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A monolayer culture of gastric mucous cells from adult rabbits

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Summary: A new method for the primary monolayer cultures of adult rabbit gastric mucous cells has been developed. Rabbit gastric mucosal cells were isolated with etylenediaminetetraacetic acid and collagenase. Cells were cultured in Coon's modified Ham's F-12 medium supplemented with 10% fetal bovine serum, 15mM HEPES buffer, antibiotics, and antimycotic. The cells reached confluency on days 3-4. Histochemically 92% of the cells contained PAS positive gramules (mucous cells), 3% of cells showed a strong reaction for succinic dehydrogenase activity (parietal cells), 2% of the cells showed positive granules by Bowie staining (chief cells), and G6PDH staining was positive in 5% of the cells (surface mucous cells). Fibroblasts were rarely seen until day 7 (<1%). Thus rabbit cultured gastric cells were considered to be mainly comosed of mucous neck cells. These cells produced prostaglandin (PG) E_2 and PGI_2. Quantitatively cultured cells synthesized 1.475±0.039 ng/mg protein/hour of PGE_2 and 0.244±0.042 pg/mg protein/hour of PGI_2. This relatively simple and convenient technique provides a useful model for the study of cellular functions of gastric mucosa. *Gastroenterol Jpn 1990;25:1–7*

Key words: *adult rabbit; cell culture; gastric cell; prostaglandin*

Introduction

Cell culture of gastric epithelial cells provides a useful model for the study of cell function without systemic factors. Successful preparations of gastric epithelial cultures have been reported using fetal rabbits[1,2]. Fetal tissue has been used to establish cell growth and to avoid contamination. However cultured cells from adult animals might be more preferrable, since they could reflect more accurately the physiological condition of mature animals. Cell cultures of chief cells from adult canine[3] and guinea pig surface mucous cells[4] have been reported. In their preparations, elutriator rotor or percoll gradients were used to enrich a specific type of cells and techniques were relatively complicated.

In the present paper, we report a simple method for the cell culture of adult rabbit gastric mucosa. The morphologic characters and the production of prostaglandins (PGs) by cultured cells were also studied.

Methods

Materials

Animals were Japanese white rabbits (Doken Laboratory, Ibaraki, Japan) weighing 2.5-3.0 kg.

Coon's modified Ham's F-12 (F-12 medium) was purchased from KC Biological Inc. (Lenexa,

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KS). Basal medium Eagle (BME), minimal essential medium (MEM) amino acid, N-2-hydroxyethylpiperadine-N-2-ethanesulfonic acid (HEPES) buffer and bovine serum albumin (BSA, fraction V) were purchased from Sigma Chemical (St. Louis, MO). Hank's balanced salt solution (HBSS) was obtained from Grand Island Biological (GIBCO; Grand Island, NY). Crude collagenase type I, indomethacin, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical. ^{125}I PGE_2 and 6-keto-PGF1α (6KF) radioimmunoassay (RIA) kits were obtained from New England Nuclear (Boston, MA).

Cell preparation

Non-fasted male white rabbits weighing about 2.5 kg were used. Isolation of gastric mucosal cells was performed using a modification of Soll's method[5]. Anesthesia was induced by intraperitoneal administration of 50mg/kg of Nembutal (Diabott Laboratories; North Chicago, IL). The fundic mucosa of the rabbit stomach was quickly separated, bluntly scraped, and minced into 2-3mm^3 pieces. The minced tissues were incubated for 15min. in BME containing 0.1% BSA, and crude collagenase type I (0.35 mg/ml). This and subsequent three incubations were done at 37°C uner 5% CO_2 and 95% O_2 in a shaker bath at 100 cycles/min. BME for these incubations contained 10mM HEPES buffer and 0.1% BSA with the pH at 7.4. After the first incubation, medium was discarded and tissues were rinsed twice with Earl's balanced salt solution (EBSS) with 2mM EDTA and incubated for 5 min. in the same solution containing 1 mM EDTA, 0.1% BSA and MEM amino acid. Mucosal fragments were then incubated in BME with crude collagenase type I (0.35 mg/ml) for 15 min. Final incubation was performed in the same solution for 50 min. Cell clumps from final incubation were collected, washed twice with HBSS containing 0.1% BSA and stored in ice. Sterile technique was not used throughout these procedures.

Cell culture

Cells were transferred to sterile tube and washed with sterile HBSS twice. Cells were resuspended with Coon's modified Ham' F-12 medium supplemented with 10% heat-inactivated (at 56°C for 30 min) fetal bovine serum (GIBCO), 15mM HEPES buffer, 100 U/ml penicillin, 100 U/ml streptomycin, and 5μg/ml fungizone and inoculated onto 24-well tissue culture plates.

