Prostaglandin E_2 Production by Dispersed Canine Fundic Mucosal Cells

Contribution of Macrophages and Endothelial Cells as Major Sources

Monica C. Chen, Martin J. Sanders, Deborah A. Amirian, Lovick P. Thomas, Gordon Kauffman, and Andrew H. Soll
Center for Ulcer Research and Education, and Medical and Research Services, Veterans Administration Wadsworth Hospital Center, School of Medicine, University of California at Los Angeles, Los Angeles, California 90073

Abstract

Endogenous prostaglandins (PGs) influence resistance of the gastric mucosa to injury, but the source of PGs is unknown. Using radioimmunoassay, we studied PG production by dispersed canine fundic mucosal cells. PGE_2 production, stimulated by bradykinin, epidermal growth factor, zymosan, and calcium ionophore, was greater in the small-cell elutriator fraction (SCEF) than in the medium and large cell fractions, which contained mucous, chief, and parietal cells. Linear density gradients of SCEF cells revealed maximal PGE_2 production in cells of light density. Mast, endocrine, and endothelial cells did not account for this PGE_2 production. Macrophages, identified by uptake of acetylated-LDL, immunoreactivity with antibodies to the human Ia antigen, and phagocytosis of fluorescent latex particles, were enriched in the SCEF and correlated with PGE_2 production in the density gradient. Magnetic separation of cells in the SCEF-ingesting iron particles enriched PGE_2 production. Fractions enriched in endothelial cells present in intact capillary fragments, but depleted of macrophages, also produced PGE_2. Regulation of PGE_2 production differed among cell types. Fibroblasts were easily cultured from submucosa, but were not detected in the SCEF. We conclude that macrophages and capillary endothelial cells are major producers of PGE_2 in the canine fundic mucosa.

Introduction

Endogenous prostaglandins play an important role in modulating mucosal resistance to injury (1). Inhibition of endogenous prostaglandin (PG) formation by nonsteroidal anti-inflammatory drugs induces upper gastrointestinal ulcers and their complications in humans (2). Immunization of rabbits and dogs with PGE_2 induces antibody formation and gastrointestinal ulcer formation. Despite the importance of endogenous PGs, there is little knowledge of the source of PGs in the gastric and duodenal mucosa. Therefore, it is difficult to study the regulation of PG production or assess the potential physiological or pathophysiological role of PGs in this complex tissue. Enzyme-dispersed canine fundic mucosal cells produce PGs at a rapid rate and, thus, provide a model for studying the cellular source of PG production. In the present study, we have separated cells by size and by density, and sought to determine the contribution to PGE_2 production by specific cell types. Fundic mucosa bluntly separated from submucosa also contained elements from the lamina propria (e.g., fibroblasts, macrophages, and endothelial cells), and we have assessed the contribution to PGE_2 production from these cells in comparison to the epithelial cells.

Methods

Cell dispersion and separation. Canine fundic mucosa and lamina propria were separated by blunt dissection from submucosa and cells were dispersed using sequential exposure to crude collagenase (0.35 mg/ml) and EDTA (1 mM), as previously described (3, 4). The present studies were performed using the large elutriator rotor (model JE-10X, Beckman Instruments, Inc., Fullerton, CA); flow rates and revolutions per minute were chosen (Table I) to elute fractions of cell composition comparable to our previous studies (3, 4). The small-cell elutriator fraction (SCEF)[1] was further separated utilizing linear-gradient (5) or step density-gradient techniques (6). Gradient solutions were modified to control osmolality. The heavy solution (Hv) was 24% BSA (Boehringer Mannheim Biochemicals, Indianapolis, IN) plus 9% Ficoll (Pharmacia Fine Chemicals, Piscataway, NJ) dissolved in Hanks' balanced salt solution (HBSS, Irvine Scientific, Santa Ana, CA) that had been diluted to 47% of the normal salt concentration. The light solution (Lt) was 12% BSA dissolved in HBSS diluted to 88% of the normal salt concentration. Optimal results were obtained when solutions were allowed to dissolve by stirring overnight in a cold room. The solutions were pH adjusted to 7.4 with 1 N NaOH, brought up to volume, and then centrifuged at 3,000 rpm for 15 min to sediment debris. Solutions were either immediately used for density gradient or transferred to new 50-ml tubes and frozen at −70°C. Density and osmolality of the Hv and Lt solutions were checked routinely.

