PREPARATION OF LARGE NUMBERS OF UNIFORM TRACHEAL ORGAN CULTURES FOR LONG TERM STUDIES.

I. Effects of Serum on Establishment in Culture

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SUMMARY

Rat tracheas were each sectioned into fourteen rings of equal size with a slicing device which holds evenly spaced razor blades in register. The razor blades were positioned to minimize shearing of tissues during sectioning so that there was no gross tissue disruption or cell death distant from cut edges. Hundreds of these fragments can be conveniently prepared for studies requiring replicate samples. The cultures can be established in McCoy's 5a (modified) medium with or without calf serum. Cultures grown in the presence of calf serum were compared with those grown in serum-free medium, using vital phase microscopy, transmission and scanning electron microscopy, and light microscopic autoradiography of thymidine incorporation. When there is calf serum in the medium, epithelization of the entire surface of the ring occurs rapidly with the cells flattening and migrating as a sheet of closely apposed cells. Until migration is complete, mitoses are limited to the original mucosa near the cut edge. Without calf serum, migration is slow. The cells do not flatten or become closely apposed. Mitoses appear later but are present on all areas of the surface before migration is complete. In both serum-containing and serum-free media, ciliated cells are included in the migrating population and differentiation into pseudostratified epithelium occurs on newly epithelialized surfaces. The differing pattern of mitotic activity makes culture in serum-containing media more suitable for studies of wound healing and culture in serum-free medium more useful for some cytotoxicity and carcinogenicity studies.

Key words: trachea; organ culture; serum; ultrastructure; bronchus.

INTRODUCTION

Fragments of trachea and bronchus have been employed as an in vitro assay system and model for study of infectious agents (1) and carcinogens (2, 3). While these experiments involved short periods of maintenance in culture, long-term culture suited to the time scale of in vivo carcinogenesis is now possible (4). For some investigations, large numbers of replicate cultures are required. A method for producing large numbers of uniform tissue fragments and establishing them in culture is presented in this report together with a description of the differential effects of calf serum on establishment of cultures.

MATERIALS AND METHODS

Preparation of cultures. Wistar/Lewis rats (Charles River Laboratories) at body weights of 150 ± 25 g were anesthetized with pentobarbital and the tracheas exposed by sharp dissection. The tracheas were transected at the level of the first cartilage ring and at the suprasternal notch. Sterile technique was maintained and care taken to avoid damage to large blood vessels. The 25 mm length of trachea was removed from the neck and placed in McCoy's 5a (modified) medium (BBL) where it was trimmed of adherent connective tissue and muscle. In order to immobilize and orient the trachea, it was pinned to extra-firm dental wax. The wax was then turned to that the trachea was dependent and could be lowered onto a cutting device in which 15 double edged razor blades were held in register (Fig. 1). Plastic spacers between the razor blades kept them separated by uniform distances and they were positioned at an angle of 20 degrees to the horizontal by two brackets.
Fig. 1. The cutting device used to section tracheas into fourteen uniform ring shaped fragments. Fifteen double edged razor blades with plastic spacers between are held in register. Disruptive shearing forces are minimized and tissue viability assured.

Fig. 2. Scanning electron micrograph of a tracheal ring after a day in culture in medium supplemented with 10% calf serum. The ring is uniform in thickness at all points of its circumference except at the cartilage-free area corresponding to the dorsal surface of the trachea. There is no debris or disruption of tissues. ×45.

Fig. 3. Light micrograph of the migrating edge of epithelium over the cut edge of cartilage after 1 day in culture medium supplemented with 10% serum. The continuous sheet of flattened migrating cells blend with those at the original cut margin. ×285.

Fig. 4. En face silver stain delineating cell borders. The sheets of migrating cells have not yet met on the outer surface in one area (arrow). Cells near this leading edge are broader but the edges of neighboring cells are continuously apposed. ×280.