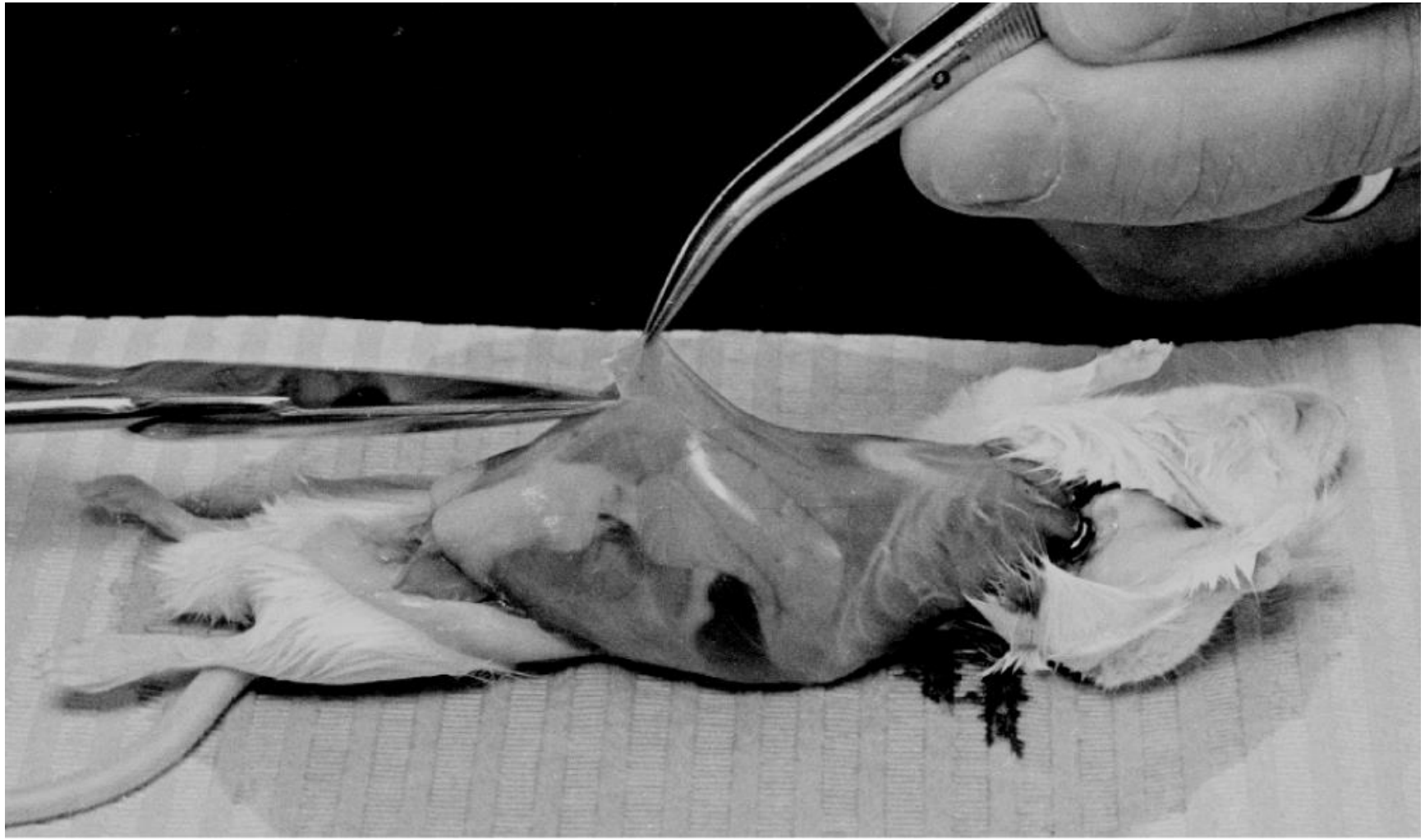
Tearing the skin to expose the abdominal wall