The linear-gradient separations were performed as described previously (5). The Hv BSA solution (100 ml) was loaded into a zonal rotor (Sorvall-DuPont, Newtown, CT) to form a cushion and a linear gradient formed between the Hv and Lt BSA (600 ml each, density 1.085 and 1.030, respectively). Cells were loaded in 8% BSA and the gradient was centrifuged at 6,000 rpm for 30 min. Fractions (20 ml) were collected, diluted with HBSS to 50 ml, and centrifuged. After resuspension in HBSS, fractions were pooled in groups of four. Specific gravity of the fractions was measured before dilution.

PGE_2 release assay. Cells were suspended (2×10^6 cells/ml) in HBSS with 2 mM glutamine, 15 mM Hepes, but without BSA. Cells were incubated at 37°C for 30 min in 20-ml glass scintillation vials in a rotary shaker bath (60 rpm). After a baseline sample (t_0), test substances or vehicles were added and the medium was sampled again after 45 min (t_{45}). Samples of cell suspension were microfuged (8,700 *g*) and the supernatant stored at −20°C for measurement of PGE_2 concentration in the medium by RIA. Data are expressed as the increment in medium PGE_2 concentration between t_0 and t_{45}, adjusted for cell count. In control studies (M. C. Chen, D. A. Amirian, and A. H. Soll, manuscript in preparation), the time course of PGE_2 production was linear between 15 and 90 min, linearly dependent upon cell number

Address reprint requests to Dr. Soll, VA Wadsworth Hospital Center, Building 115, Room 215, Los Angeles, CA 90073.

Received for publication 6 April 1987 and in revised form 29 June 1989.

The Journal of Clinical Investigation, Inc.
Volume 84, November 1989, 1536–1549

1. *Abbreviations used in this paper:* acLDL, acetylated low density lipoprotein; DiL, dioctadecylindocarbocyanine; GS-1, griffonia simplicifolia; 5-HT-LI, 5-hydroxytryptamine (serotonin)-like immunoreactivity; Hv, heavy; Lt, light; SCEF, small-cell elutriator fraction; β-TPA, β-12-*O*-tetradecanoylphorbol 13-acetate.

Table I. Separation of Canine Fundic Cells with the JE-10X Rotor

Elutriator fraction	Flow rate*	Speed	Apparent cell diameter‡
	ml/min	*rpm*	*μm*
1	30.0	1,400	6.5
1.5	30.0	1,200	7.5
2	55.0	1,200	10.0
3	75.0	1,200	11.6
4	87.5	1,200	12.6
5	100.0	1,200	13.6
6	142.5	1,200	16.1
7	185.0	1,200	18.5
8	192.5	1,100	20.5
9	200.0	1,000	23.0
REST	>200.0	—	>23.0

* The flow rates and speed were calculated from the nomogram provided with the Beckman Instruments, Inc. elutriator manual. The rest fraction was collected by turning off the centrifuge and collecting the remaining cells as the rotor came to a stop.
‡ Apparent cell diameter was determined from the nomogram noted above.

between 1×10^6 and 6×10^6 cells/ml. Indomethacin blocked PG production with an ID_{50} of ~ 0.1 μM.

Radioimmunoassay for PGE_2. Preliminary studies using both RIA for 6-keto-$PGF_{1\alpha}$, PGE_2, and thromboxane B_2 and thin-layer chromatography (Boughton-Smith, N., M. C. Chen, D. A. Amirian, B. J. R. Whittle, and A. H. Soll, manuscript in preparation) after loading with [^{14}C]arachidonic acid (Amersham Corp., Arlington Heights, IL) indicated that PGE_2 was a major product. For the present studies PGE_2 was measured by RIA as described (7). In brief, 100-μl aliquots of supernatant from the cell suspension or PGE_2 standards were added to 12 × 75-mm glass tubes. [^{3}H]PGE_2 (Amersham Corp.) and PGE_2 antibody (Seragen, Inc., Boston, MA) were added (100 μl each) and the mixture was incubated at 4°C for 18–24 h. After adding dextran-coated charcoal, tubes were centrifuged (1,000 *g*, 12 min), and radioactivity in the supernatant was counted. The interpretable range for PGE_2 was from 0.07 to 20 ng/ml. 10 ng/ml of neither 6-keto-$PGF_{1\alpha}$ nor thromboxane B_2 inhibited antibody binding of tracer, indicating < 0.01% cross-reactivity.

