Internal Observation of Rabbit Uterus

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A PLASTIC CHAMBER has been developed for studying the interior of the pregnant rabbit uterus *in vivo*, under low magnification. It is believed that its principle is applicable to the short-term study of the interior of any hollow organ. A description may therefore be of interest to other experimental biologists.

The organ is transected or incised to expose its lumen. Several millimeters of the organ wall surrounding the opening are then pulled through a tapered hole in a sheet of plastic. While the organ is held in that position, the small end of a tapered, transparent plastic "stopper" is inserted into the lumen and pushed farther until the organ wall becomes compressed between the stopper and the plastic "bottleneck" provided by the tapered hole (Fig. 1).

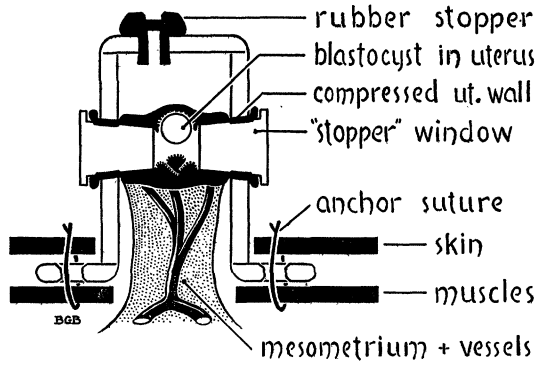

FIG. 1. A segment of rabbit uterus is shown in sagittal section in the position occupied when the chamber is assembled and ready for observation. Natural size.

This compression holds the organ in position and provides hemostasis. In addition, it serves as a seal that allows maintenance of the pressure of the fluid within the organ and offers partial protection against bacterial contamination from the outside. The stopper itself serves as a window, since both its ends are plane and polished (Fig. 1).

A large stopper naturally allows a better view than a small one. A cylindrical organ accepts a larger stopper if the lumen is entered at a transection rather than through a lateral incision. A second consideration is that the interior of a thick-walled organ is visualized best and illuminated with least danger of overheating when two such stopper windows are employed. (This, incidentally, provides a plain visual background and adds the possibility of oblique or dark-field illumination [Figs. 4 and 5].) These two limitations were imposed by the pregnant rabbit uterus and were accommodated by using a segment of uterus mounted in a pair of bottleneck holes, with stoppers, placed on two opposite walls of a box which formed a plastic hernia, or abdominal chamber (Figs. 1 and 2).

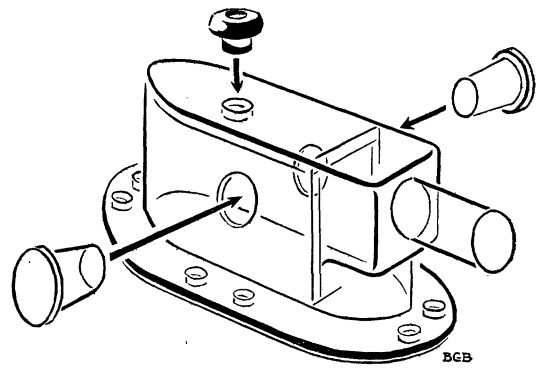

FIG. 2. Plastic chamber for *in vivo* observation of the interior of a rabbit uterus. Natural size.

General dimensions are not critical, but chambers about $10 \times 25 \times 30$ mm inside, made from 1.5 mm thick plastic,[2] have been found somewhat better than larger ones. The ends are rounded, and the bottom is open and surrounded by a brim—suggesting a high hat with extreme stretching (Fig. 2). Eight suture holes of 3 mm diameter are drilled in the brim. A rubber stopper, of the vaccine-vial type, is inserted into a hole drilled in the top of the chamber, preferably off-center. An easily clamped, cylindrical handle is attached firmly at one end. The tapered bottleneck holes for mounting the specimen are drilled in the front and back with centers 17 mm above the brim. Each is enlarged with a taper reamer until the particular stopper for that hole snaps into it easily, but does not fall out. The edges are then rounded and polished to prevent cutting of tissue. Stoppers are turned to taper from about 6 to 7 mm, approximating the external uterine diameter. A convenient length is 8 mm. A small flange around the outer edge favors a firm grasp by the manipulator, and a circumferential groove in the tapered portion near the flange allows the stopper to snap gently into position and thereby to oppose extrusion by the uterus (Fig. 1). If turned

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[2] Lucite or Plexiglas.

from plastic rod, the ends are ground and polished; if from thick sheet plastic, the polishing is avoided, but the turning is more difficult.