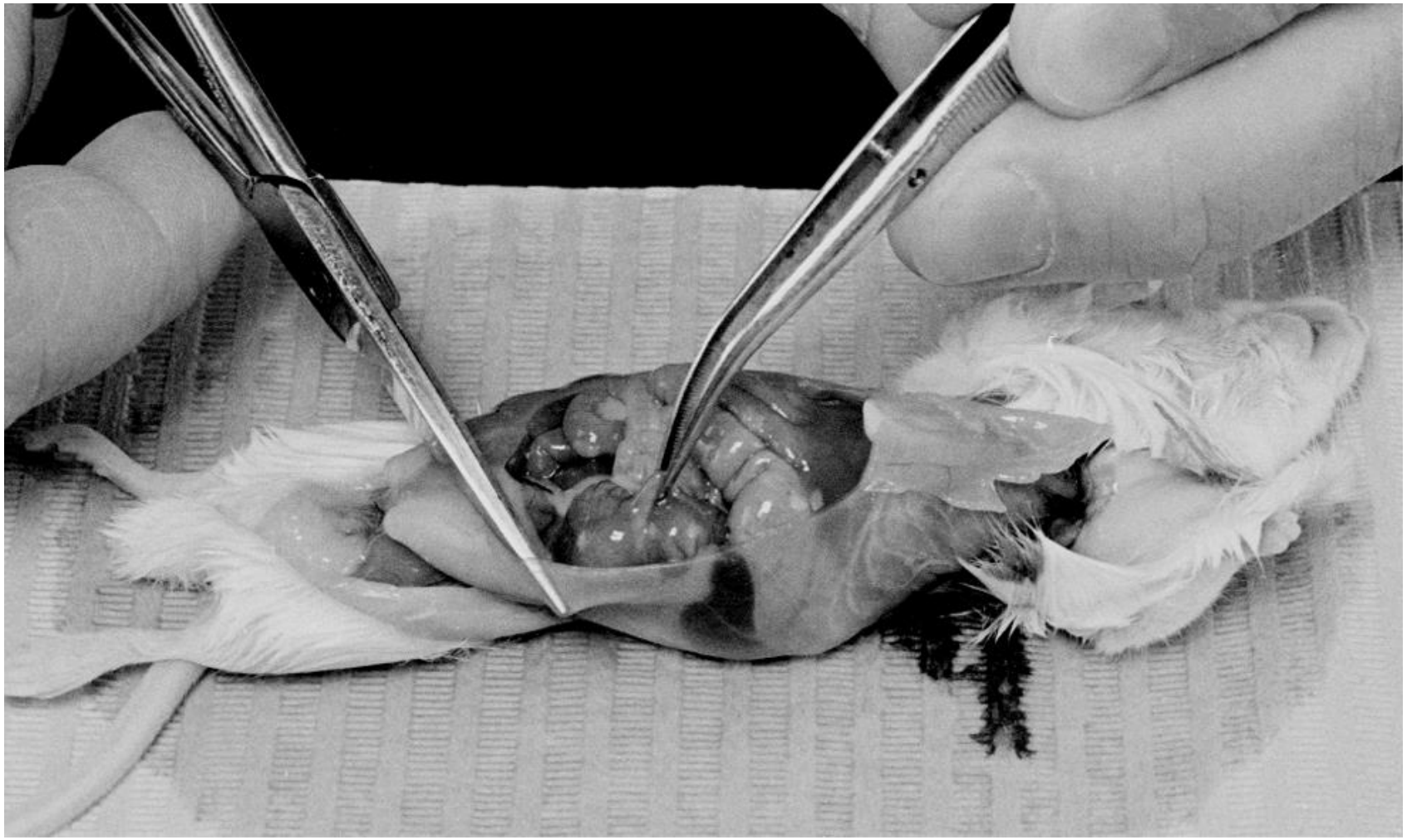
Completely teared view



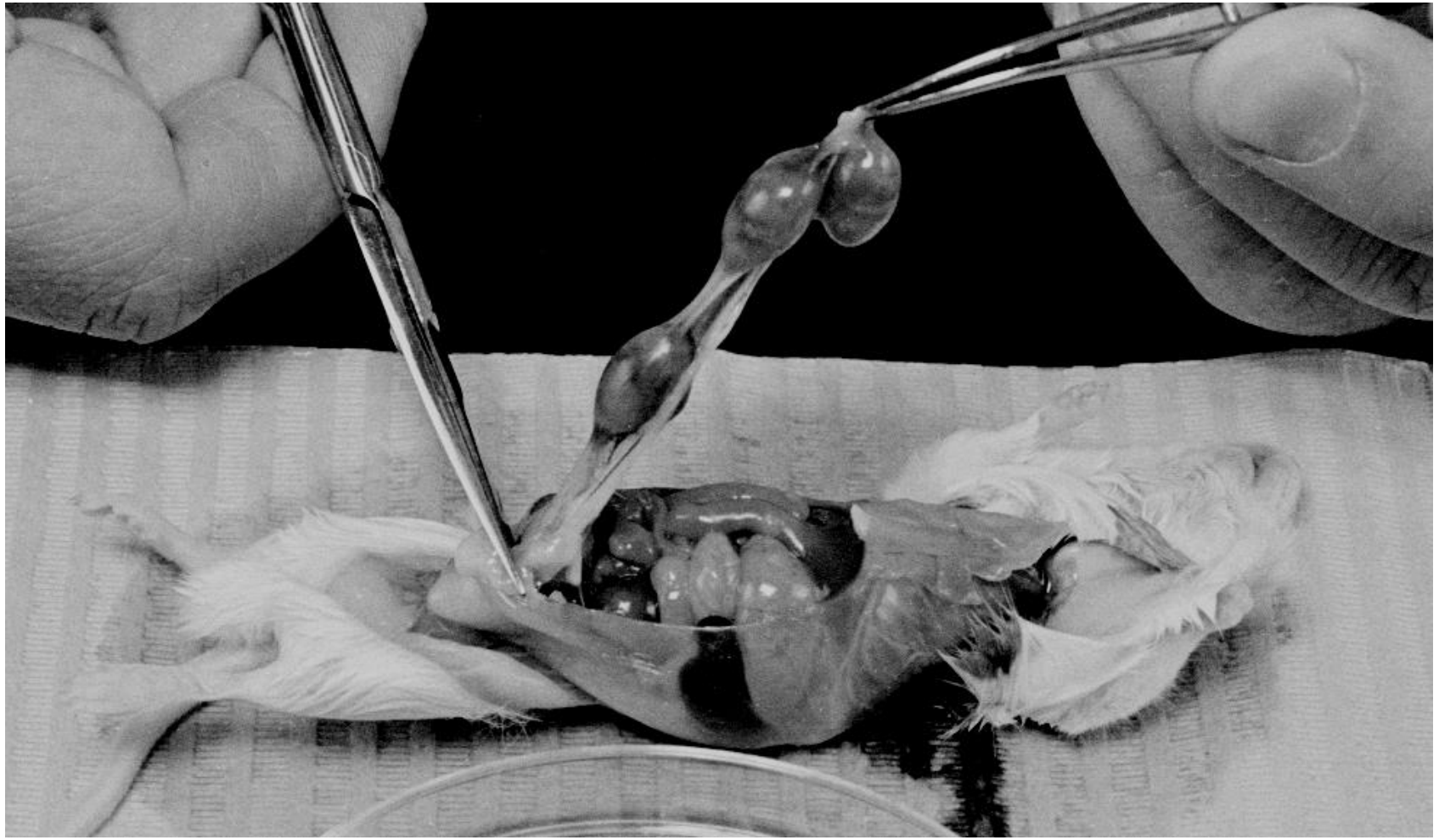
Opening the abdomen



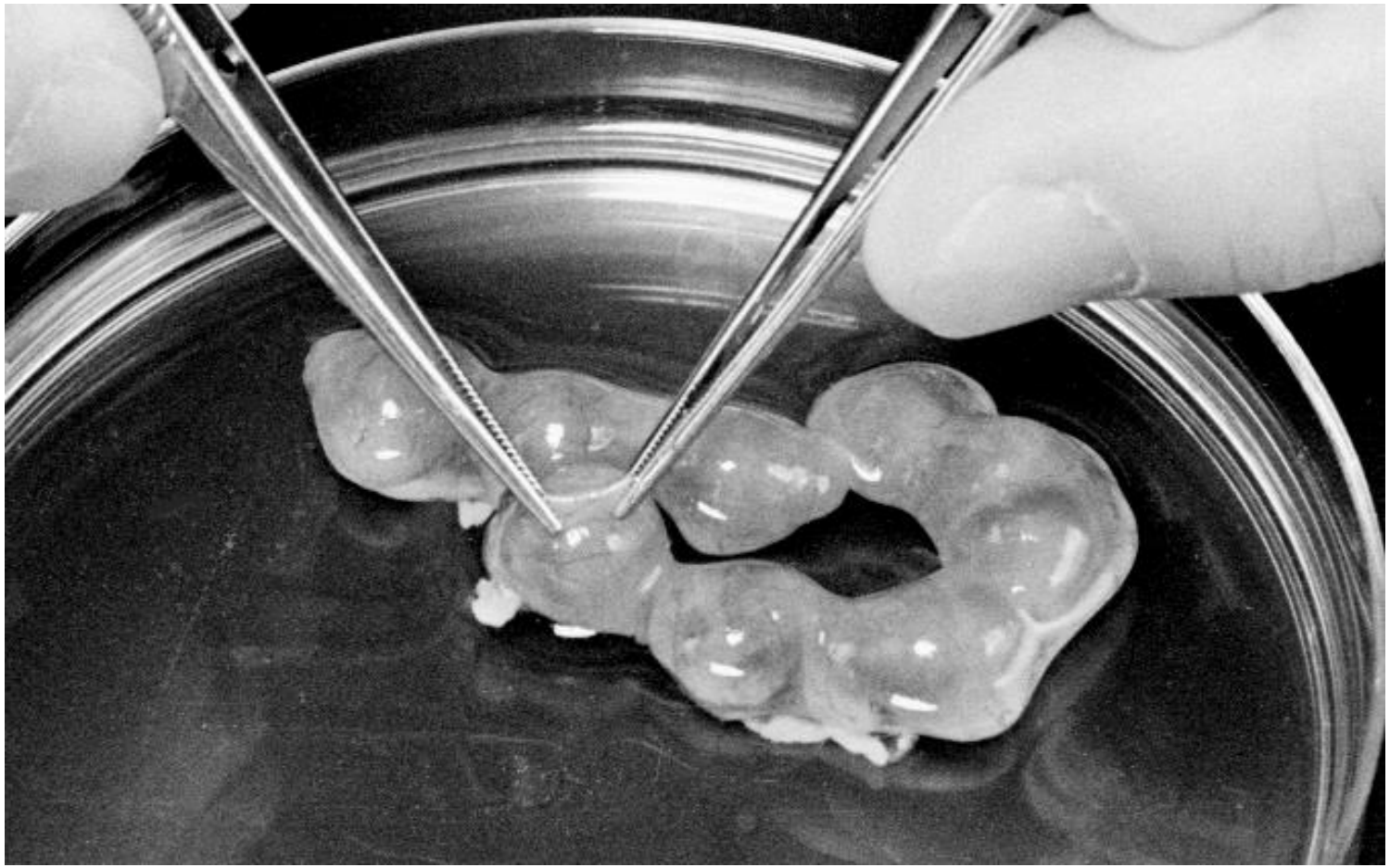
