Organ Culture of Normal and Carcinogen-treated Rat Bladder

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Summary
A system for organ culture of rat bladder in defined medium, without serum, is described. Transitional epithelial morphology of normal bladder was well maintained in Waymouth's Medium MB 752/1, while Ham's Medium F12 caused marked epithelial hyperplasia. Hyperplastic epithelial states induced by in vivo administration of the carcinogen, N-methyl-N-nitrosourea, were well maintained in vitro during organ culture in Medium MB 752/1. The combination of hydrocortisone and insulin markedly inhibited the proliferative response of normal bladder epithelium grown in Medium F12.

Introduction
The maintenance of cells and tissues in culture provides an opportunity to study various aspects of their biology under rigidly controlled conditions. The technique of organ culture has special advantages in this respect in that it allows normal tissue architecture to be maintained in culture while causing a minimum of cellular trauma. The ability to organ culture transitional epithelium of normal and carcinogen-treated bladders should be of considerable use in carcinogenesis research, especially in relation to the problem of preneoplastic lesions and their reversal. Organ culture of normal bladder, for example, provides the opportunity for studying, in vitro, the morphological and biochemical effects (and their reversal) of known bladder carcinogens such as MNU and butyl(4-hydroxybutyl)nitrosourea. One of these (MNU) appears to be a direct-acting bladder carcinogen (2). The ability to culture bladder epithelium that has been exposed to a well-characterized bladder carcinogen prior to explanation to culture provides the opportunity for studying in vitro the progression and reversal of known precancerous and cancerous lesions.

In this paper techniques are presented for the organ culture of epithelia from normal bladders and bladders rendered hyperplastic by carcinogen treatment in vivo.

Materials and Methods
Carcinogen Treatment of Animals. Female Wistar and Wistar-Lewis rats (125 g body weight) received an intravesicular dose of 1.5 mg MNU in sterile 0.9% NaCl solution at biweekly intervals. A total of 2 doses was given. After the last dose, animals were held 4 weeks before sacrifice.

Organ Culture Techniques. Bladders were removed and cut in half longitudinally. Preliminary experiments indicated that bladders could not be cultured on the surface of plastic dishes because of the tendency for the epithelium to migrate off of the explant onto the plastic surface. Hemibladders were placed, epithelium up, on stainless steel grids in 60-mm Falcon plastic culture dishes containing 2 ml of culture medium. These conditions maintained the epithelium at the gas-medium interface. Cultures were maintained at 35°C in a gas phase containing 5% CO₂ and 20 or 50% O₂. Medium was changed every 2 or 3 days. All culture media, MEM, Waymouth's MB752/1, and Ham's F12 were obtained from Grand Island Biological Co., Grand Island, N. Y., and contained penicillin, 100 units/ml, and streptomycin, 100 μg/ml. Serum supplements were not used. All media were completely defined.

Histology. Tissues were fixed in buffered neutral formalin, embedded in glycol methacrylate and sectioned at 2 μm, or embedded in paraffin and sectioned at 5 μm. Sections were stained with hematoxylin and eosin.

Results
Culture of Normal Bladder Epithelium. Bladder epithelia have been cultured successfully from the following rat strains: Fischer, Osborne-Mendel, Wistar, and Wistar-Lewis. The results presented below on normal bladder were from 7- to 8-week-old male Fischer rats. The bladder epithelium of the Fischer rat is normally composed of 2 to 3 cell layers. After 6 days in culture, the epithelium displayed varying degrees of hyperplasia and atypia depending upon the culture conditions used. Epithelia cultured with a gas phase of 95% air and 5% CO₂, in MEM (Fig. 1) or Waymouth's MB752/1 (Fig. 2) showed transitional cell hyperplasia. In Ham's F12 medium, epithelia displayed moderate to marked hyperplasia with loss of transitional cell morphology (Fig. 3). Epithelia cultured in F12 also showed a tendency for extensive nodular downgrowth into the stroma. These areas of nodular proliferation seemed to be associated with epithelial clefts.

