ISOLATION AND SEPARATION OF HIGHLY ENRICHED
FRACTIONS OF Viable MOUSE GASTRIC
PARIETAL CELLS BY VELOCITY SEDIMENTATION

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ABSTRACT

Methods of tissue dissociation and cell separation have been modified to obtain highly enriched fractions of mouse gastric parietal cells. Suspensions of gastric mucosal cells are prepared by pronase digestion of the glandular portion of the stomach from adult mice. By utilizing the velocity sedimentation technique to separate cells of different sizes, it is possible to recover parietal cells, which are larger than the other cell types, in fractions with purity of 75–95%. The homogeneity of cell fractions has been assessed by light and electron microscopy. The ability of the isolated cells to exclude the dye trypan blue, to incorporate labeled substrate, to consume oxygen, and to retain their structural integrity indicates that they are viable and still capable of functional activity.

Numerous attempts have been made to assign specific functions to the individual cell types in the gastric mucosa. The classical approach to this problem has been that of Holter and Linderstrom-Lang (4) correlating quantitative variations in biochemical activity with the numbers of a particular cell type in the mixed population in frozen sections cut at different levels of the gastric glands. Analysis of the functions of the respective cell types could be accomplished with greater validity and precision if it were possible to isolate relatively pure fractions of the different kinds of cells in the gastric mucosa. Recent advances in gastric cell isolation methods and the unit gravity sedimentation method seemed to us to hold considerable promise of achieving this objective. We are reporting here the results of a modification of the isolation technique of Blum et al. (1) used in conjunction with the velocity sedimentation method of Miller and Phillips (10). This procedure provides a means of recovering highly enriched fractions of gastric parietal cells which are still viable as indicated by biochemical, physiological, and ultrastructural parameters.

MATERIALS AND METHODS

Suspensions of mouse gastric mucosal cells were prepared by adapting the method described by Blum et al. (1) for separation of cells from amphibian stomach. Male CD-1 mice, 60–90 days old, were killed by cervical dislocation. Stomachs were excised and all but the area containing the gastric glands proper was discarded. This glandular portion of the stomach, including its muscularis and serosa, was washed free of adhering lumenal content by grasping the organ with forceps and vigorously shaking in a Krebs-Ringer buffer solution.

All of the in vitro steps used in this study employed a Krebs-Ringer bicarbonate buffer solution (122 mM NaCl, 4.8 mM KCl, 25.2 mM NaHCO₃, 1.2 mM...
KH₂PO₄, 1.2 mM MgSO₄·7H₂O, and 1.3 mM CaCl₂, pH 7.2-7.4) enriched by addition of 11 mM glucose, 1 mM glutamine (Sigma Chemical Co., St. Louis, Mo.) and Eagle's (3) minimal and nonessential amino acids (Difco Laboratories, Detroit, Mich.). This medium was aerated with 95% air:5% CO₂. The enriched Krebs-Ringer bicarbonate solution (EKRB) was adopted after it was noted that the incorporation of [³H]acetate and [³H]leucine by the isolated cells was linear for periods up to 4 h when in a medium supplemented with amino acids. In a nonsupplemented Krebs-Ringer solution no further uptake of labeled substrate was observed after the first 60 min. Furthermore, structural integrity of the cells was maintained for longer periods in the supplemented medium.

The glandular portions of five stomachs were stretched and pinned mucosal side up in a 8.75-cm diam stainless steel photographic developing tank which had been lined with dental wax. This was done while the stomachs were submersed in EKRB. When the pinning was completed, the EKRB was replaced with 10 ml of 0.15% (1.5 mg/ml) pronase E (70,000 PUK U/g, EM Laboratories, Inc., E. Merck, Darmstadt, Elmsford, N. Y.) in EKRB. The chamber was covered and gassed continuously with 95% air:5% CO₂ and incubated in an Eberbach water-bath shaker (Eberbach Corp., Ann Arbor, Mich.) set at 37°C and shaken at 80-100 cycles/min. After 60 min the enzyme solution containing some free cells was again replaced with 10 ml of fresh solution. Incubation was continued for 15 min; then the enzyme solution was again replaced with 10 ml of fresh pronase solution. At this time the stomachs were unpiined, held with forceps, and vigorously shaken manually at 200-250 cycles/min for 2 min each in the pronase solution. This step released the remaining epithelial cells and many intact gastric glands from the connective tissue and muscle. All three fractions contained epithelial cells. Since the first two samples were not as rich in parietal cells as the shake fraction, only the latter was used for the present study.