Revealing the uterus *in situ*



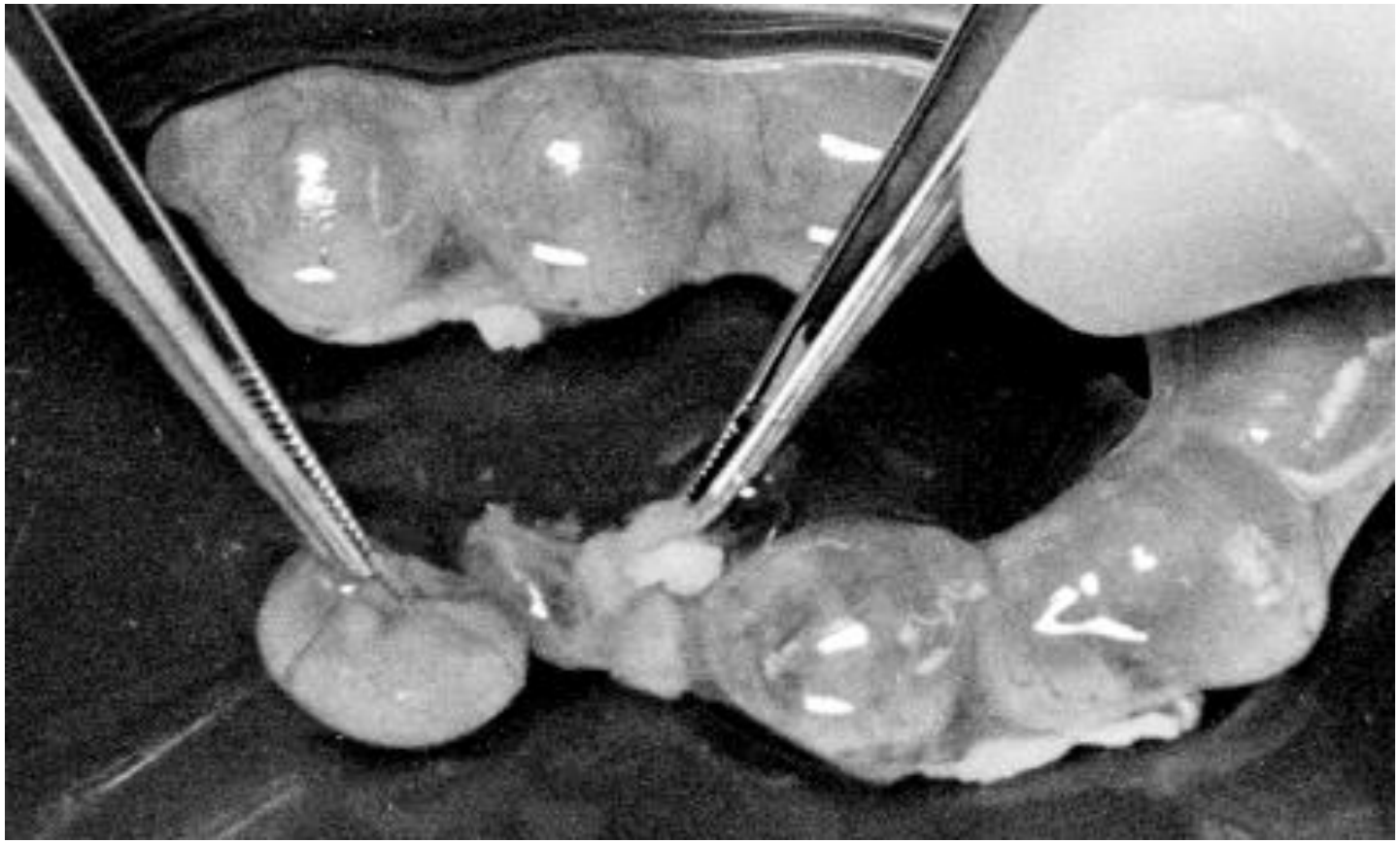
Removing the uterus



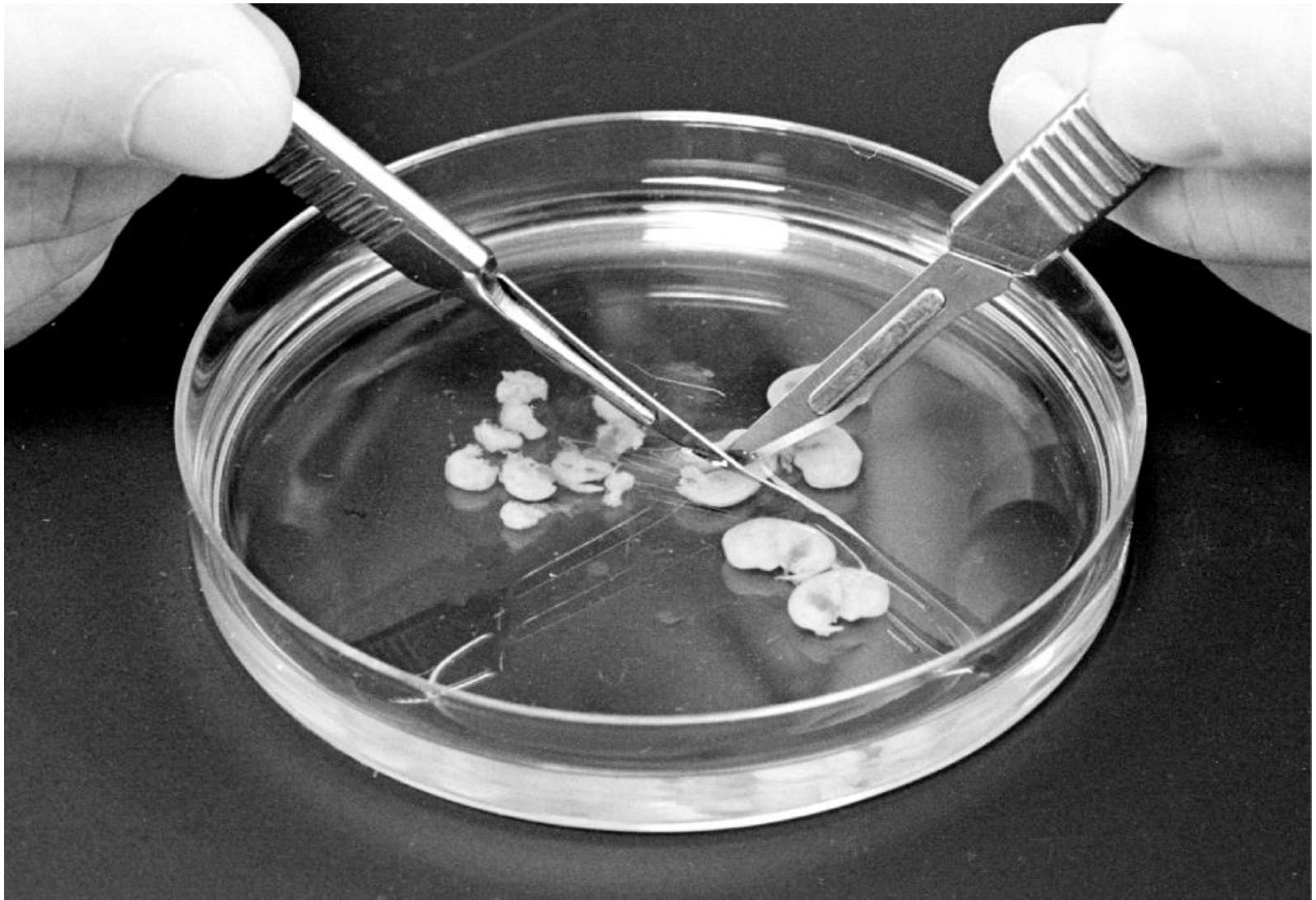
Dissecting the embryos from the uterus