If desired, one of the stoppers may be intubated. A small hole is drilled parallel to the line of vision but at one side of the visual field. The tip of a piece of polyethylene tubing[3] is stretched to a point, threaded through the hole, pulled until tight, and trimmed off at the small end of the stopper. A convenient accessory is a 90° silvered prism or first surface mirror mounted on a microscope in place of the stage. It allows a vertical microscope position and better clearance between the microscope and the animal. A compound microscope with a 48 mm objective and a ×10 ocular gave about × 20 magnification and ample working distance for general observation. Records were made by colored motion pictures at normal speed and approximately normal size.

Mounting of the rabbit's uterus in the chamber follows aseptic midline abdominal section under Nembutal and ether anesthesia. Drying of exposed tissues is prevented by the use of wet sponges. Six or seven days after coitus the uterus is locally distended and rendered translucent by each blastocyst within it. About 1 or 2 cm proximal and distal to such a site, at points which permit the least possible disturbance of its blood supply from mesometrial vessels, the uterus is doubly ligated and cut between ligatures (Fig. 3). One or more control segments may be pre-

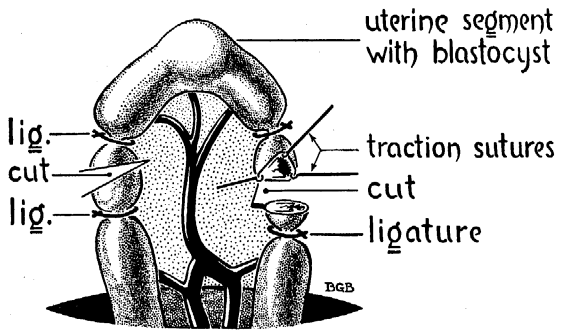

FIG. 3. Selection and preparation of the specimen with respect to blastocyst and blood vessels. Natural size.

pared in the same manner. The chamber and stoppers are then removed from 70 per cent ethanol, in which they have been immersed at least an hour, wiped with sterile gauze, and rinsed in Tyrode solution. If the ligated uterine segment has retained good color, it is inserted through the open bottom of the chamber, oriented to minimize twisting of the mesometrium, and maneuvered so that one end projects from one tapered bottleneck hole and the other from the opposite hole. Held by hand or in an Allis clamp, a stopper is then pushed into the uterine lumen, the constricting ligature is cut, and the stopper is pushed until it snaps gently into position. During the manipulation, instruments or traction sutures are used only near the cut edge of the uterine segment. After the stopper is in its final position, this traumatized edge projects beyond the stopper and is trimmed off. The mounting procedure is repeated on the other end of the uterine segment, with care to leave the segment without kinks, yet without undue longitudinal tension. While the chamber projects out through the abdominal incision, the brim is sutured into position between the muscles and skin (Fig. 1) or, alternatively, in contact with the peritoneum. Any portion of the incision which remains open is then closed. Thereafter anesthesia may be discontinued.

The rubber stopper is inserted in the hole in the top of the chamber. Tyrode solution is injected and air aspirated through that stopper by a hypodermic syringe until no tissue in the chamber remains exposed to air. Gloves and drapes are then removed. A clamp is applied to the handle for immobilization of the chamber, and observation is attempted. If folds of mucosa obscure the view, the rubber stopper is wiped with an alcohol sponge, and a sterile hypodermic needle is directed through it, into the chamber and into the uterine wall near one end of the uterine segment. Then, under microscopic observation, mucosal folds may be pushed or pulled aside gently. If this be insufficient, the uterine lumen may be entered and the uterine segment dilated slightly by a slow injection of Tyrode solution. If it is desired to avoid needle puncture of the uterus, the injection may be made through an intubated stopper. Although this stopper modification interferes somewhat with observation from its side, it is convenient in experiments that require repeated injections of test materials or frequent aspirations of fluid for smears or biochemical tests.

