EXPLANT CULTURE OF RAT ESOPHAGUS IN A CHEMICALLY DEFINED MEDIUM

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SUMMARY

Esophagus from adult male CDF rats was cultured for a period of 28 d in CMRL-1066 medium supplemented with pyruvic acid, HEPES buffer, β-retinyl acetate, and antibiotics. Morphological, radioautographic, and biochemical studies indicated that the survival of the tissue in serum-free medium was equivalent to that in medium containing 5% heat-inactivated fetal bovine serum. There was a relatively constant uptake of [3H]thymidine into DNA and [3H]leucine into protein of the esophageal explants during the incubation. Only the basal cells of the epithelium incorporated [3H]thymidine into their nuclei. The normal morphology of the tissue was preserved when the explants were maintained at both 37 and 30°C, and in either 50 or 20% O₂. Ninety-five percent O₂ was highly toxic to the cells of the explants. This culture system should be suitable for a variety of investigations in esophageal cell differentiation and carcinogenesis.

Key words: esophagus; rat; explant; in vitro; culture.

INTRODUCTION

Esophageal cancer in humans occurs worldwide with the highest incidence in Chile, Puerto Rico, the Soviet Union, Sweden, France, Iran, and in certain areas of Africa and Japan (1,2). The disease occurs most frequently in the lower socioeconomic groups, which may reflect the poor nutrition of these groups inasmuch as dietary factors have been associated with esophageal cancer (3). Additional factors, other than nutritional, that have been linked to esophageal cancer are tobacco smoking, alcohol consumption, and certain plant products (4–6). With respect to alcohol, the origin of the alcohol may be as important as the amount consumed; in Africa, beer produced from maize that may have been contaminated by nitrosamines has been implicated in the development of esophageal cancer (7).

The rat has been used the most extensively for animal model studies in esophageal carcinogenesis. The early studies with dialkyl nitrosamines, summarized by Druckrey (8), indicate that the asymmetrical N-nitroso compounds are more active carcinogens for the esophagus than are the symmetrical. High incidences of esophageal cancers, most carcinomas, were induced by several routes of carcinogen administration. Since these studies, several nitroso compounds have been shown to elicit esophageal cancer in rats.

To our knowledge there have been only two reports describing the culture of esophageal tissues. Rosztoczy et al. (9) demonstrated that cultured explants of human esophageal tissue support replication of influenza virus for more than 4 d. In addition, Stenn and Stenn (10) reported on the organ culture of mouse esophagus for a period of 3 d in a chemically defined medium. In the present study, we report on the culture of explants of rat esophagus in the chemically defined medium, CMRL 1066. Viable, morphologically normal-appearing tissues were maintained in culture for at least 28 d.

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MATERIALS AND METHODS

Animals. Five to six-week-old male CDF rats were obtained from Charles River, Wilmington, MA. The animals were kept in cages with pine-wood sawdust bedding in well ventilated rooms with 12 h dark/light cycles (7 am to 7 pm). They were fed Wayne Lab-blox obtained from Allied Mills, Inc., Chicago, IL.

Explant culture. The rats were killed by asphyxiation, the esophagi removed by aseptic technique and placed in ice-cold Liebovitz L15 (11) culture medium (GIBCO, Grand Island, NY) for transport to the tissue culture laboratory. While bathed in L15 medium, the esophagi were opened longitudinally with surgical scissors, laid flat with the epithelium uppermost, and cut into pieces each approximately 3 mm². The pieces, with the epithelium uppermost, were placed on Metricel membrane filters (47 mm width; pore size, 0.8 μm; Gelman Instrument Co., Ann Arbor, MI) in 60-mm tissue culture dishes (3 to 4 pieces/dish). Each dish was fed with 3 ml chemically defined CMRL 1066 medium (GIBCO) supplemented with pyruvic acid (1 mM); HEPES buffer (20 mM; Ultrol, Calbiochem, LaJolla, CA); β-retinyl acetate (0.1 μg/ml; Hoffman-LaRoche, Nutley, NJ); amphotericin B (GIBCO) (0.25 μg/ml); and gentamicin (Schering Corp., Kenilworth, NJ) (50 μg/ml). The explants were incubated in an atmosphere of 50% O₂, 45% N₂, 5% CO₂ at 37°C on a rocker platform (Bellco Glass Co., Vineland, NJ) (5 cycles/min) so that the explants were submerged approximately one-half of the time (12). The medium was replaced with fresh medium every 2 d. After 7 d, amphotericin B was omitted from the culture medium.

Using morphological, radioautographic, and biochemical methods the maintenance for 28 d of

![Fig. 1. Explant of rat esophagus just prior to culture. ×282.](image1)
![Fig. 2. Explant cultured for 7 d in serum-free CMRL-1066 medium. ×340.](image2)
![Fig. 3. Explant cultured for 28 d in serum-free medium. ×282.](image3)
![Fig. 4. Explant cultured for 15 d in serum-free medium. Note migration of epithelial cells around edges of explant. ×37.](image4)