Removing the membranes

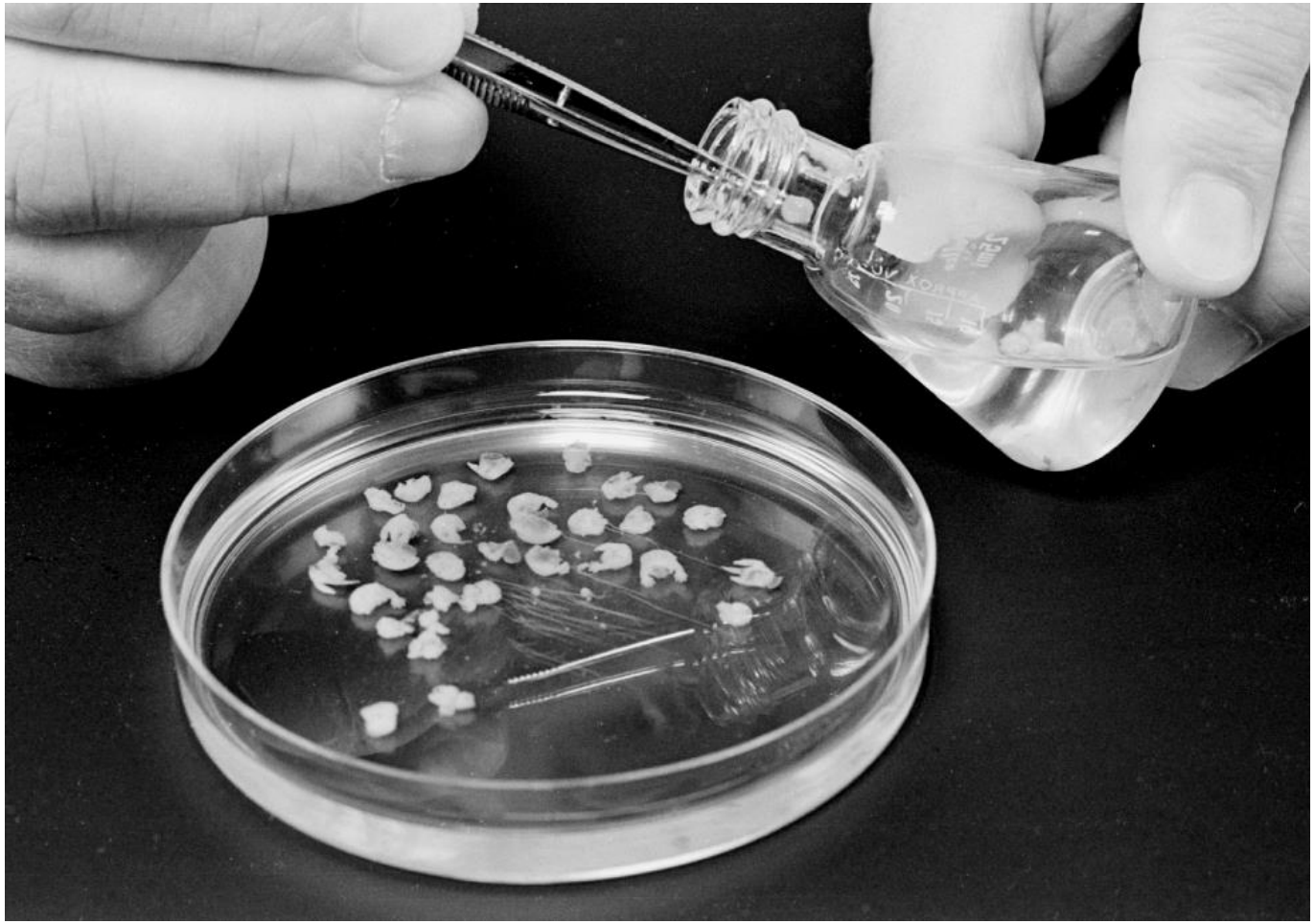


Chopping the embryos

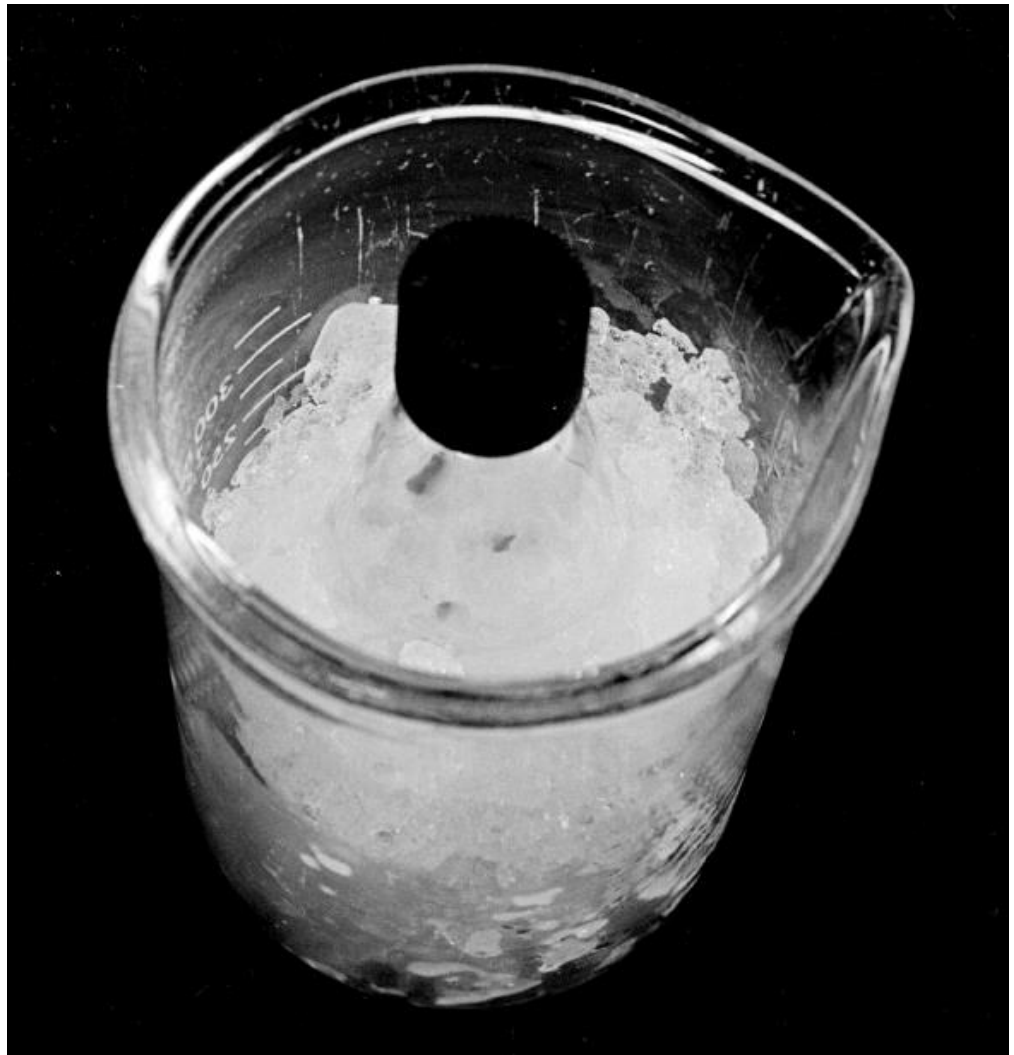


Transferring pieces to trypsinization flask