The chamber technique introduces several biological, chemical, and physical changes which should be recognized. The ligation and cutting of a segment of rabbit uterus stop the blood supply from anastomotic longitudinal vessels. Therefore, the detailed vascular pattern of the mounted segment cannot be assumed to be entirely normal even if the mesometrial vessels are successfully preserved and continue to provide an apparently adequate circulation. The segmentation procedure may disturb nerve supply in some organs, but it is not a problem in the rabbit uterus (*1*). Despite standard aseptic surgery, and cleansing of the chamber in 70 per cent ethanol, an increase of polymorphonuclear leucocytes was usually evident in fluid aspirated from uterine lumina 24 to 30 hours postoperatively. Infection became macroscopically evident after two or three days. This suggests the use of antibiotics when prolonged observations are desired. The injection of Tyrode solution almost certainly alters the pH and osmotic value of the uterine contents. This procedure must be assumed to alter pressure as well, since it is done for the purpose of distending the uterus slightly. Overheating of tissues occurred when the thick lateral wall of a 7-day pregnant rabbit uterus was transilluminated in an earlier single window chamber. However, with the double window chamber, no such trouble has been encountered with light

[3] Polyethylene tubing, PE 20, Clay-Adams Co.

intensities sufficient for observation and color motion photography. In view of the several changes mentioned and possible additional, unrecognized ones, it is considered desirable to accompany each chamber experiment with one or more controls. One control segment may be mounted for observation parallel with the experimental segment. This is accomplished in a chamber provided with an extra pair of bottleneck holes and stoppers. An additional segment of uterus may be ligated and cut like the others but simply left in the peritoneal cavity until autopsy.

Six or seven days after copulation, blastocysts were seen, each in a ballooned-out, antimesometrial "dome" formed by local decrease of uterine muscle tone. Elsewhere the uterine muscle was in "contracture" (*2*). Changes in tone affecting the dome produced surging motion of detritus in the uterine fluid as the blastocyst was carried in somewhat pistonlike motion in the mesometrial-antimesometrial directions. The cycles varied in duration, but many lasted about half a minute. The period of minimum tonus occupied about 5–10 seconds of this cycle. Occasionally a rotational shift of a blastocyst was also observed. However, detection of this phenomenon required continuous observation of markers (such as a grouping of individual trophoblast cells), and, unfortunately, it was often quite difficult to follow them for an appreciable time.

The chamber may be used not only for observation (and thus for obtaining specimens for fixation at stages described by behavior as well as age), but also for simple experimental procedures. Injection of fluid into mounted segments of uterus was intended to improve visualization. However, the procedure easily caused excessive distension, which arrested the motion. Release of pressure was eventually followed by a resumption of motion. In five cases, however, the overdistension also dislodged the blastocyst from the

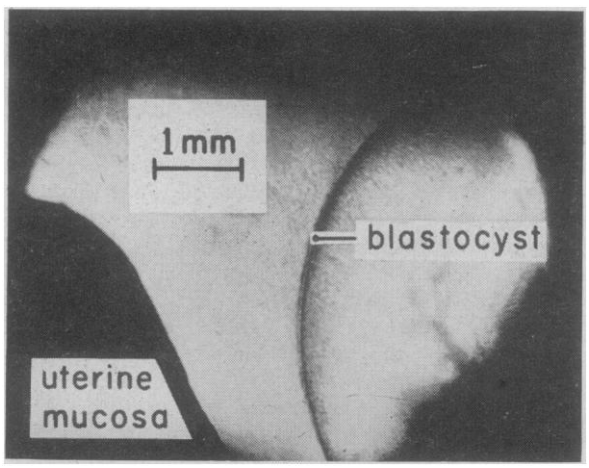

FIG. 4. This view through the lumen of a segment of rabbit uterus mounted in the chamber shows the edge of a spherical blastocyst which lies low and to the right of the center of the field. Earlier, this blastocyst was at the top of the field, but distension of the uterine segment dislodged it from that antimesometrial position, about 7 days after copulation. The thin line just outside the blastocyst is the unwrinkled zona pellucida.

