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ORGAN CULTURES OF RAT AND HAMSTER COLON

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SUMMARY

Human fibrin foam as a matrix for three-dimensional organ cultures was successfully employed for the cultivation of newborn rat and hamster colon tissue. Colonic tissue was maintained under different oxygen atmospheres and with various synthetic media. Explants of rat and hamster colon, maintained in Leibovitz's L-15 medium buffered with HEPES in the presence of 5% CO_2 and air, retained their normal columnar epithelial architecture for 24 days in culture. Outgrowth of epithelial cells from colonic explants similar to those of the mother fragments grew into the foam. Viability of the explants was indicated by radioactive thymidine incorporation, and by the ability of the fragments to support viral replication.

Key words: human fibrin foam; organ culture; rat colon; hamster colon.

The use of a three-dimensional matrix for cultivating human and animal tissues was first introduced by Leighton (1) using cellulose sponges and plasma clots. Leighton et al. (2), using collagen-coated cellulose sponge as a matrix, were able to maintain the histological pattern of Walker tumor 256 with three-dimensional growth of cells similar to those seen in the original specimen. Studies by Kalus and Klement (3, 4) indicated that human fibrin foam is also a suitable matrix for a three-dimensional organ culture system for primary embryonic and tumor tissue explants. This matrix system provided free exchange of gases and an adequate supply of nutrients through capillary action. Past attempts with the three-dimensional systems to establish intestinal explants in relatively long-term culture have met with varying success (4, 5). Comparative studies of various media, and oxygen requirements for long-term maintenance of colon organ cultures have not been investigated. The present study was undertaken in a effort to evaluate the parameters necessary to establish long-term organ cultures of rat and hamster colon.

MATERIALS AND METHODS

Colon organ cultures. Colon derived from newborn male and female Sprague-Dawley rats and Syrian hamsters, Cr:RGH(SYR), were used. The colonic tissue was removed under aseptic conditions, opened longitudinally, and washed for 15 min in each of three changes of cold Hanks' balanced salt solution containing penicillin, streptomycin, and amphotericin B. Organ cultures were prepared following the technique of Kalus and O'Neal (4). Human fibrin foam (Institute of Sera Vaccines, Sarisske Michalany, Czechoslovakia) was cut into cubes measuring 1 × 1 × 0.3 cm. Each fibrin foam cube was placed separately in a 35 × 10-mm Falcon Petri dish and approximately 2.5 ml of culture medium added. Colonic tissue was cut with a sharp scalpel blade into pieces 2 mm square. Four colonic fragments were gently placed on the surface of each fibrin foam cube and the medium adjusted until capillary action caused the bottom of the explant to be level with the culture fluid. The basic culture media used in this study were medium 199, Leibovitz's L-15 medium, and Dulbecco's modified Eagle's medium (Gibco), each containing 0.2% bovine serum albumin, 2.0 mM glutamine, 250 units per ml of penicillin, 250 μg per ml of streptomycin and 0.5 μg per ml of amphotericin B, and buffered with either 0.026 M $NaHCO_3$ or 0.025 M HEPES (6). In all cases the pH was adjusted to 7.2 to 7.4 with 0.2 N NaOH or 0.1 N HCl. Cultivation was carried out in either 5% CO_2:95% O_2, or 5% CO_2:air at 37°C and 98% relative humidity. The media were changed three times a week. Cultures were termi-

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nated at 7 14, 18, 21, 24, and 28 days, and examined fo viability by assessing morphological appearanc , by measurement of [^{3}H]thymidine incorporat on into trichloroacetic acid-precipitable mater l, and by ability to support viral replication Three dishes of colonic explants, making a otal of 12 explants, were used at the various tin e intervals for each experimental condition.

Indices of viability. Histological examinations were made to determine the morphological integrity of the colonic mucosa. Explants were fixed in 10% neutral buffered formalin, and paraffin sections were stained with hematoxylin and eosin for routine examination, and by the periodic a id-Schiff reaction for mucin.

Incorpo ation of [^{3}H]thymidine into acid-precipitable r aterial was measured after incubating the colon explants with medium 199 containing 1 μCi [^{3}H]t ymidine per ml for 4 hr. Explants were was ed with medium until the washings were devo d of radioactivity. The tissue was precipitated with 5% trichloroacetic acid, rinsed in methanol, solubilized with Nuclear-Chicago Solubilizer (Amersham/Searle) and counted in Bray's liquid scintillation fluid. Control explants were boiled for 10 min and cooled to room temperature, after which [^{3}H]thymidine was added. Measurements were made in triplicate on pools of four explants. Radioactivity incorporated into DNA was expressed as disintegrations per min per four explants.

