AN IMPROVED CULTURE SYSTEM FOR SECONDARY PALATAL ELEVATION

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SUMMARY

An organ culture system devised for studying the development of the secondary palate was modified so that it retained high partial pressures of oxygen and supported total anterior and posterior palatal elevation. The cultured tissues appeared healthy as judged by histological examination. Medium was continuously recirculated through the culture system in which Day 13 embryonic mouse heads, with the brain and tongue removed, were totally submerged and suspended. The medium was constantly gassed via hollow fiber devices. A motor-driven stirrer, run at a low rate, agitated the medium so that the boundary layer surrounding the tissue was dispersed. Embryonic mouse heads were cultured in each of four media: Eagle's basal medium, Ham's F-12 medium, Fitton-Jackson's modified BGJb medium, and Waymouth's MB 752/1 medium. Elevation of the palate in both anterior and posterior regions with excellent tissue viability was achieved in all heads grown in BGJb medium.

Key words: organ culture; palate; embryonic mouse.

INTRODUCTION

The secondary palatal shelves on the midgestation rodent embryo initially grow downward from the maxillary processes and assume vertical positions on either side of the tongue. The shelves subsequently reorient to a horizontal position superior to the tongue where they fuse with each other and with the nasal septum anteriorly. Elevation of the palate above the tongue is apparently due to two different types of movements. The anterior part of the shelf appears to rotate as if attached by a hinge (1,2). Whereas, in the most posterior area, elevation seems to involve a medial protrusion and lateral regression of shelf tissue, suggesting an active remodeling of tissue (3). Palatal development occurs in a rapidly growing head in which many factors are at work (4). Studies of palatal closure would be facilitated by the use of an in vitro system in which extrinsic factors could be controlled and the developing secondary palate directly manipulated either physically or chemically.

Trowell-type organ culture has been successfully used to investigate the fusion of excised, opposed palatal shelves (5-8). However, with this system the crucial process of elevation cannot be studied. Recently, a culture system has been described in which elevation does occur (9). We have improved the design and function of this system such that in vitro palatal closure more closely approximates that seen in vivo. A different culture medium, increased partial pressures of oxygen, and improved medium circulation have resulted in a higher percentage of palatal closure and enhanced tissue vitality in the system described in the present report.
MATERIALS AND METHODS

A schematic diagram of the organ culture system is shown (Fig. 1a). Two such systems were housed in a 3.5 MP isolator (Germfree Laboratories, Miami, FL) provided with ultraviolet (UV) fluorescent lighting and individual ports of entry. The following is a detailed description of the operation of the system.

Culture chamber. The water jacketed, cylindrical glass culture chamber retained two optically ground parallel “windows” for optimum visual and photographic observation (Fig. 1b). Medium entered the bottom of the 30-ml chamber and flowed past the tissue and out the side-arm. This configuration allowed a small gas phase (about 3 ml) at the top of the chamber. Temperature of the medium was controlled at 34°C by a 10-gallon water bath connected to jackets around the culture chamber and the humidifier where the largest volumes of medium and gas, respectively, resided.

To eliminate fluctuations of pH at the gas-to-medium interface, the gravity held lid was sealed with silicone grease. The stainless steel lid rode on an autoclavable silicone rubber gasket. The head-support assembly projected from the lid. It consisted of stainless steel posts and a ring where nine stainless steel hooks were placed (Fig. 1c). The hooks were secured by sliding a complementary bracket over the ring. Flags extended the length and width of the hooks for easy handling with forceps. Heads were hooked through the foramen magnum and could be added and removed at any time during an experiment.

Medium circulation. Medium was pumped through the recirculating system by a variable speed, two channel Holter pump (Extracorporeal Medical Specialties Inc., King of Prussia, PA). All connections were made using medical grade Tygon tubing, which is relatively impermeable to oxygen and carbon dioxide. The medium was pumped above the chamber to a reservoir where a constant head of pressure was maintained. It then flowed through the gassing device back to the culture chamber. The entire volume of culture medium required was about 40 to 45 ml.

Because of a slow flow rate of medium, 5 to 6 ml/min, it was necessary to disrupt the boundary layer of nutrients, wastes, and gases surrounding the embryos. This was accomplished by a small detachable stainless steel stirrer projecting into the center of the culture chamber lid with the heads hanging in a circle around it. The two stirrers were powered by a model 402 dual power supply, (Semiconductor Circuits, Inc., Haverhill, MA) each using about 0.02 A (6.0 V) so that the medium and heads were slightly agitated but not shaken loose.

Transport of oxygen to the tissues. This aspect of the culture system will be discussed in two stages. First, preanalyzed 95% O₂:5% CO₂ gas (Matheson Gas Products, East Rutherford, NJ) was humidified at the culture temperature and delivered to the medium through highly permeable, silicone polycarbonate, hollow fiber membranes housed in a minitube (Biomedical Engineering and Instrumentation Branch, Division of Research Services, NIH, Bethesda, MD). The minitube gas outflow and the medium pressure head overflow were vented to atmospheric pressure. This method enabled the system to equilibrate to 600 ± 30 mm Hg in approximately 15 min.

![Schematic diagram of components of the culture system.](image-url)