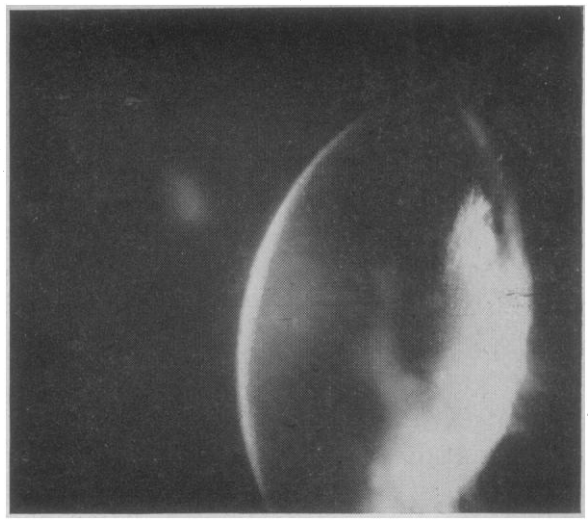

FIG. 5. Same subject as Fig. 4, with oblique illumination.

"grasp," as it were, of its dome (Figs. 4 and 5). This suggested a rather fine balance of mechanical factors. On the other hand, in three preparations the blastocysts were not dislodged by such distension. Furthermore, in one case the distension was made deliberately severe, and the blastocyst was not dislodged, despite obvious loss of contact with all parts of the mucosa that could possibly have transferred a muscular grasping or lifting force. The blastocyst was instead held in the dome, as if it were somehow attached to the antimesometrial wall of the uterus. Surprisingly enough, all three cases of attachment had occurred despite the fact that the membranes surrounding the blastocysts had not yet been shed. A comparable observation has been made histologically, and it appears that the zona pellucida or the albuminous envelope is in fact involved in the attachment (Fig. 6).

During the injection that caused the severe distension, the last contact between blastocyst and mesometrial or lateral uterine mucosa was seen to become stretched and finally broken. The distension was released, a rest period was allowed, and a second distension was performed. On this occasion motion pictures recorded a repetition of the previous events at exactly the same, quite restricted, location (Fig. 7). It seemed curious that the adhesiveness should not be apparent in immediately adjacent areas. This blastocyst did, however, adhere to one of the polished plastic stopper windows. One may therefore probably consider the implantation adhesiveness to be a property acquired by the zone pellucida or albuminous envelope rather than by uterine epithelium. About the time this adhesiveness becomes evident, one observes that the blastocyst and its coverings no longer appear to be distended concentric spheres as in Figs. 4 and 5. They have become creased, or even flaccid (Fig. 7).

The cause of these changes in the blastocyst coverings is currently being investigated by biochemical procedures *in vivo* with the chamber and *in vitro*. In the hemisphere of the blastocyst opposite the em-

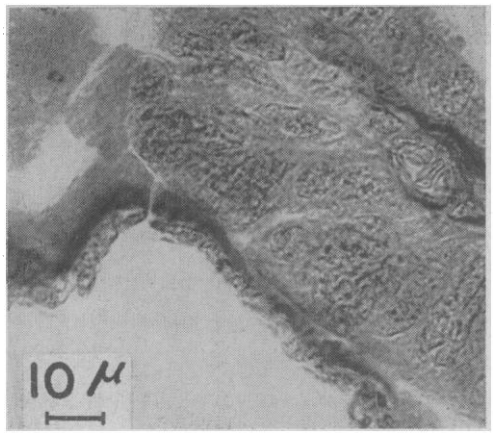

FIG. 6. An adhesion between blastocyst and uterine epithelium involves the noncellular blastocyst coverings about 7 days after copulation. Ten-micra sections, azan stain, and a red filter show a pale (red) layer of blastocyst cells above the clear area. Next is an inner dark (blue) layer tentatively interpreted as zona pellucida, and then a paler (blue) layer believed to be the albuminous envelope. Pale (red) uterine epithelium occupies the upper and right part of the field.