Transferring the pieces to a small Erlenmeyer flask

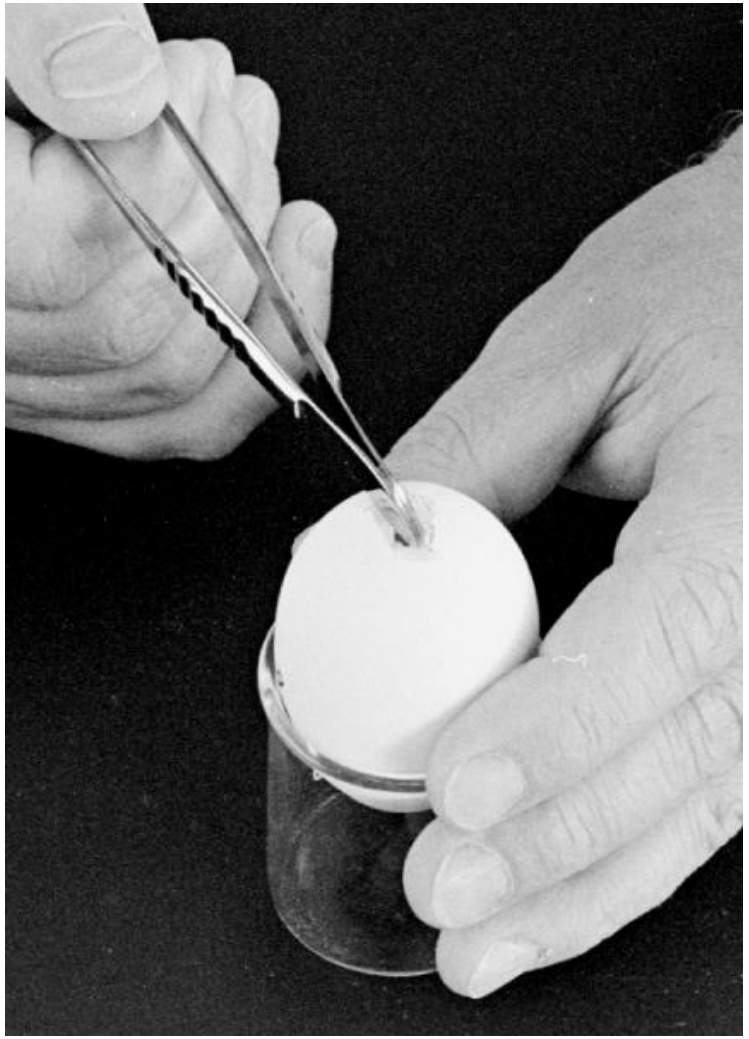


Chopped pieces in Erlenmeyer flask

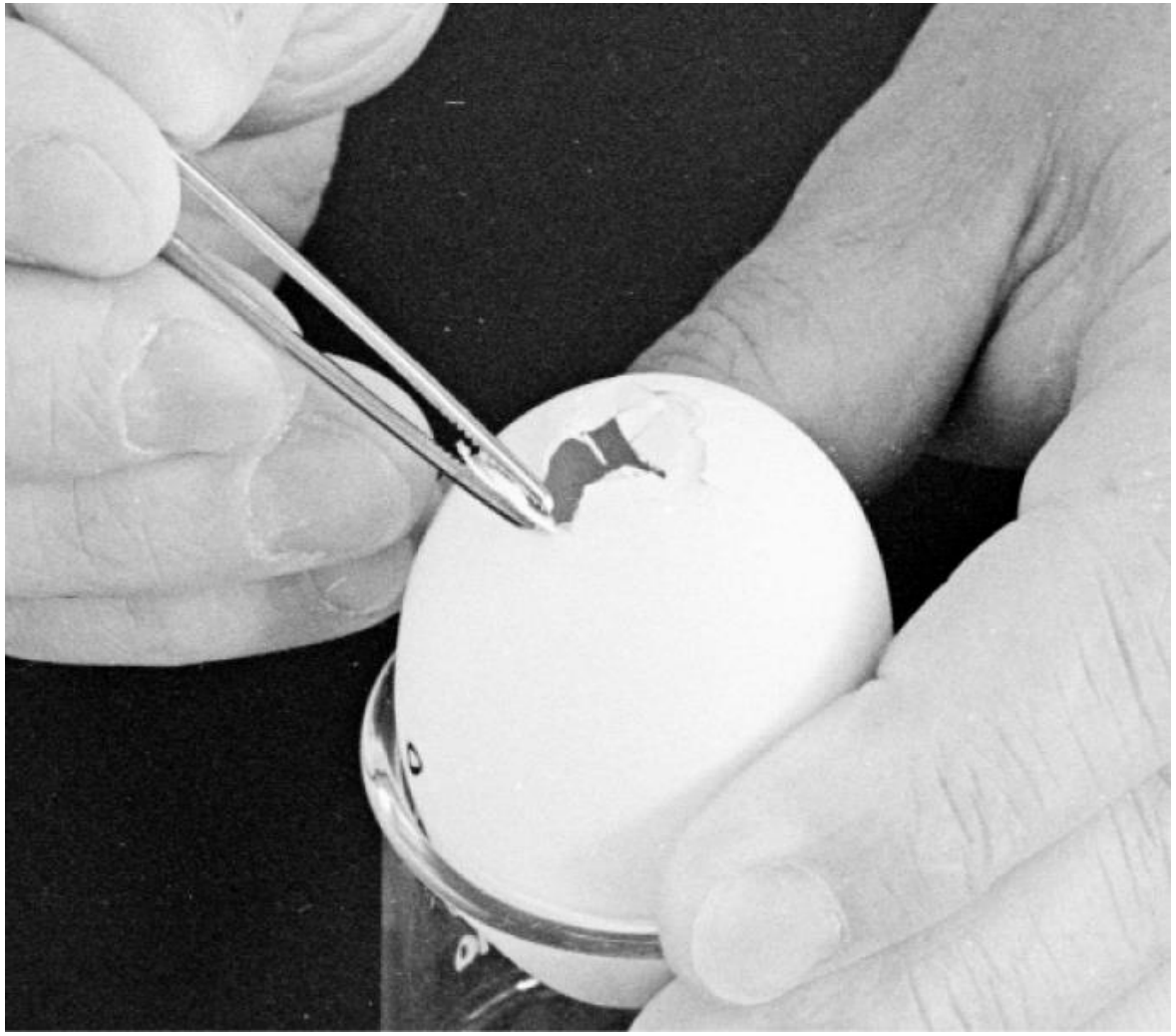