Evidence of viability of organ cultures was also provided by the ability of explant to support viral replication. The Abney strain of reovirus type 3, and parainfluenza virus type 1, strain D/Sendai/52, which have been shown to replicate in human and mouse colon organ cultures (7), were used for colonic infection. Reovirus infectivity was determined by production of cytopathogenic effects in BSC-1 strain of monkey kidney cell cultures. Sendai virus infectivity was determined in primary rhesus monkey kidney cells and the 50% end point titration was calculated, based on hemadsorption of guinea pig erythrocytes.

Experimental Results

In the presence of 5% CO_2:air, colon explants cultured in Leibovitz's L-15 medium buffered with HEPES retained their normal columnar epithelial architecture, with mucin production, for periods of 24 days (Fig. 1). Explants of

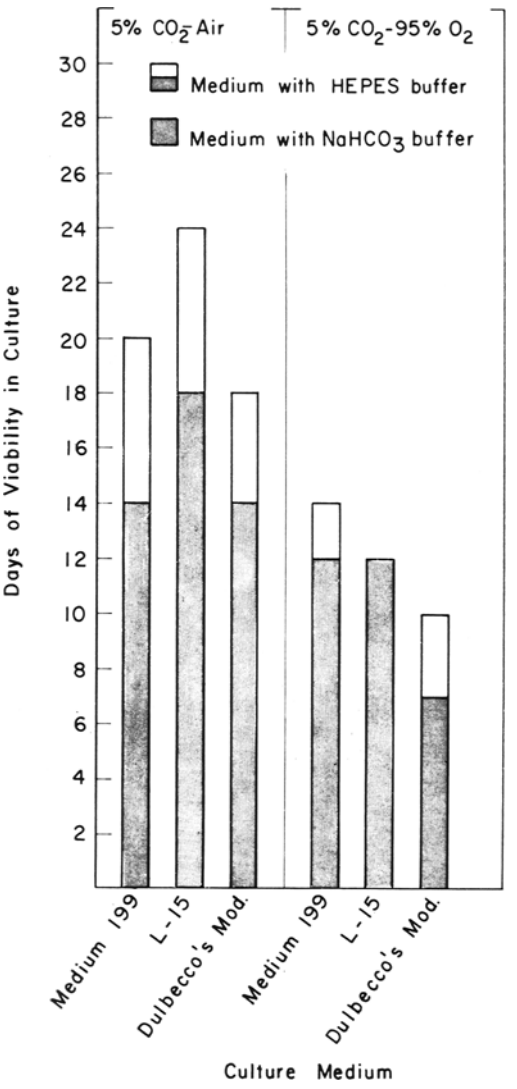

FIG. 1. Influence of culture media buffered with 0.026 M $NaHCO_3$ or 0.025 M HEPES and gaseous phase on maintenance of rat colonic organ cultures.

hamster colon were also maintained in culture for periods up to 24 days. Columnar epithelium that covered the surface of both rat and hamster explants also grew into the lacunae of the foam (Fig. 2). In 5% CO_2:air atmosphere, preservation of the epithelial surface was also observed after 20 days in medium 199, and 18 days in Dulbecco's modified medium. Survival of explants in the three culture media containing HEPES buffer, cultured in 5% CO_2 in air, was better than that of explants cultured in similar media in 5% CO_2:95% O_2.