bryonic disk there apparently develop one or more areas that release an unidentified substance with a pH over 9. The blastocyst coverings affected by it become adhesive, increasingly permeable, flexible, histologically degenerated, and eventually perforated. A pertinent detail is that 4-day-old blastocysts with thick albuminous coverings tended to stick to their containers when placed in solutions more alkaline than pH 9 or 10. Subsequent acidification strengthened the bond. If torn loose, however, the blastocysts did not re-establish an adhesion in the acid solution. It may therefore be significant that Tyrode solution with phenol red, when injected into the lumen of a 6½-day pregnant uterine segment in the chamber, promptly changed from rose-red to orange-red, indicating a shift to about pH 7.3.

The transparent plastic chamber described in this paper has facilitated observational and experimental studies of rabbit blastocyst implantation. About 6½ days after coitus, when a blastocyst has enlarged to 4 or 5 mm, it is accommodated by a local decrease in uterine muscle tone which forms an antimesometrial dome. The blastocyst is thereby held in prolonged and intimate contact with attenuated antimesometrial mucosa and permitted only passing contact with mesometrial epithelium. At the same time, changes in tonus impart a pistonlike motion to the blastocyst and a surging motion to the fluid in the uterine lumen. A rotational motion of the blastocyst has also been detected, and it is speculated that eventually each portion of the blastocyst surface has a sojourn in the dome. Alternatively, there may be an orientation mechanism which depends on the oblate spheroid blastocyst accepting a position determined by internal uterine contour, as suggested by Assheton (*3*). Whatever the mechanism, the abembryonic, alkali-emitting portion of the blastocyst eventually comes to lie in the dome. The abembryonic alkaline substance is considered able to confer adhesiveness upon the overlying zona pellucida or the albuminous envelope. However, this ability is believed to be counteracted by the vigorous circulation of much less alkaline fluid in the uterine lumen—*until* the abembryonic portion of the blastocyst comes to lie in the antimesometrial dome.

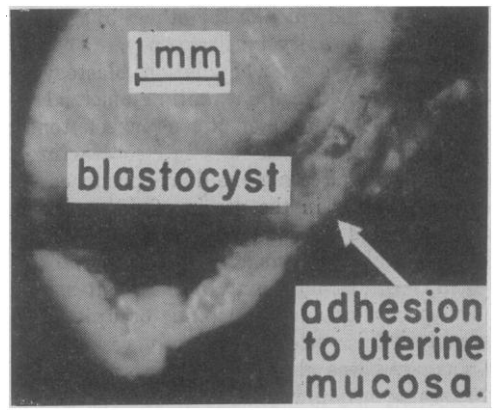

FIG. 7. This view should be compared with Fig. 4. It was photographed during the dilatation of a uterine segment 7½ days after copulation. This blastocyst could not be dislodged from its antimesometrial attachment. An area of adhesion between blastocyst, zona pellucida, and lateral uterine epithelium is shown under tension about 1 second before it tore loose. This blastocyst has acquired wrinkles and adherent detritus.

There, its close apposition to attenuated uterine mucosa protects it from the continual rinse of uterine fluid, and the abembryonic alkaline substance is in a uniquely favorable situation for effecting adhesiveness of the overlying zona pellucida or albuminous covering. Geometrically, too, the situation is singularly favorable for an adhesive attachment between the blastocyst coverings and uterine epithelium, since that part of the blastocyst which is in the dome has the least possible motion with respect to epithelium touching it. Adhesion is believed to occur between 7 and 7½ days after copulation.

When the first attachment occurs, the orientation of the blastocyst becomes established. It is therefore suggested that antimesometrial dome formation and abembryonic alkali emission may be considered not only mechanisms of rabbit blastocyst attachment, but also agents of regional preference contributing to the characteristic (*3, 4*) abembryonic, antimesometrial orientation of early rabbit blastocyst implantation.

References

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