Cell markers. Immunohistochemical and cytochemical markers were utilized to identify as many cell types from the fundic mucosa and lamina propria as possible. The sources and methods for these markers are listed in Table II. Parietal, chief, and mucous cells were identified on Bouins-fixed, periodic acid Schiff-stained cytocentrifuge slides (6). Mast cells were identified with toluidine blue staining after glutaraldehyde fixation (5). 200 cells in each of four to six randomly selected fields per slide were counted; clumps were not included in the count. Two investigators counted each coded slide.

Identification of macrophages. Markers used to identify macrophages included immunohistochemistry with two antibodies to the human Ia antigen that cross-react with canine macrophages: antibody B1F6-16 (lot 84-1, Dr. R. Alejandro, Diabetes Research Institute, University of Miami, FL [8]) and antibody 7.2 (Dr. H. J. Deeg, Division of Oncology, University of Washington, Seattle, WA [9]). Both of these antibodies also may interact with Ia antigen present on lymphocytes and, therefore, three lymphocyte-specific markers were also utilized: DLy6 (Dr. H. J. Deeg [10]) and 1A1 and 6C6 (Dr. D. R. Kraweic, Veterinary Clinical Medicine, University of Illinois at Urbana-Champaign). Use of these antibodies is described in Table II. Uptake of dioctadecylindocarbocyanine (DiL)-labeled-acetylated-LDL (acLDL) (11, 12) (Table II) and lucifer yellow (13) were evaluated as markers for macrophages.

Phagocytosis. Phagocytosis was tested using polybead-fluorescent microspheres (2.5% solids-latex, diameter 0.83 μm; Polysciences, Inc., Warrington, PA). Beads were serum-activated by adding two drops of beads to 10 ml of HBSS containing 1% heat-inactivated dog serum and 0.1% BSA. Cells were incubated with beads (4:1) in HBSS containing 0.1% BSA and glucose (3 g/liter) at 37°C for 30–60 min, with or without 40 nM β-12-*O*-tetradecanoylphorbol 13-acetate (β-TPA) (Molecular Probes Inc., Junction City, OR). The cell suspension, diluted 1:1 with Lt BSA solution, was layered over the Lt BSA solution and centrifuged at 430 *g* for 20 min to remove free beads, which floated over the Lt BSA solution. In a second protocol, phagocytic cells were further enriched by density gradient: cells diluted 1:1 with Lt BSA were layered over a 100% Lt BSA step and a 40% Hv/60% Lt step and centrifuged in a Sorvall RC-5 rotor at 430 *g* for 30 min. Cells were examined for latex particles using a phase-contrast fluorescent microscope. Canine peripheral blood monocytes (14), separated from red blood cells and granulocytes by single-step gradient of Ficoll-Hypaque (Pharmacia Fine Chemicals) (15) were used as a positive control.

Phagocytosis of iron (16) was evaluated using antibody-coated particles (BioMag M4400, Advanced Magnetics Inc., Cambridge, MA). Particles were washed using the BioMag Separator, resuspended in a similar volume of HBSS, and added to a cell suspension ($4–8 \times 10^6$ cells/ml) at a 1:4 (vol/vol) ratio. After a 30-min incubation at 4°C or 37°C, the suspension was exposed to magnetic field for 2 min using a BioMag Separator taped onto a plastic petri dish. After decanting the nonattracted cells, the BioMag Separator was removed and the attracted cells were resuspended in HBSS. Cells enriched by step-density gradient were also tested for iron phagocytosis; cells from the SCEF (60×10^6 cells) were loaded onto a density gradient consisting of a 10-ml step of 50% Ht/50% Lt BSA layered over 10 ml of 70% Ht/30% Lt and centrifuged for 20 min at 2,200 rpm.