After 14 days of culture, epithelia in MEM and MB752/1 were less well organized but were still recognized as transitional. Epithelia in F12 showed foci of marked hyperplasia which no longer displayed transitional type cells. Nests of Brunn (Fig. 4) were observed frequently. Preliminary evidence indicates that the hyperplasia and disorganized growth characteristic of epithelia in F12 medium can be reduced by hydrocortisone. In the presence of 1 μg hydro-
Cortisone and 1 μg insulin per ml of culture medium, epithelia were only slightly hyperplastic and had transitional cell characteristics. Insulin alone (1 μg/ml) did not inhibit hyperplasia.

High O₂ tensions in the gas phase have been found necessary in organ culture of some tissues. In the case of bladder epithelium, however, elevation of the O₂ concentration in the gas phase to 50% can be toxic. Epithelia cultured in MB752/1 and F12 were not affected by the higher O₂ concentration, while the combination of 50% O₂ and MEM proved toxic. If, however, the glucose concentration of MEM was increased so that it equaled that of MB752/1 (27.8 mM), the toxic effect was no longer observed.

Under certain conditions, the bladder epithelium is capable of squamous metaplastic changes accompanied by cornification (1). Bladders were cultured for up to 21 days in MB752/1 without displaying any of these changes. In all media examined, occasional foci of cells with intercellular bridges were seen in epithelia cultured for 7 and 14 days, but clear-cut squamous lesions were not observed.

Culture of MNU-treated Bladders. Although it is not known if the 2-MNU-treatment protocol used in these experiments is carcinogenic in our animals, a 25 to 50% tumor incidence has been reported by others with this protocol (R. M. Hicks, personal communication). Half of each MNU-treated bladder was fixed immediately upon removal from the animal. The other half was cultured for 6 days. By comparing the 2 halves it was thus possible to assess to some degree the ability of the various culture conditions to support MNU-induced hyperplasia and atypia.

MEM was inconsistent in its ability to support MNU-treated bladders in culture. In most cases, extensive epithelial degeneration was observed. Ham's F12 supported a degree of hyperplasia comparable to that observed in control bladder halves but it did not allow preservation of the characteristic epithelial morphology seen in control bladder halves. MNU-treated epithelia displayed varying degrees of heterogeneous morphology; considerable nuclear pleomorphism and dysplasia were observed (Fig. 5). After 6 days in culture in F12, however, the epithelium lost these characteristics and assumed a homogeneous morphology (Fig. 6). This behavior was not observed in MB752/1. Both hyperplasia and characteristic morphology were preserved by this medium (Figs. 7 and 8). In the case of both media, a gas phase containing 50% O₂ gave best results with MNU-treated bladders.

When MNU-treated bladders were removed from the animal, they frequently displayed squamous metaplastic changes accompanied by cornification. In no case were these lesions still found after 6 days of culture in either F12 or MB752/1. However, in a few cases, cornified lesions were seen in MNU-treated bladder maintained in culture for 14 days in F12, MB752/1, and MEM (Fig. 9).

Discussion

Before neoplastic lesions can be studied effectively in culture, it is essential that their characteristic morphology be successfully maintained in vitro. This is especially important in studies that deal with the alteration of lesions in culture. Whether the lesions induced by MNU in this study can be classified as neoplastic is questionable. The important point, however, is that Waymouth's MB752/1 is capable of preserving, in culture, the characteristic hyperplasia and atypical growth pattern of lesions induced by a known bladder carcinogen.

There is obviously a limitation on the degree of hyperplasia that can be successfully organ cultured. In the case of severely hyperplastic lesions, higher O₂ concentrations in the gas phase may be necessary for the survival of cells in the basal part of the lesion. In conjunction with higher O₂ concentrations, elevated glucose levels may also be needed.

Because of the characteristic growth pattern it imposed on MNU-treated epithelia, F12 medium is obviously not a good choice for the culture of neoplastic lesions. Whether the morphology of MNU-induced lesions is preserved in F12 in the presence of hydrocortisone is being investigated. The ordered transitional cell growth pattern of epithelia cultured in F12 in the presence of hydrocortisone is probably due largely to inhibition of cell proliferation. It would be of interest to know what effect hydrocortisone has on carcinogen-induced lesions.

The present culture conditions do not seem to be suitable for the preservation of cornified lesions. The failure to observe any such lesion after a 6-day culture period suggests to us that these lesions may be sloughed in culture and that those few lesions that were observed after 14 days of culture were formed secondarily during the culture period.

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References


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