Swabbing the egg with alcohol

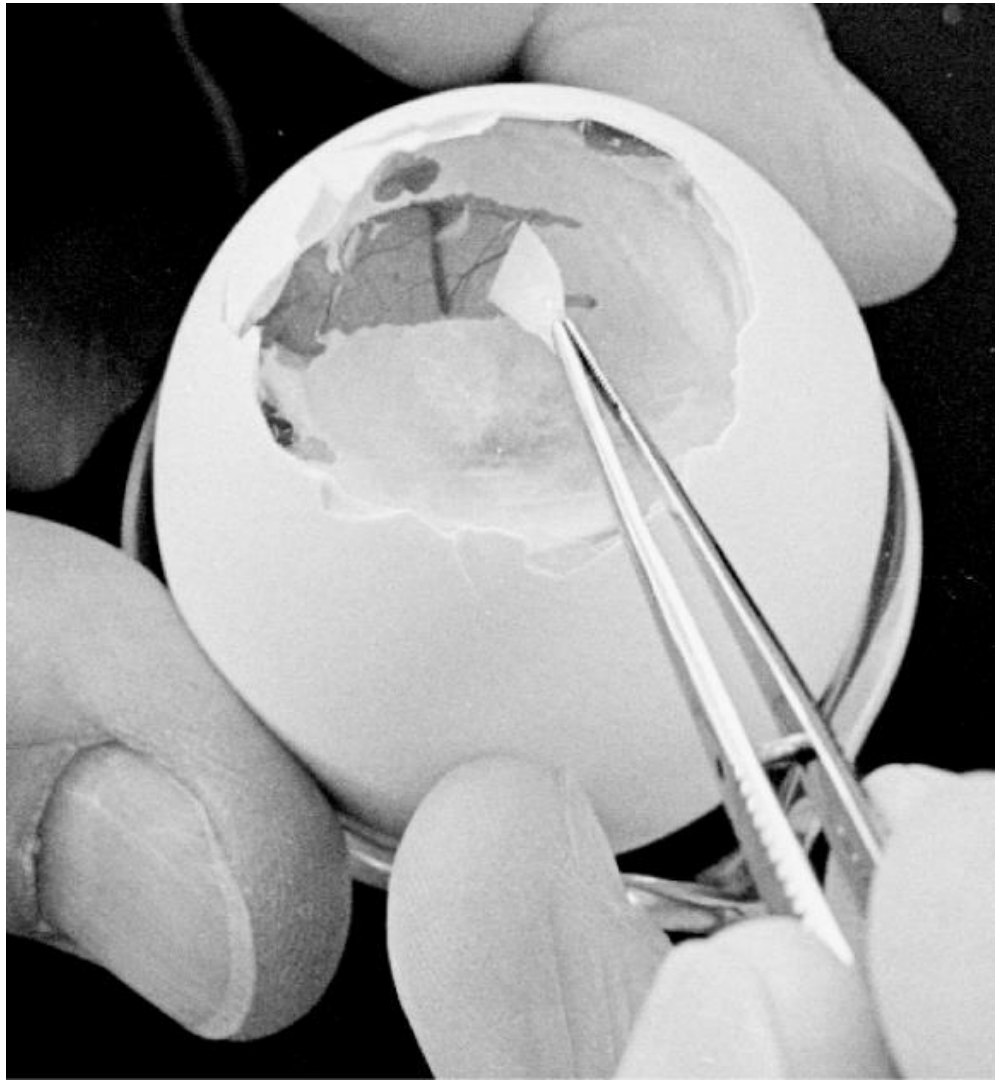
Cracking the shell



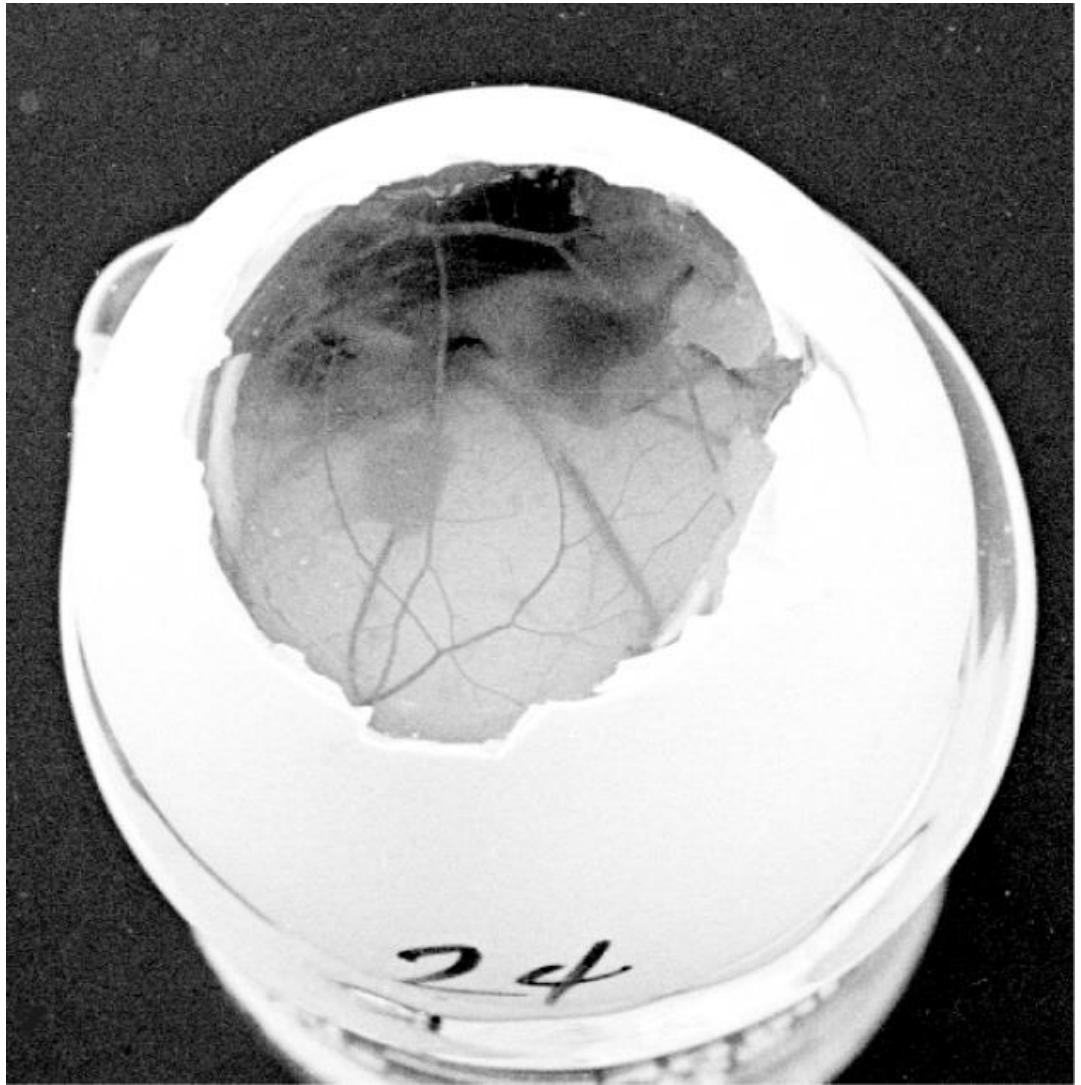
Peeling off the shell