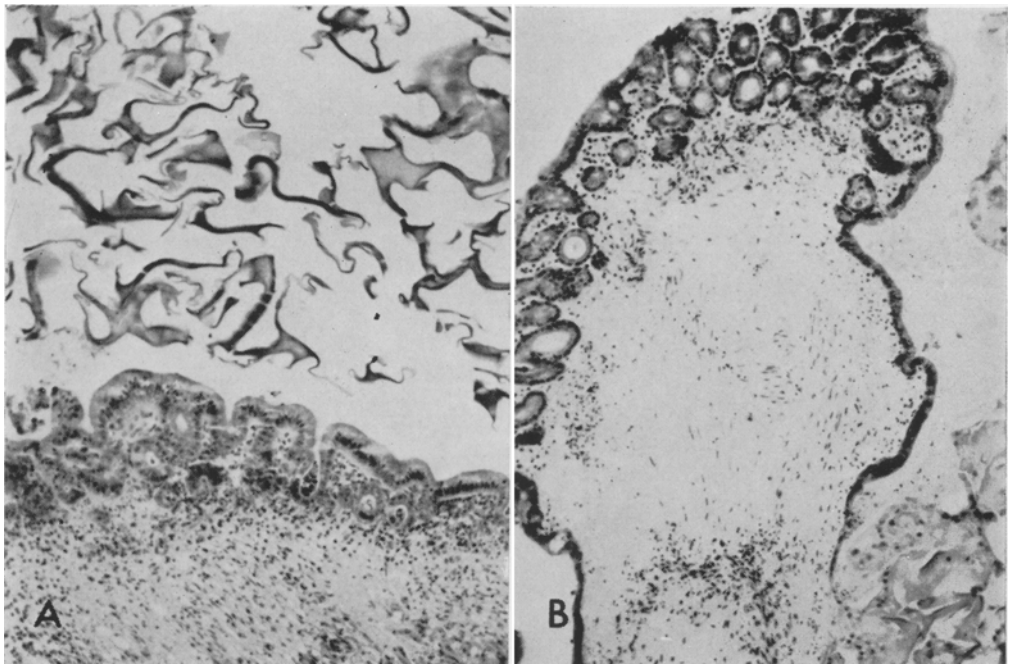

FIG. 2. Colonic organ cultures in matrix culture. *A*, section of 24-day culture of rat colon with well maintained mucosal architecture. Hematoxylin and eosin. ×58. *B*, section of 24-day culture of hamster colon with proliferation of epithelium into the fibrin foam. Hematoxylin and eosin. ×22.

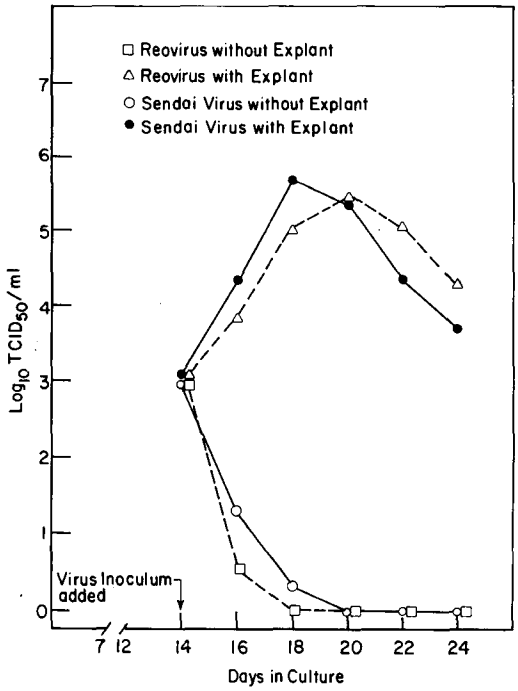

FIG. 3. Growth curve of reovirus type 3 and Sendai virus in rat colon organ cultures.

Viability was indicated histologically by retention of normal colonic mucosal architecture, with epithelial cell migration away from the surface of the tissue explants. Radioactive thymidine incorporation of the heat-inactivated control cultures was approximately 0.01% of that in the normal incubated specimen (18 to 25 cpm per four explants versus 3365 to 3442 cpm per four explants). Although the [^{3}H]thymidine incorporation does not give an indication of localized differential viability, it does indicate the over-all viability of the tissue. Organ cultures from both rat and hamster supported the replication of reovirus and Sendai virus. The growth curves of both viruses in rat colonic explants infected with an inoculum of 10^8 $TCID_{50}$ per ml, after 14 days of maintenance, showed peak titers, $10^{5.5}$ $TCID_{50}$ per ml for reovirus, and $10^{5.7}$ $TCID_{50}$ per ml for Sendai, 4 days after infection, and the titers persisted at a level greater than that of the inoculum through the 24th day, when tissue necrosis began to appear (Fig. 3). Similar growth curves were seen with both viruses in hamster explants.

Discussion

The use of three-dimensional matrix organ culture systems to study the histotypic organization of human tumors is well established (7, 9). Very litle information, however, is available on the maintenance of gastrointestinal tissue in matrix organ cultures. Kalus and O'Neal (4) reported cultivation of human embryonic and fetal mouse gastrointestinal tract in a matrix of human fibrin foam for 21 days, with excellent maintenance of columnar epithelium in the mother fragment. Their studies suggested that human fibrin foam could be used as a matrix for cultivation of human and animal tissues.

In our expriments, we have extended the use of human fibrin foam to the study of various media and oxygen requirements in relation to relatively long-term maintenance of newborn rat and hamster colonic organ cultures. The present data have shown that colonic explants retain their original properties with outgrowth for a period of 24 days in Leibovitz's L-15 medium, in conjunction with the zwitterionic buffer, HEPES, in an atmosphere of 5% CO_2:air. (Although Leibovitz's L-15 medium is specifically designed for use without additional buffering or an enriched atmosphere, in an attempt to prolong the viability of our cultures, we experimented with a variety of combinations of media, buffer systems, and gaseous atmospheres. The cultures maintained in the Leibovitz:HEPES medium remained viable 3 days longer than those maintained in L-15 alone, but a number of factors other than culture medium could be involved.) This indicates the importance of the buffering system in organ cultures and the ability to maintain cultures at physiological pH. The cultures appeared normal when observed by light microscopy, and appeared to be physiologically viable in their ability to incorporate [^{3}H]thymidine, and support virus replication. The importance of in vitro cultivation of colon explants is apparent when one considers their use in chemical carcinogenesis, and as a system in which intestinal viral infections may be studied.

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