Glass adherence. Glass adherence by monocytes (14) and fundic macrophages was tested. Cells ($4–6 \times 10^6$ cells/ml) were incubated with or without fluorescent microspheres on tissue culture chamber/slides (eight chambers, Lab-Tek, Miles Scientific Div., Naperville, IL) for 1–4 h at 37°C. Nonadherent cells and beads were gently washed away with phosphate-buffered saline. Adherent cells were fixed with one drop of 1% glutaraldehyde for 5 min. Plastic chambers were removed, a coverslip was applied, and the slide was examined with phase-fluorescent microscopy.

Isolation and identification of mucosal endothelial cells (microvessels). We initially identified capillary endothelial cells by the presence of factor VIII antigen (17, 18), a specific marker known for endothelial cells (Table II). Subsequent studies with a panel of lectins revealed that the FITC-labeled lectin isolated from griffonia simplicifolia (GS-1, E.Y. Lab, Inc., San Mateo, CA) selectively bound to the morphologically distinct capillary fragments. The lectin marker offers the advantage of rapid identification of microvessels using unfixed material. Dispersed cells were filtered through coarse nylon mesh (62 threads per inch) and then through a finer nylon mesh (195 threads per inch). Capillary fragments retained on the latter mesh were freed by gently scraping of the mesh immersed in HBSS. We attempted enrichment of microvessels in this suspension by elutriation; a poor yield obviated routine use of this technique.

Isolation and identification of gastric fibroblasts. After separation of the submucosa from the mucosa and removal of residual mucosa fragments, the fundic submucosa was bluntly separated from the muscularis. Submucosal tissue was minced with a McIlwain tissue chopper (Brinkmann Instruments Co., Westbury, NY) and placed into digestion flasks for two sequential 60-min incubations in 1.25 mg/ml of crude collagenase under similar conditions to those described previously (3). After digestion, the suspension was washed twice in HBSS and filtered through coarse nylon mesh. Cells were loaded into an elutriator rotor (model JE-6, Beckman Instruments, Inc.) at 2,500 rpm and a 25 ml/min flow rate and the fibroblast fraction was eluted at a 50 ml/min flow rate. Cells ($10–15 \times 10^6$) were cultured in 25-cm^2 T flasks

Table II. Identification of Cell Markers

Target cell	1° Ab or stain	Source	Incubation Time/temperature	2nd Ab (if applicable)	2nd Ab source	Incubation Time/temperature
			min/°C			*min/°C*
TdB lymphocytes*	Mouse anti-canine IgM DLy-6, 1:100	Dr. H. Joachim Deeg (Seattle, WA)	30/4	Rabbit anti-mouse IgG or IgM FITC, 1:50	American Qualex (La Mirada, CA)	30/4
TdB lymphocytes‡	Mouse anti-canine IgM (1A1), IgG (6C6) 1:250 (1A1) 1:1,000 (6C6)	Dr. D. R. Kraweic (Urbana-Champaign, IL)	30/4	Rabbit anti-mouse IgG FITC 1:50	Accurate Chemical (San Diego, CA)	60/20
Somatostatin§ cells	Sheep anti-somatostatin, 1:500	Dr. Greg Aponte (UCB, S.F., CA)	Overnight/4	2° Ab, rabbit anti-sheep IgG, 1:10	Cappel (Cochranville, PA)	30/20
				3° Ab, sheep PAP, 1:100	Cappel (Cochranville, PA)	30/20
Glucagon§ cells	Rabbit anti-glucagon VI, 1:500	Dr. Tache Yamada (Ann Arbor, MI)	Overnight/4	2° Ab, goat anti-rabbit IgG, 1:10	Cappel (Cochranville, PA)	30/20
				3° Ab, rabbit PAP, 1:100	Cappel (Cochranville, PA)	30/20
Serotonin§ cells	Rabbit anti-serotonin RS1-1, 1:600	Immuno-Tech (Chapel Hill, NC)	Overnight/4	2° Ab, goat anti-rabbit IgG, 1:10	Cappel (Cochranville, PA)	30/20
				3° Ab, rabbit PAP, 1:100	Cappel (Cochranville, PA)	30/20
Macrophage*	Mouse 7.2 anti-human Ia IgG ascites, 1:100	Dr. H. Joachim Deeg (Seattle, WA)	30/4	Rabbit anti-mouse IgG FITC, 1:50	Accurate Chemical (San Diego, CA)	30/4
Macrophage*	DiL-acLDL, 10 μg/ml	Biomedical Technologies, Inc. (Cambridge, MA)	Overnight/37			
Macrophage*	Lucifer yellow 0.5 mg/ml	Sigma Chemical Co. (St. Louis, MO)	30/37			
Macrophage*	Mouse anti-human Ia B1F6-16 (Lot 84-1), 1:500	Dr. R. Alejandro (Miami, FL)	60–90/4	Rabbit anti-mouse IgG FITC, 1:50	Accurate Chemical (San Diego, CA)	60/4
Endothelial‖ cells	Rabbit anti-canine factor VIII related antigen, 1:50	Drs. Roger Benson/ Dodds, (Dept. of Health; Albany, NY)	60/20	Swine anti-rabbit IgG FITC, 1:75	Accurate Chemical (San Diego, CA)	60/20
Endothelial¶ cells	FITC-GS-1 lectin	E-Y Lab Inc. (San Mateo, CA)	15/25			

