A Simple Method for the Isolation of Viable Epithelial Cells of the Gastrointestinal (GI) Tract[1] (37040)

CHARLES F. A. CULLING, PHILIP E. REID, LINDA S. TRUEMAN, AND WILLIAM L. DUNN
(Introduced by S. W. French)

Department of Pathology, University of British Columbia and Vancouver General Hospital, Vancouver, British Columbia, Canada

Our current research involves a study of the chemistry and histochemistry of the epithelial glycoproteins of the large intestine of man in health and disease. Preparations of isolated epithelial cells provide an ideal starting material for such a study since the glycoproteins obtained should not be contaminated with: (a) connective tissue elements as would be expected in mucosal scrapings (1–4) or whole minced organs (5); (b) glycoproteins degraded by bacterial or other metabolic action which could well be present in irrigates (6–8) or mucous plugs (9).

A number of techniques have been employed to remove cells from the small intestine (*e.g.*, proteolytic enzymes (10, 11), hyaluronidase (12, 13), lysozyme (14), sodium citrate (15) but little attention has been given to large intestine (10), although in man it is the site of several diseases of unknown etiology. We have studied the removal of epithelial cells from everted rat colon and small intestine by shaking with physiological saline, EDTA (16) or hyaluronidase (12, 13).

EDTA provided a simple rapid method for the removal of all the epithelial cells; while leaving the underlying basement membrane histologically intact. Hyaluronidase treatment gave a poorer total cell yield but the cells isolated have been successfully maintained in tissue culture. Histological examination of the isolated cells from both types of preparation showed only an occasional leukocyte. Preliminary experiments indicate that cells can be successfully removed from human colectomy specimens.

[1] Supported by Medical Research Council of Canada Grant No. MA 4375.

Methods. Wistar strain male rats (100–250 g) were anesthetized with ether, their colons were resected into cold isotonic saline and the fecal contents were removed by flushing with fresh, cold isotonic saline.

A plastic coated solid copper electrical wire with one end bent like a "shepherd's crook," was passed through the colon, hooked onto the distal end, and pulled upon until the colon was everted (17).

One end was ligated and the colon was inflated with ice-cold saline, using a 10 ml syringe without a needle. When the colon was under mild pressure, it was tied off as close to the end as possible. It was then placed in 100 ml of the appropriate solution in a 250 ml screw-capped jar (made watertight by using plumbers' Teflon tape on the thread).

The following solutions were evaluated:

1. Isotonic saline or phosphate buffered saline (PBS).

2. EDTA (tetrasodium salt). The following concentrations in PBS were used: 1 and 2×10^{-4}, 1 and 5×10^{-3}, 1 and 2×10^{-2} g/100 ml; also 1, 2, and 5×10^{-3}, 1 and 2×10^{-2} g/100 ml were used in PBS containing 0.1% glucose.

3. 1.25% hyaluronidase (obtained from Nutritional Biochemicals Co.) (300 units/mg) in isotonic saline.

A total of 120 colons were shaken in the following solutions: 22 in saline or PBS, 34 in hyaluronidase, 54 in EDTA in PBS and 10 in EDTA in PAS with added glucose. The procedures were all carried out at 37° with the solutions being aerated. The following experimental parameters were evaluated:

1. Total time of shaking (30–120 min),

TABLE I. Summary of Results Obtained by the Use of Various Solutions for the Removal of Epithelial Cells from Rat Colons.

Solutions employed	No. of spec.	Cell removal	Viability	Basement membrane intact
Saline (or PBS)	22	Poor	—	—
Hyaluronidase	34	Moderate	Good	Yes
EDTA, 0.01%	23	Good	Poor	Yes
0.0002%	12	Good (with frequent changes)	Poor	Yes
Others	29	—	—	—

2. Time interval between solution changes (10–30 min),

3. The number of solution changes (2–6 per hr),

4. Mechanical or manual shaking.

The following technique was finally adopted: the specimens were treated with either hyaluronidase or 1×10^{-2} EDTA in PBS for a total period of 1 hr with changes of solution after 10 and 30 min (from start). Specimens were alternately shaken manually for 5 min and incubated in a 37° waterbath for 5 min; during the incubation a steady stream of air was bubbled through the solution at such a rate that fluid was not lost by frothing.

The cells were recovered by centrifugation at 2000 rpm for 10 min in a refrigerated centrifuge. The viability of the isolated cells was tested with trypan blue.