The suspension of epithelial cells was transferred to a 40-ml conical centrifuge tube, and 500 µg DNase (bovine pancreas deoxyribonuclease I, Sigma Chemical Co.) was added. The suspension was incubated in the water bath shaker for an additional 30 min with continuous aeration. All glassware and pipettes were siliconized to minimize loss of cells by disruption and adhesion. After the final enzyme incubation step the cell suspension was gently pipetted about 50 times over a 3-min period with a Pasteur pipette attached to a soft 2-ml rubber bulb. This

![Figure 1](image-url)
Mouse gastric mucosa after 75-min digestion in 0.15% pronase. Surface mucous cells have been removed, leaving the still intact gastric glands. The lamina propria and submucosa are markedly loose and expanded. The smooth muscle cells appear contracted and are no longer organized as distinct layers. Scale equals 29 μm. × 350.
FIGURES 3-5 Interference (Nomarski) photomicrographs of isolated mouse gastric mucosal cells. Scale equals 10 μm. × 1,000.

FIGURE 3 Dissociated cells before separation by velocity sedimentation.

FIGURE 4 A fraction containing cells recovered after velocity sedimentation. The fraction contains less than 2% parietal cells.

FIGURE 5 A fraction containing parietal cells recovered after velocity sedimentation.
FIGURE 6  A photomicrograph of a parietal cell fraction. Many of the parietal cells demonstrate extensive intracellular canaliculi surrounded by numerous mitochondria. A large chief cell with prominent dense granules is also seen in the field. Only two of the remaining cells in the field are not parietal cells. They are either mucous or endocrine cells. Scale equals 10 µm. × 1,000.

step dissociated the remaining cell clusters into individual cells. This cell suspension was layered over 10 ml of 0.5% bovine serum albumin (BSA) fraction V (Sigma Chemical Co.) in EKRB and centrifuged for 10 min at 200 g in a refrigerated PR-J International centrifuge set (International Equipment Co., Needham Heights, Mass.) at 5°C. The cell pellet was resuspended by gentle pipetting in 10 ml of EKRB with 0.5% BSA and DNAse (50 µg/ml). The cell concentration was determined with a hemacytometer. The suspension was then diluted in the same medium to a final concentration of 2 × 10⁶ cells/ml. The normal yield from the five mouse stomachs was 50–75 × 10⁶ cells.

Cell suspensions were carefully checked by phase-contrast and Nomarski interference-contrast microscopy at 100 and 400 magnification for the absence of pairs and clusters of cells before being used for unit gravity velocity sedimentation at 5°C in a cold room. A 10-ml sample containing 2 × 10⁶ cells was filtered through a nylon mesh with a pore size of approximately 70 µm to remove any large pieces of tissue debris and introduced into a STA-PUT SP-120 sedimentation chamber (Johns Scientific, Toronto, Ont.), which has a loading capacity of 10 ml of fluid per vertical millimeter in the cylindrical portion of the chamber. The cells were introduced after 20 ml of EKRB and a linear 2–4% BSA gradient was formed under the cell layer. To form the gradient, two reservoirs were used; one contained 275 ml of 2% BSA in EKRB, and the second contained a similar volume of a 4% BSA in EKRB. The standard STA-PUT system was slightly modified. The original special chamber used for the introduction of the cell suspension was replaced with a 20-ml glass syringe barrel attached to a stainless steel three-way valve. This modification minimizes cellular contamination of the gradient, results in rather distinct cell bands, and markedly improves the final purity of the isolated fractions. A sedimentation chamber loading rate of 10 ml/min was used for the cell suspension and for the initial loading of the linear gradient. After 5 min the loading rate was increased to 40 ml/min. Complete loading of the chamber (with EKRB, cell suspension, and BSA gradient) to a total volume of 580 ml was completed in about 20 min.

1 h after the introduction of cells into the chamber, when the cells had sedimented into bands, the chamber was drained and 10-ml samples were collected in silicon-
ized glass test tubes in a Fractomat automatic fraction collector at a rate of 2 tubes per minute. The total time period for all of the cell separation steps in the sedimentation chamber was about 90 min. The samples were kept in the cold room or on ice at 4–5°C before centrifugation at 200 g for 10 min. After centrifugation, all but 0.5 ml of the supernate was discarded from each sample and the pellets were resuspended and checked for cell types by phase-contrast microscopy. After determining the distribution of cell types, appropriate samples were pooled before being used for further study.

Oxygen depletion rates were determined for cell suspensions with a Clark oxygen probe (Yellow Springs Instrument Co., Yellow Springs, Ohio) in a 20-ml glass and stainless steel chamber (12) maintained at 37±0.01°C. The incubation medium for the O₂ depletion studies was EKRB with streptomycin sulfate (100 μg/ml) and penicillin-G (100 U/ml). All solutions were aerated with 95% air:5% CO₂. Samples of cells introduced into the chamber contained 0.5–4 x 10⁶ cells.