Morphological studies

Cultures were examined daily with a phase-contrast microscope. The trypan blue dye exclusion test was performed by the method of Phillips[6] to assess the viability of isolated cells.

Histochemical studies

To distinguish parietal cells succinic dehydrogenase activity was determined by the method of Nachlas et al[7]. To distinguish chief cells, Bowie stain[8] was used. To stain mucous cells, periodic acid Schiff (PAS) staining was employed. The techniques for different stainings were described elsewhere[9]. Glucose-6-phosphate dehydrogenase (G6PDH) was also performed by the method of Andersen and Hoyer[10].

Electron microscopy

Cultured cells were fixed with 2% glutaraldehyde for 20 min and post-fixed with 1% OsO_4 for 20 min. The cells were dehydrated with graded alcohol and embedded in Epon 812 and polymerized at 60°C overnight. The sections were stained with uranyl acetate and lead citrate.

PG production by cultured gastric cells

Media contents of PGE_2 were measured by RIA. The effect of indomethacin on PG production was tested. Cells were washed 3 times and incubated with EBSS (control) or indomethacin (IND, 10^{-6}M-10^{-4}M) for 1 hour under culture conditions. Media were stored at -20°C until assayed. Cells were scraped and protein was determined with the dye binding assay according to Bradford[11]. PGE_2 and 6KF content of

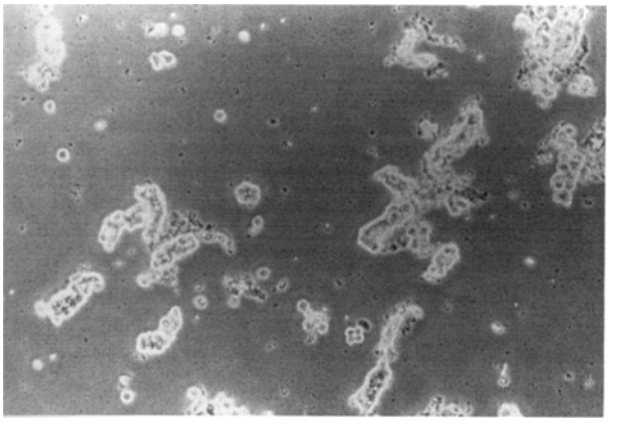

Fig. 1 Phase contrast photomicrograph of isolated gastric cell clumps and glands (×130).

media were assyed directly with PGE_2 and 6KF RIA kits.

Statistics

Data are presented as mean ± standard error of the mean (SE). Analysis of variance and Student's t-test were used to assess the significance of difference; $P<0.05$ was considered significant.

Results

Light microscopy

Cell isolation: the cells from the final incubation were mainly composed of cell clumps or glands. The viability of the cells were >90% (**Fig. 1**).

Cell culture: the cells attached to the bottom of culture dishes within 1 day. Cells began to grow and reached confluency on days 3 or 4 (**Figs. 2a, b**). In most cases fibroblasts were not seen at this stage. Rarely, after day 8, fibroblasts started growing. Bacterial or fungal contamination was not observed up to 2 weeks.

Histochemical studies

In 4-day old cultures, 93% of the cells contained PAS positive material in the cytoplsm (mucous cells, **Fig. 3a**). Three percent of the cells showed a strong reaction for succinic dehydrogenase activity (parietal cells, **Fig. 3b**). Two percent of the cells had positive granules in Bowie staining (chief cells, **Fig. 3c**). Four percent of the cells were G6PDH-staining-positive cells (surface mucous cells, **Fig. 3d**). Thus about 90% of the cultured cells were considered to be mucous neck cells.

Transmission electron microscopy

Ultrastructurally, micro-villous-like projections were apparent on the surface of the cells. The majority of the cells had electron dense granules which are characteristic of mucous cells (**Fig. 4a**). A cell with abundant mitochon-

