AUTO-TRANSPLANTATION OF RABBIT MYOMETRIUM INTO EAR-CHAMBER AND RECORDING OF ITS ACTIVITY

By

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ABSTRACT

A transparent ear-chamber and its installation in the rabbit is described as well as the subsequent autografting of myometrial tissue into the connective tissue formed in the chamber. Of the rabbits initially provided with ear-chambers 65% were found to be suitable for grafting while finally only 40% of these presented activity either spontaneously or induced by stretch or oxytocin. Qualitative evaluation of the mechanical activity was obtained by photoelectric recording of the transmission of light through the graft. An evaluation of the electrical activity was made possible by recording volume conducted potentials from wire electrodes in contact with the grafted tissue. A close time interrelationship between electrical potentials and change in transmission of light of the graft was found. Oxytocin produced electrical activity and blanching of the graft. It is concluded that recording of the electrical activity provides the most reliable way of expressing the activity of grafted myometrium.

With the introduction of the transparent ear-chamber technique (Sandison 1924; Clark et al. 1930) and its subsequent development (for review see Williams 1967) a new tool for direct observation of living grafted tissue was obtained. This technique was applied to the study of myometrial physiology when Cliff et al. (1964) and Michael & Cliff (1964) placed myometrial autografts in pre-
inserted transparent ear-chambers in 5 rabbits. By this means they studied the change in transmission of light in the graft caused by oxytocic and vaso-motor drugs by means of photo cell recordings.

As grafting of the myometrium thus seemed to be a possible way of studying the function of the tissue under well controlled conditions, a large series of experiments was carried out. The method of grafting into a rabbit’s ear-chamber as well as the results of simultaneous registration of the mechanical and electrical activity of the graft is presented here.

![Diagram of ear-chamber parts](image)

**Fig. 1.**
The single parts of the ear-chamber. A: upper celluloid cover plate. B: upper acryl part of chamber. C: nickel plated copper ring. D: filter paper ring. E: mica plate. F: acryl base. G: lower celluloid cover plate. Cross section of the chamber is shown in the lower part of the figure with the bolts and nuts. All dimensions are given in mm.
Fig. 2.

The base of the ear-chamber (viewed from the outside and in cross section) with the three permanent platinum electrodes (diameter 0.2 mm) protruding above the centrally placed circular table. The electrodes are connected to the plates to which leads are soldered.

MATERIAL AND METHODS

The ear-chamber

Fig. 1 shows the single parts of the ear-chamber used at the beginning of this study. It is a modification of the chambers described by Williams & Roberts (1950) and by Williams (1961).

The chamber consists of an upper and a lower part (base) of acryl (B and F, Fig. 1) placed on each side of the ear (upper on the inside and lower on the outside) and fixed by four through-going bolts of nickel-plated brass. The base (F) has a centrally placed circular table of 8 mm diameter, elevated 0.8 mm above the surroundings. In the upper part (B) the central hole of 12 mm diameter is surrounded by a 2.5 mm wide and 1.5 mm deep groove that supports a thin mica disc (E), a filter paper ring (D) and a nickel-plated copper ring (C). These three parts are kept in place by two spring-bronze holders. For protection two celluloid plates (A and G) are placed on each side of the chamber after its placement.

Being transparent the chamber offers the possibility of visual observation. Registration of electrical activity was made possible by modifying the chamber. Through the central table of the base (F in Fig. 1) three holes were drilled and a platinum thread (0.2 mm in diameter) was drawn through each protruding about 0.2 mm into the chamber (Fig. 2). On the outside each thread was led to a metal plate and soldered to it. A highly flexible Plastosyn lead (Type 1360, Dätwyler containing
65 twisted copper threads, 0.05 mm in diameter) was soldered to another part of the plate. The whole area was then coated with an insulating lacquer.

**Installation of chamber**

Female rabbits (2400–2900 g) with long, wide ears were selected. The skin should preferably be loosely bound to the underlying perichondrium.