Tissue and cell samples were fixed and prepared for light and electron microscopy. Cell suspensions were fixed in a mixture of 1.25% paraformaldehyde, 2.5% glutaraldehyde, and 0.02% trimetrexol in cacodylate buffer, pH 7.2 (7), postfixed in 1% OsO₄ in the same buffer, dehydrated with ethanol, and embedded in low viscosity Epon (9). Tissue samples were handled similarly and embedded in Epon-Araldite (11). 1-μm sections were stained with toluidine blue and examined by light microscopy. Thin sections were stained with uranyl acetate and lead citrate and examined with a JEM 100B electron microscope.

RESULTS
The glandular gastric mucosa resembles that of other mammals. A typical area from the greater curvature of the stomach is illustrated in Fig. 1. After 75-min incubation of the mucosa in the 0.15% pronase solution (Fig. 2) the surface epithelium and much of the gastric pits, which are composed of surface mucous cells, have been removed, but the remaining gastric glands are still intact. However, most of the epithelial cells have a clear surrounding space and appear loosely associated. The connective tissue of the lamina propria and submucosa are markedly loosened and thickened. The smooth muscle cells of the muscularis and muscularis mucosae are contracted and
rounded and seem to have lost their organization into definite layers.

After this incubation period numerous individual cells and remnants of glands can be dislodged from the connective tissue by vigorously shaking the tissue. Cells that are still not isolated from each other are held together by the complex of tight junctions. Complete separation of the epithelium into isolated individual cells (Fig. 3) is achieved by continued incubation in pronase and gentle aspiration and expulsion of cells through a pipette. Most of these isolated cells retain their structural integrity when examined by light (Figs. 3–6) or electron microscopy (Figs. 7–11). Over 95% of these isolated cells exclude the vital dye trypan blue (5), indicating intact plasma membranes.

These isolated cells, illustrated as a monolayer by Nomarski optics (Fig. 3), are a heterogeneous population of rounded cells, which can be classified according to size and morphology and designated as the crude suspension. The population of large cells which have a granular cytoplasm and a diameter of 18–25 μm are the parietal cells. They account for 10–15% of the cells in the crude suspension. Chief cells which have large prominent cytoplasmic granules and an average diameter of approximately 15 μm account for 5–10% of the cells in the crude suspension. The smaller, smooth contoured, nongranular cells are, for the most part, surface mucous cells, mucous neck cells, and occasional endocrine cells, connective tissue cells, and erythrocytes. This mixed population of smaller cells, varying in diameter between 10 and 15 μm, accounts for 80–85% of the cells in the crude suspension.

Separation of an enriched parietal cell fraction from the crude cell suspension is achieved by unit gravity sedimentation at 4°C. 20 min after loading 2 × 10⁷ cells into the STA-PUT chamber, two distinct bands of cells are apparent. The small cells form an upper band that sediments at a rate of 5–10 mm/h, while the parietal cells form the lower band that sediments at a rate of 15–20 mm/h. The chief cells are generally found in the leading edge of the upper band, but the larger chief cells do sediment with the parietal cells. During some separations, an intermediate band can be seen between the two more prominent bands. After 60 min of separation, 60 10-ml aliquots are collected at a rate of 2 samples per minute. The last tube
collected, containing only the EKRB that was originally layered over the cell layer in the STAPUT chamber, is discarded. The next sample is designated "sample 1," and the rest of the samples are numbered in sequence. After centrifugation and resuspension of the samples in 0.5 ml of EKRB, small aliquots of each are checked for cell type and purity by examination by phase-contrast microscopy. Samples containing the same cell types are pooled as needed for further study. The small-cell fraction (Fig. 4), fraction I, is typically obtained by pooling samples 5-10. Fraction I contains primarily mucous cells, with a few chief and endocrine cells. These cells have diameters of 9-15 μm and an average volume of about 1,000 μm³ (400-1,400 μm³). About 5-10 × 10⁶ cells are recovered in fraction I, of which less than 2% are parietal cells. The parietal cell fraction (Figs. 5 and 6), fraction II, contains cells usually from samples 18-30. This fraction contains primarily parietal cells which have diameters of 18-24 μm and an average volume of approximately 4,400 μm³ (2,800-6,000 μm³). Occasional clusters of other cell types, usually surface mucous cells, are also present in fraction II. The enriched parietal cell fraction has a purity of 75-95% parietal cells with a yield of 1-1.5 × 10⁶ cells.