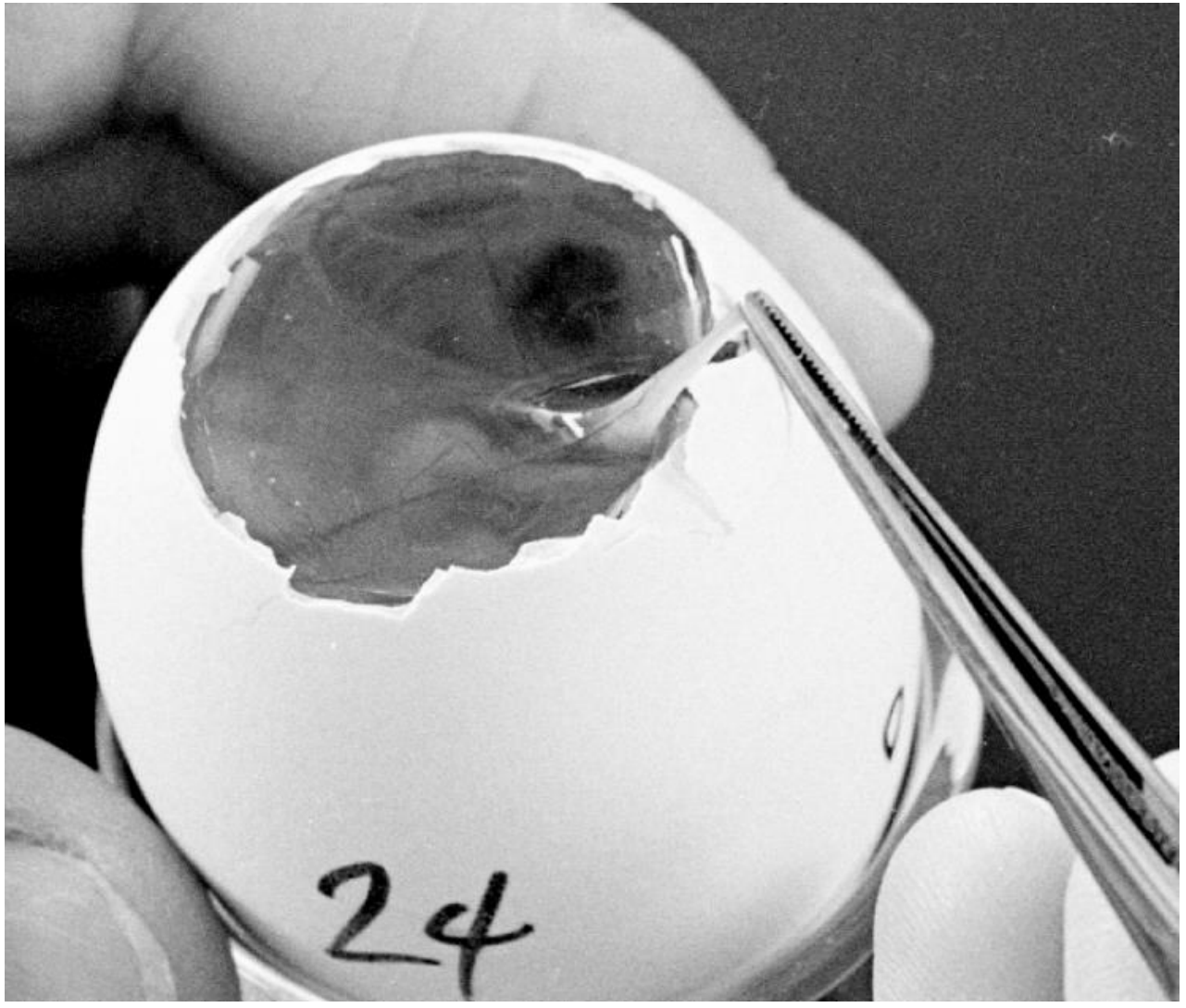
Peeling off the shell



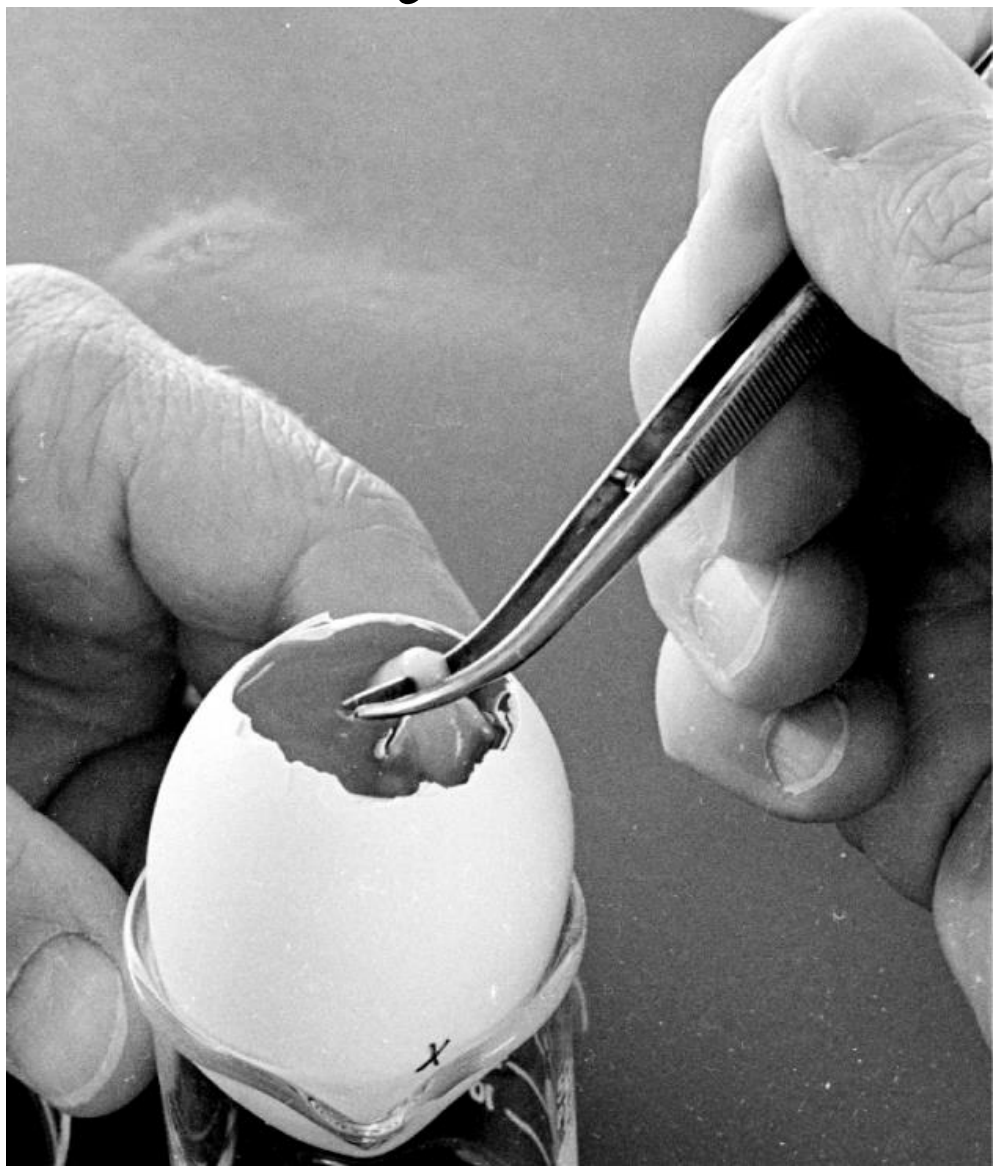
Chorioallantoic membrane (CAM) and vasculature revealed