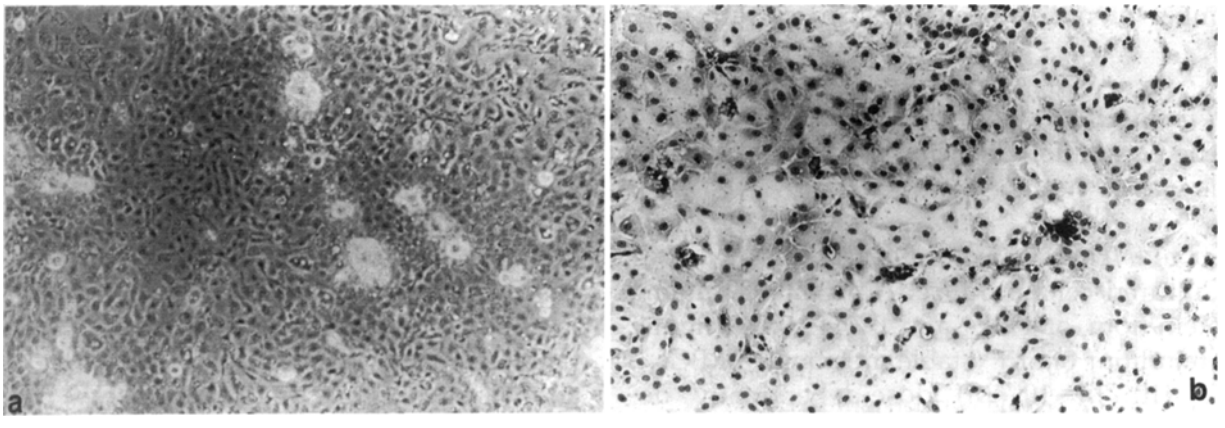

Fig. 2 Cultured rabbit gastric cells. Four day old culture.
(a): Phase contrast photomicrograph (×65).
(b): Giemsa staining (×65).

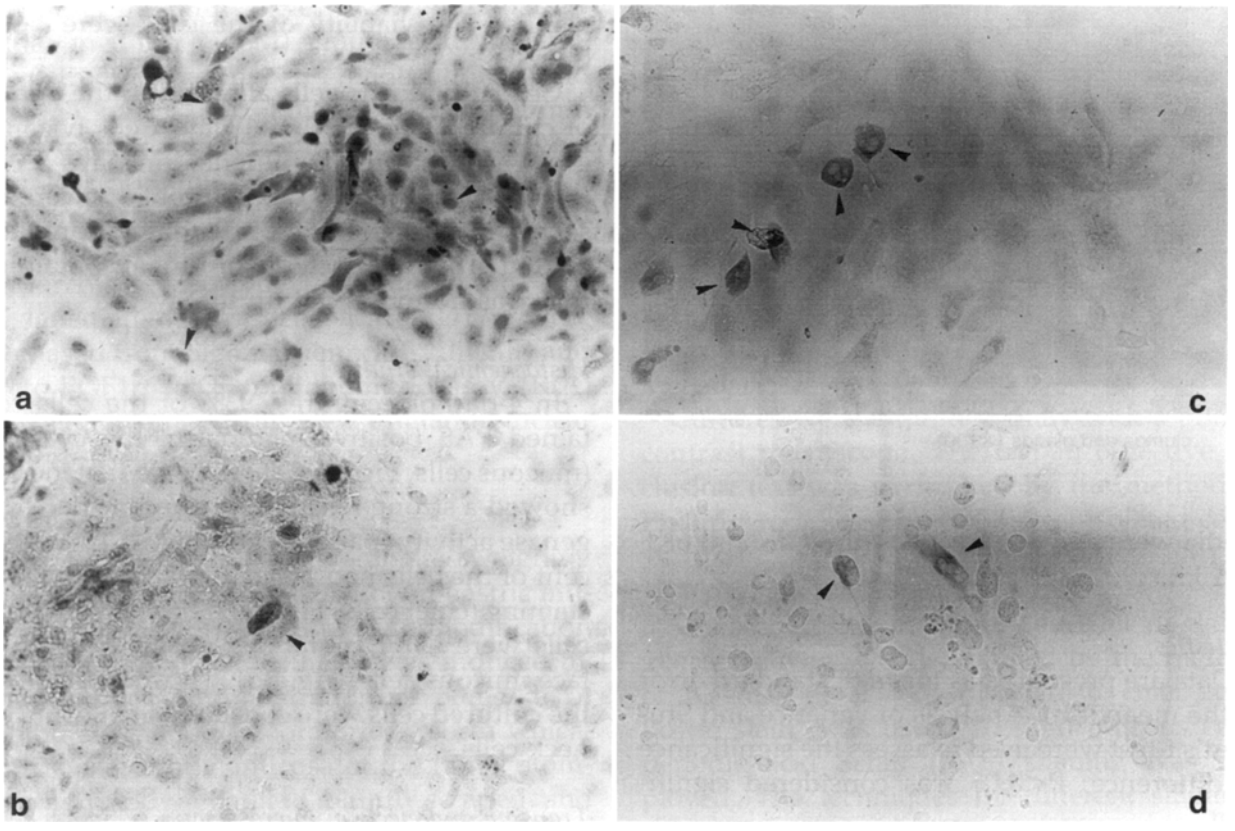

Fig. 3 Histochemical stainings of cultured rabbit gastric cells. Four day old culture.
(a): PAS staining (×65). Dark granules in the cytoplasm are mucous granules (arrows).
(b): Succinic dehydrogenase staining (×65). A drakly stained cell is a parietal cell (arrow).
(c): Bowie staining (×130). Darkly stained cells are chief cells (arrows).
(d): G6PDH staining (×130). Cells having dark cytoplasm are surface mucous cells (arrows).

dria was also detected, suggesting that small number of parietal cells exist in culture (**Fig. 4b**).