A central hole of 6.2 mm and four corner holes each of 2.6 mm in diameter were punched in the ear, followed by blunt dissection. The surgical procedure was carried out aseptically in the fully anaesthetized animal and the chamber sterilized. The base of the chamber (Fig. 2) was placed on the outer side of the ear with the central plateau in the large hole. The elevated corner areas fit into the four peripheral holes. The through-going bolts were put into position and the upper part of the chamber (B, Fig. 1) was placed on the inside of the ear. Before placing the filter paper ring it was wetted in a 1% chloramphenicol solution.

**Grafting in chamber**

After 4–6 weeks a thin, vascularized layer of connective tissue had grown into the central hole and grafting could be undertaken. This was performed in non-pregnant as well as in pregnant animals. The copper ring, mica cover, and filter-ring were cautiously lifted from the chamber, as the tissue may stick to the mica. The cavity of the chamber was then filled with a 1% chloramphenicol solution (in physiological saline) to protect the tissue from drying out. Through a sterile, low midline laparotomy a small piece of myometrium providing a 1/2–1 mm³ large graft was excised. The graft was placed as close as possible to the tip of an electrode and close to a vessel, into a hole pricked with a knife into the newly formed connective tissue. Immediately after this a new sterile piece of mica was placed in position (together with a new chloramphenicol treated filter paper ring and a new copper ring) in order to keep the graft fixed. The abdominal incision was closed in two layers.

**Photo cell recording**

For examination the rabbit was either held down on a special table or left sitting in a pillory box with the ear fixed by a specially designed holder.

On one tube of the Zeiss stereo microscope a cylindrical tube with a black inner surface and provided with a photo cell (Siemens, Type BPY 44) was placed. In this way changes in transmission of light could be recorded. A stabilized DC incandescent lamp was used as light source, as the AC (or changes in the AC) would interfere with the registration. The signals from the photo cell were fed directly into one channel of an oscilloscope (Tektronix 502 A). The differential amplifier had an input impedance of 2×1 Megohm, each shunted by 47 pF. The input selector was in DC position and the vertical amplifier of the oscilloscope was connected to a pen-recorder.

**Electromyographic recording**

Once the graft had healed, it was possible to pick up changes in electrical potentials using the electrodes upon which the graft had been placed as the 'direct' and one of the others protruding into the connective tissue, as the 'reference' electrode. The base of the ear was earthed. The leads were fed into the other channel of the oscilloscope with the input selector in AC position giving a lower frequency limit of 1.5 Hz. The output from this channel was likewise connected to a multi-channel pen-recorder (Offner, RB Dynograph).
**Fig. 3 A.**

Photo taken through a microscope showing a living, resting graft (circular pale area with visible capillaries) and surrounding connective tissue transilluminated. 24 x.

**Fig. 3 B.**

Same as Fig. 3 A, but during stretch induced contraction. No capillaries are seen in the graft. 24 x.
Fig. 4.
Photomicrograph of myometrial graft 4 weeks after grafting, and 9 weeks after insertion of chamber. The musculature (arrows) is of normal appearance and vascularisation. No invasion of connective tissue is seen. No reaction in the surrounding connective tissue can be detected. Note cartilage layer above graft. Perfusion fixation *in vivo* with glutaraldehyde. 250 x.
RESULTS

General

Far from all animals with a chamber in position had connective tissue growing into the central hole. Of the rabbits initially equipped with ear-chamber 65% were found fit for grafting. Out of these less than half (about 40%) were suited for recording of activity.

During the days immediately following the transplantation it is usually not possible to make visual observations. In a few cases it was possible to observe circulation in the grafts as early as 3 days after embedding. Usually 8-10 days elapsed before circulation was started. Observation was best undertaken at a 20 to 40 times magnification (Fig. 3 A).