More than 95% of the cells in the enriched fraction exclude the vital dye trypan blue. Furthermore, when fixed and examined by electron microscopy the cells appear to have retained their ultrastructural integrity (Fig. 10). Most of the cytological features of parietal cells in the intact stomach are also found in these isolated cells. The major differences are their rounded shape and the loss of all cell-to-cell junctions (Figs. 10 and 11). The isolated cell in Fig. 11 has numerous large mitochondria, characteristic tubulovesicular membranes, and an intracellular canaliculus lined with microvilli. A few profiles of rough endoplasmic reticulum can be identified and the nucleus with marginated chromatin is not atypical of parietal cells in situ. Blebs of cytoplasm attached to some isolated cells may be fragments of neighboring cells still attached by junctional complexes.

The analysis of O₂ consumption by these cells is
FIGURE 10 Electron micrograph of an isolated parietal cell. The cell contains numerous large mitochondria, sparse endoplasmic reticulum, scattered profiles of tubulovesicular membranes, and a characteristic intracellular canaliculus lined with microvilli. Scale equals 1.4 μm. × 7,000.

Further indication that these isolated cells are viable (Table I). The crude suspension containing about 15% parietal cells had an O₂ consumption rate of 4.8 nmol O₂/min per 10⁵ cells. Periodic checks of samples from the cell suspensions demonstrate that the rate remains constant for periods up to 4 h. The average O₂ consumption rate for enriched parietal cell fractions was 11.9 nmol O₂/min per 10⁶ cells. Since the percentage of parietal cells in any fraction can be readily determined, it is possible to calculate the O₂ consumption rate for isolated parietal cells. It was found that the rate of O₂ consumption for isolated parietal cells is significantly higher than the rate observed for the small-cell fraction, 15.2 nmol O₂/min per 10⁶ parietal cells vs. 2.8 nmol O₂/min per 10⁶ small cells.

DISCUSSION

With the method described we have been able reproducibly to separate and purify a parietal cell fraction and a mixed “small-cell” fraction in
milligram quantities. A high degree of viability of the isolated cells is indicated by the fact that (a) most of the cells (95%) exclude the dye trypan blue; (b) the isolated cells incorporate labeled substrate; (c) O$_2$ consumption rates are maintained over periods of hours; and (d) ultrastructural integrity is retained. It is also important to note that the isolated cells retain their capacity to transport chloride.

To compare our data with those of previous studies, we undertook to convert O$_2$ consumption rate to a base of milligrams protein. The protein content of the isolated crude suspension was determined (8), and it was found that 10$^4$ cells (10–15% parietal cells) contained approximately 250 µg protein. We prefer, however, to express the rate on a per cell basis since ultimately we are interested in metabolic activity at this level.

The results of the O$_2$ consumption rates of these isolated cells indicate that isolated parietal cells had a rate 5.4 times greater than that of the small-cell fraction (Table 1). Blum et al. (1) made a similar observation on isolated cells from the Necturus gastric mucosa. They reported that the pure oxyntic cells have a rate five times that of the nonoxyntic cells of the tissue. A direct comparison of the respiration rates of mouse parietal cells and frog oxyntic cells is not possible since we express

![Figure 11. Electron micrograph of a juxtanuclear region of a parietal cell. Characteristic tubulovesicular membranes and intracellular canaliculus are shown. Scale equals 0.36 µm, x 28,000.](image-url)
our rates on a cell count basis. It would appear, however, that the rate for mouse parietal cells is about four times greater than that for the Necturus oxyntic cells. The rate of O₂ consumption by mouse parietal cells also compares favorably with the data reported by Croft and Ingelfinger (2) on isolated canine gastric parietal cells. Their calculated parietal cell O₂ consumption rates were 10.4 and 12.5 μl O₂/h per 10⁸ cells. If we convert the rate for mouse parietal cells of 15.2 nmol O₂/min per 10⁶ cells to these units, we obtain a rate of 20.4 μl O₂/h per 10⁶ cells. In studies on the respiratory activity of rat liver parenchymal cells, Howard et al. (6) report that the average rate of endogenous respiration in Krebs-Ringer phosphate solution of liver cells was 30.0 μl O₂/h per 10⁶ cells.

Since the O₂ consumption data do compare favorably with those of other studies on isolated cells, it seems reasonable that this is a valid indication of cell viability. In addition to respiration and chloride transport studies, we are exploring the possibility of stimulating these isolated cells with drugs and hormones. Purified cell fractions should be useful in further studies of gastric physiology and should permit a more definitive assignment of secretory and metabolic roles to specific cell types.

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