Cell proliferation

The population doubling time of the cultured cells was 19 hours (**Fig. 5**).

Effect of IND on PGE_2 production

Cultured cells produced 1.475±0.039 ng/mg protein/hour of PGE_2 and 0.244±0.042 ng/mg protein/hour of 6KF (stable metabolite of PGI_2). IND (10^{-6}M−10^{-4}M) dose-dependently decreased PG E_2 and 6KF production (**Fig. 6**).

Discussion

We previously reported methods for the culture of gastric fundic cells from baby rats[12], adult rats[9], and humans[13]. We utilized mainly cultured gastric cells from baby rats to investigate PG production[14], cytoprotection by PG[15,16] and other factors[9,17-20], and oxygen metabolite-induced cytotoxicity[21]. In the present study, gastric cells from adult rabbits were used. This method provides several advantages. Since rabbit stomach is much larger than that of other animals such as rats or guinea pigs, we can get large amount of gastric cells from one stomach and prepare many cultures from one rabbit. Virtually, bacterial or fungal contamination was

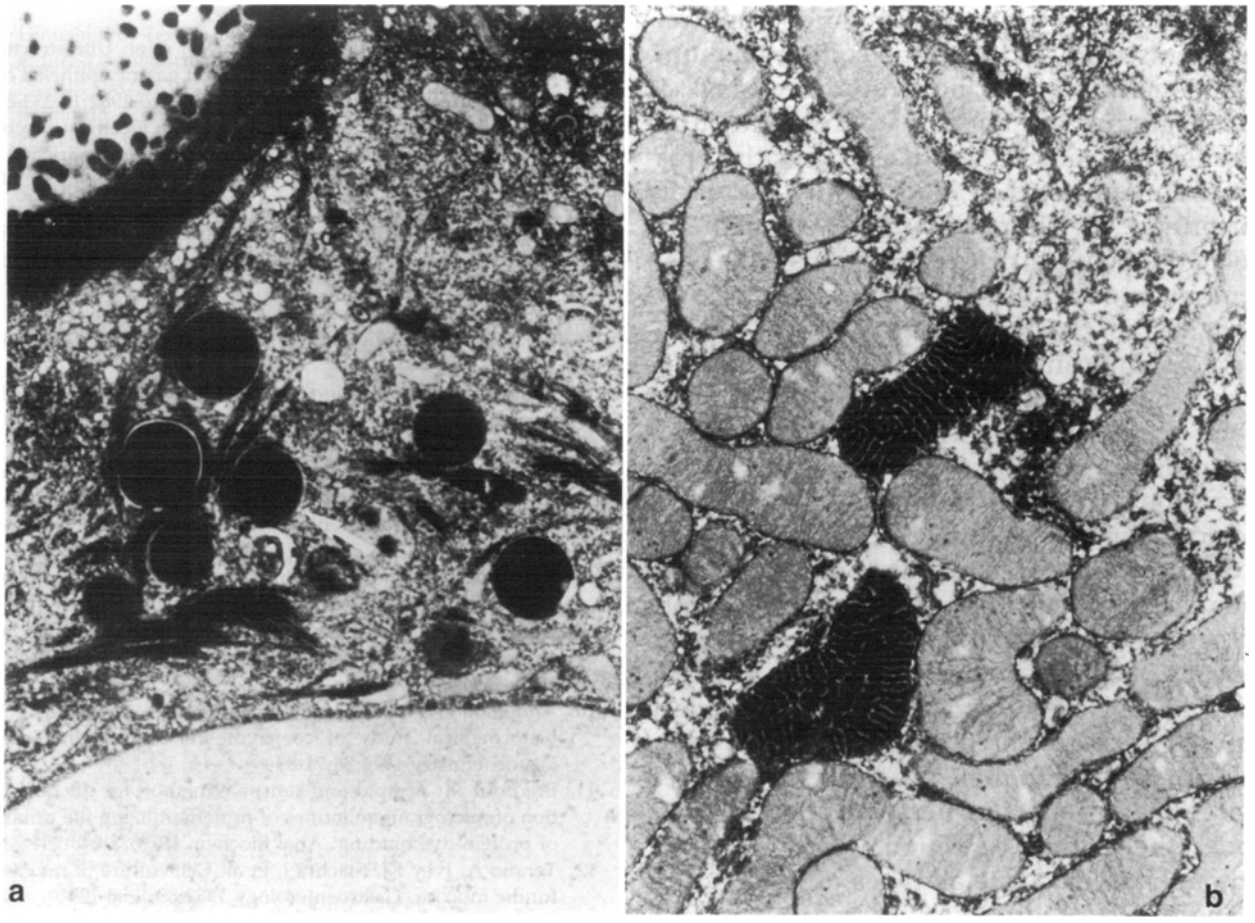

Fig. 4 Transmission electron micrograph of cultured rabbit gastric cells (×10000). Four day old culture.
(a): Mucous granules (arrow) are observed in the cytoplasm.
(b): A cell with abundant mitochondria (parietal cells) was observed.

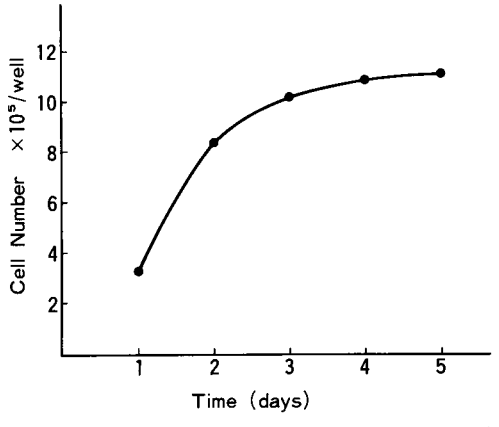

Fig. 5 Growth curve of cultured rabbit gastric cells. Each point represents the mean of four cultures.

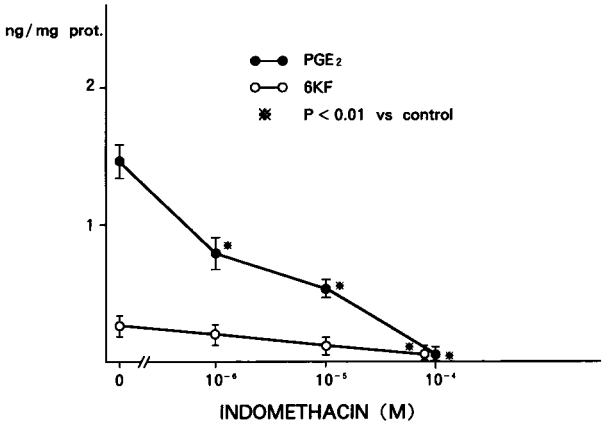

Fig. 6 The effect of indomethacin on the production of PGE_2 and 6KF of cultured rabbit gastric cells. Each point represents mean±S.E.M. from four cultures.