Cells for tissue culture were obtained by manual shaking in 1.25% hyaluronidase in 0.9% sodium chloride or in EDTA 1×10^{-2} g/100 ml PBS, for two periods of 10 and 20 min duration. The cells were resuspended in medium 597 (Connaught Laboratories) containing penicillin G (660 units/ml) and 1.6 mg/ml of streptomycin sulfate and recentrifuged at 500 rpm for 20 min.

The cultures were set up in 25 ml Falcon plastic screw-capped tissue culture bottles containing 5 ml of medium 597 with 20% fetal calf serum to which was added 7.5% sodium carbonate to pH 7.4. Penicillin G (660 units/ml), streptomycin sulfate (1.6 mg/ml) and Mycostatin (200 units/ml) were added to control contamination: Mycostatin was added when it was noticed that fungi grew almost invariably in its absence. Cultures were incubated at 37° in an ordinary incubator; they were then left undisturbed for 24 hr before examination with an inverted microscope.

Tissues for histological examination were taken before and after each time interval (to check degree of cell removal), fixed in formol–calcium and paraffin processed. Sections were cut at 5 μm and stained with hematoxylin and eosin and by the PAS/alcian blue technique (18). Isolated packed cells were examined in a similar manner.

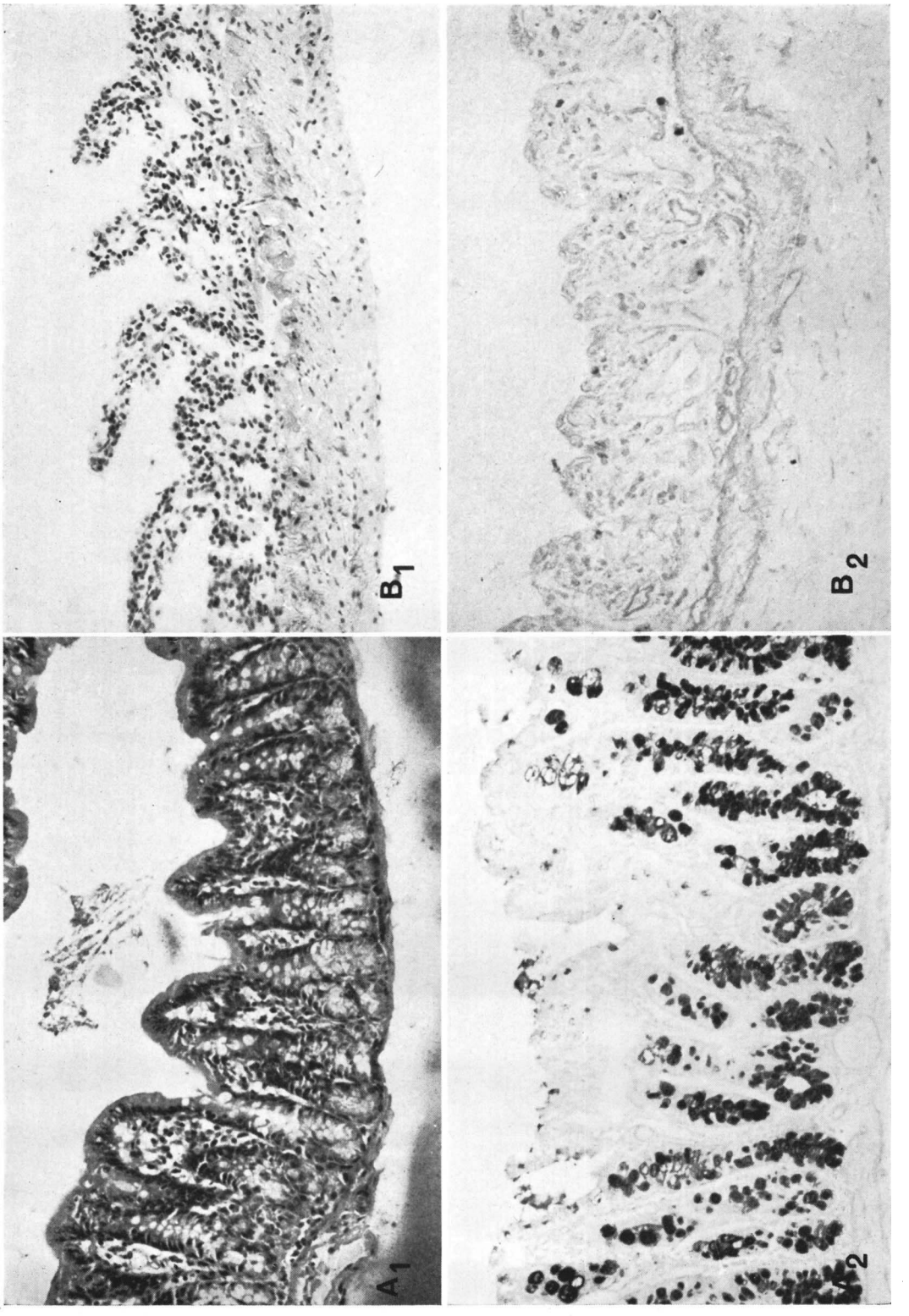
Results and Discussion. A summary of results is given in Table I.