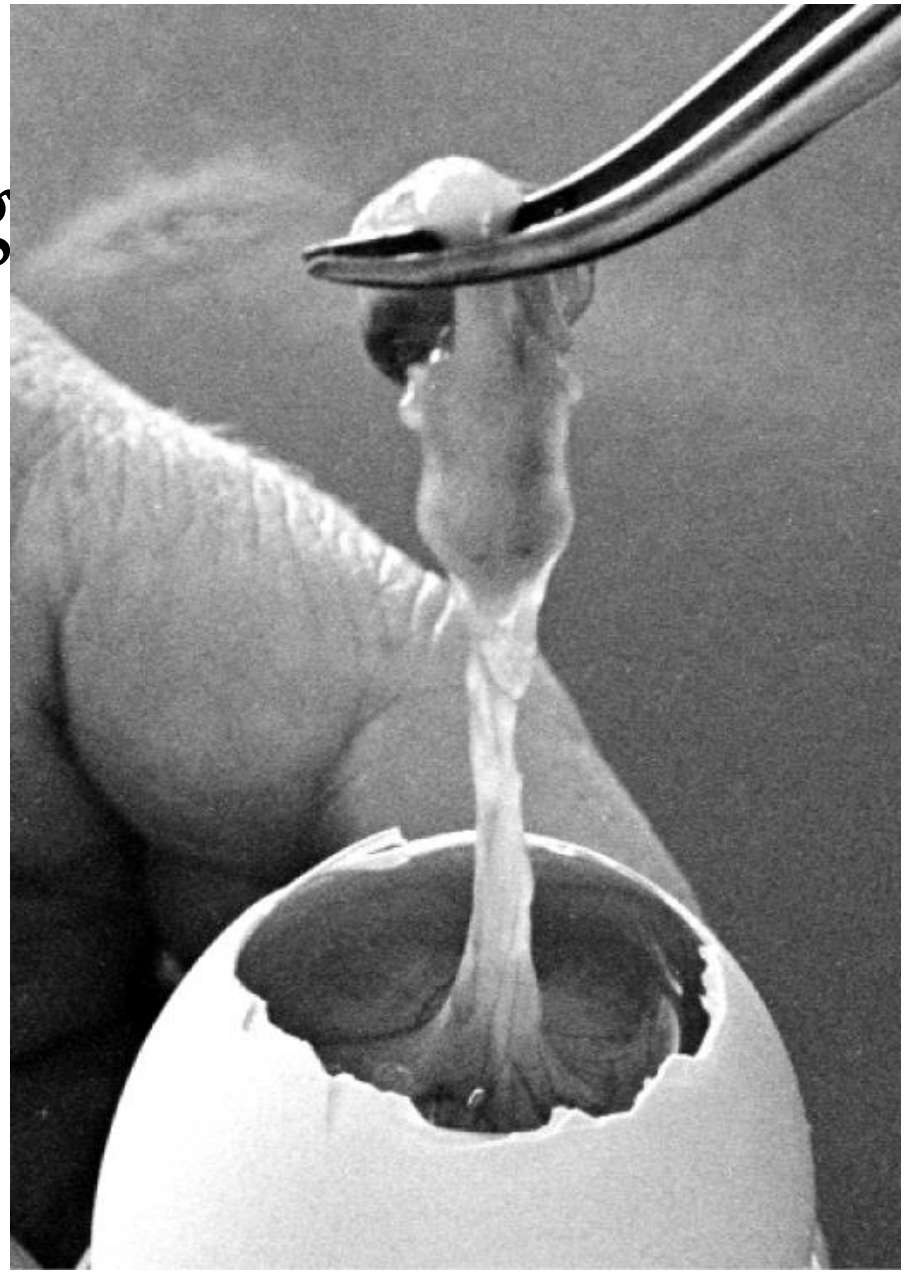
Removing CAM with forceps



Grasping the embryo round the neck



Withdrawing
the embryo from the egg



Isolated 10-day embryo in Petri dish