During the weeks and months following the first observation the field quite often got less clear and gradually quite opaque. This eliminated the use of photo cell recording but did not interfere with the electrical activity. When such cases were examined histologically, it was found that a layer of cartilage had grown into the chamber (Fig. 4). The longest observed period with a functioning graft was 13 months.

![Fig. 5.](image)

Photoelectric records showing the increasing transmission of light following quick stretch of the ear close to the chamber in 2 different rabbits. Note the different time scales. Ordinate: Photo cell current in arbitrary units.

773
Fig. 6.

Photoelectric records from 4 different rabbits. Same ordinate as in Fig. 5. a. 20 min record with a single spontaneous increase in transmission of light. b. 14 min spontaneous and oxytocin accelerated excursions. c. 10 min registration showing a positive response to 5 mU oxytocin (Syntocinon®) injected iv. d. 5 min record of spontaneous activity. e. 35 min record of response to 150 mU oxytocin injected iv. Same rabbit as d., but with 6 days’ interval.

Mechanical activity

In order to make certain that the graft was excitable, a quick stretch was applied to the ear close to the chamber. Within 1/2–1 1/2 seconds this resulted in a blanching of the graft due to disappearance of corpuscular elements (blood cells) from its vessels (Fig. 3 B). Fig. 5 shows two examples of stretch-induced increase in transmission of light. When observed under the microscope, movement or a slight distortion of the tissue could be seen quite often. A slight decrease in diameter of the area could be measured. As exactly the same
phenomenon could be observed following oxytocic or electrical stimulation, it seemed reasonable to interpret blanching of the graft as caused by mechanical activity, i.e. contraction of the myometrium.

Fig. 6 presents results of photo cell recordings from different animals. It

![Graph](image)

*Fig. 7.*

Simultaneous recordings of transmission of light and electrical activity in 4 different animals. a. effect of stretch. b. effect of iv injection of 15 mU oxytocin. c. effect of 100 mU oxytocin injected im 1 h previously. d. effect of 10 μg noradrenaline injected iv 20 seconds before first spike.
is seen that the excursions may be mono-, di-, or even multiphasic within the same period of contraction. Often a sudden increase in transmission of light is seen followed by a more or less pronounced fall or plateau before a final slow but more intense increase occurs (Fig. 6, d). The duration of a single contraction may vary from about 5 to 100 seconds often with a slight fluctuation in the “pale” phase. The decrease in blanching may occur as suddenly as the initiation, but may be of longer duration. The blanching phase is usually followed by a period of less transmission of light as compared to that of the resting level recorded immediately before the blanching. The appearance of the curves obtained from one rabbit to the other varied very much. However, in the same animal all blanchings were of the same amplitude and duration whether they occurred spontaneously or were induced by oxytocics and the reproducibility was good.

Simultaneous recording of mechanical and electrical activity

When simultaneous recording of electrical and mechanical activity was carried out blanching of the graft was always preceded by and/or occurred simultaneously with spike potentials (Fig. 7 a–d). The coincidence between electrical and mechanical activity was found both in cases where the activity was induced (by oxytocin, noradrenaline, stretch, electrical stimulation) and when it occurred spontaneously.

When contractions were sustained a great number of spikes occurred while the contractions of short duration were accompanied by a few spikes only.

CONCLUSION

The photoelectrical recording of changes in light transmission is cumbersome, and is often hampered by a layer of cartilage. Additional information is obtained by recording of the electrical potential variations.

In the experiments reported no electrical activity was found when no graft was present in the chamber, and furthermore a close correspondence between the mechanical and electrical activity always was found. This has convinced the author that for physiological studies the recording of the electrical potential variations is the method of choice for description of activity of myometrial grafts in ear-chambers. A definite advantage is the long distance to other excitable tissues which diminishes interference of bioelectrical signals from irrelevant structures. A radio-telemetry system suitable for picking up the small potential variations has been constructed (Wagner 1957a) and its use documented in physiological studies (Wagner 1975b).
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