1. Saline or PBS. Saline or PBS solutions alone did not remove all the epithelial cells, even in the most successful cases the cells in the bases of the crypts remained undisturbed.

2. Hyaluronidase. While hyaluronidase was at times completely successful it did not consistently remove all the cells from the bases of the crypts. In all cases examined histologically the basement membranes were intact.

In 5/5 experiments viable cells were seen in tissue cultures when examined after 24 hr. In 3/5 of the experiments cell cultures were viable after 10 days, one culture was still viable after 4 mo, and has been successfully subcultured.

When hyaluronidase-treated cells were tested for viability by the addition of trypan blue, the whole cells were stained blue-purple instead of the nucleus alone staining which normally indicates nonviability. After 24 hr tissue culture the cells no longer stained when exposed to trypan blue; therefore we con-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sider that the morphology of the freshly isolated cells under the phase contrast microscope and after 24 hr in tissue culture are better criteria of cell viability in this instance.

3. EDTA in PBS. Shaking with EDTA 1×10^{-2} g/100 ml PBS for 10 min followed by a further 20 min, gave good to excellent removal of the cells from the bases of the crypts with the basement membranes being histologically intact in all cases examined (see Fig. 1). Histological examination of the packed cells showed the virtual absence of leukocytes. Investigation with concentrations of EDTA from 2×10^{-4}–2×10^{-2} g/100 ml showed that cells were removed more rapidly as the concentration of the EDTA was increased. Low concentrations of EDTA (with more frequent changes of the solution) were apparently equally effective in removing the cells. In these experiments the great majority of the isolated cells were nonviable with trypan blue. Tissue cultures of cells isolated with 1×10^{-2} g/100 ml EDTA in PBS showed that only occasional cells were viable after 24 hr in 4/5 experiments. In 1/5 experiments there was a much better survival of cells after 24 hr but in this case, as in the others, no cells survived past 48 hr.

Rat small intestines. The successful methods described above were applied to rat small intestine with similar results. One sample of cells, removed by hyaluronidase treatment, have remained viable in tissue culture for 2 mo.

Preliminary experiments on human large intestine. Surgical specimens, both normal and diseased, have been treated with hyaluronidase or with EDTA (either 1 or 2×10^{-2} g/100 ml). In the first experiments the specimen, after washing in ice-cold saline, was sutured so as to form a blind ended sac with the mucosal surface on the outside and the sac was inflated with saline. The specimen was shaken with saline (0.9%) for 10 min and then treated with hyaluronidase. In these experiments the inflated specimen leaked continually and considerable quantities of red blood cells contaminated the isolated epithelial cells.

A technique was then devised which in most cases avoids both these problems. The specimen was thoroughly washed in saline, everted, and sewn securely into a tube-like structure with the mucosal surface outermost. A sausage-type balloon was passed through the specimen, the latter was then stitched at each end so as to constrict the piece of ⅜ in. rubber tubing which had been threaded over the visible ends of the balloon (and to approx 1 in. within the specimen). The rubber tubing was used to prevent the over inflation of the balloon outside the specimen. The balloon was at first inflated with compressed air until the specimen was well stretched, tied off and the end was pushed into the rubber tubing. This procedure proved to be unsatisfactory in that the specimen floated; we now stretch the specimen by filling the balloon with tap water under gentle pressure. In general this has the effect of preventing blood leakage in addition to exposing the epithelial cells of the crypts to the shaking action.

Summary. A simple convenient method for the complete removal of epithelial cells with EDTA from the large or small intestine is described. When using hyaluronidase the cells obtained remain viable in tissue culture over a long period.

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FIG. 1. Sections of rat colon stained (A) before, and (B) after, treatment with 1×10^{-2} EDTA in buffered saline. Note the complete removal of epithelial mucus secreting cells in (B_1) and (B_2) without apparent damage to basement membrane (B_2). Sections (A_1) and (B_1) are stained by H & E and sections (A_2) and (B_2) are stained by alcian blue–PAS technique (×100).

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Received Oct. 5, 1972. P.S.E.B.M., 1973, Vol. 142.