not observed, when antibacterial and antifungal agents were added to the culture medium. Additionally no specific procedure, such as percoll gradients or an elutriator rotor, to purify specific types of cells was used. Thus the present technique is much more simply and easily performed, compared with other methods. Isolated rabbit gastric cells or glands have been demonstrated to show good responses to secretagogues as previously reported[22,23]. We observed significant effects of histamine and carbachol on aminopyrine uptake using isolated rabbit parietal cells[24]. Therefore cultured mucous cells from rabbit might be a good tool to assess the effects of secretagogues on the physiological response of mucous cells.

Cultured cells were mainly composed of mucous cells, particularly mucous neck cells, without any purification of isolated gastric cells. Mucous neck cells are relatively more undifferentiated cells than parietal, chief, or surface mucous cells. We obtained similar results using baby rats and adult rats[9,12]. Thus it is suggested that immature mucous neck cells mainly grow under the culture condition which we used, although we inoculate all types of gastric cells.

These cells produced PG E_2 and PGI_2. Previously we demonstrated that cultured mucous cells from baby rats produced similar PGs[14]. It has been suggested that parietal cells produce more PG than non-parietal cells[25-27]. PGs produced by mucous cells, however, might play an important role in maintaining gastric mucosal integrity, since mucous cells form the first lining of the gastric mucosa against the changes in the gastric lumen. In summary, we have described a method for successful monolayer culture of adult rabbit gastric epithelial cells, which were mainly composed of mucous neck cells. Cultured monolayers produced PGE_2 and PGI_2, and provide useful tools for the physiological study of gastric mucosa.

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