Fixation and staining: * No fixation needed. Fresh cells were incubated with first Ab in microfuge tube. Cells were spun and washed and then incubated with second Ab. After washing off the unbound second Ab, cytospins were made for evaluation. ‡ Cytospins were fixed in acetone, 10 min, 20°C; transferred to 0.05 M Tris, pH 7.4, for storage and staining. § Cytospins were fixed in Bouins with 1% glacial acetic acid, 20 min, 20°C; transferred to 70% ethanol for storage and staining. ‖ Cytospins were fixed in acetone, 10 min, 20°C; transferred to 0.1 M PBS for storage and staining. ¶ Fresh cells incubated with lectin, cytofuged and wet mounted for evaluation.

(Corning Glass Works, Corning, NY) in 10 ml of Ham's F12:DME (1:1) (Irvine Scientific), supplemented with 10% calf serum (Irvine Scientific), 20 mM Hepes, 8 μg/ml insulin, and 50 μg/ml gentamicin sulfate. After 2–3 d, the attached cells were removed from the T flasks by a trypsin-EDTA solution (Gibco Laboratories, Grand Island, NY) and passed to 75-cm^2 T flasks at 1:3 dilution in fresh medium. These cells were then passed weekly for 2–3 mo. We have identified fibroblasts on the basis of their ability to be plated and maintained on untreated plastic culture substrates, their morphology in culture, and their ability to be subcultured by trypsinization.

DNA assay. We measured the DNA fluorometrically (19) using 4',6'-diamidine-2-phenylindole as the intercalator.

Statistical analysis. In all experiments, data were analyzed by Student's paired t test in which n refers to the number of separate cell preparations used, which equals the number of animals used.

Results

Stimulation of PGE_2 production. PGE_2 was produced basally by unfractionated fundic mucosal cells at a rate of 0.25±0.01 ng/10^6 cells per 45 min. PGE_2 production was stimulated by bradykinin (1 μM, 1.27±0.41 ng/10^6 cells per 45 min, n = 5). The present studies are focused upon identification of the major PGE_2-producing cells.

PGE_2 production: cell separation by elutriation. Examining the elutriator fractions, basal PGE_2 production was found to be greatest in the small cell fractions 2, 3, 4, and 5 (Fig. 1 *A*). PGE_2 production, in response to bradykinin (1 μM) (Lys-bradykinin from Peninsula Laboratories, Inc., Belmont, CA) and A23187 (1 μM) (Calbiochem-Behring Corp., San Diego, CA), was also maximal in fractions 2, 3, and 4 (Fig. 1 *A*).[2] Using this elutriator protocol, mucous, chief, and parietal cells are

2. The pattern of maximal PGE_2 production occurring in fractions 2–4 was found in seven of nine gradients. In two elutriation separations a different pattern was found in that maximal PGE_2 production occurred in fraction 1, collected at an rpm of 1,200 and a flow rate of 35 ml/min (Table I). In one of these latter separations cells taking up acLDL were also found in maximal numbers in fraction 1, suggesting that the PGE_2 production reflected the presence of macrophages that eluted in this fraction. Whether the differences in these two cell preparations reflect differences in cell size, density, or some other factor was not established.

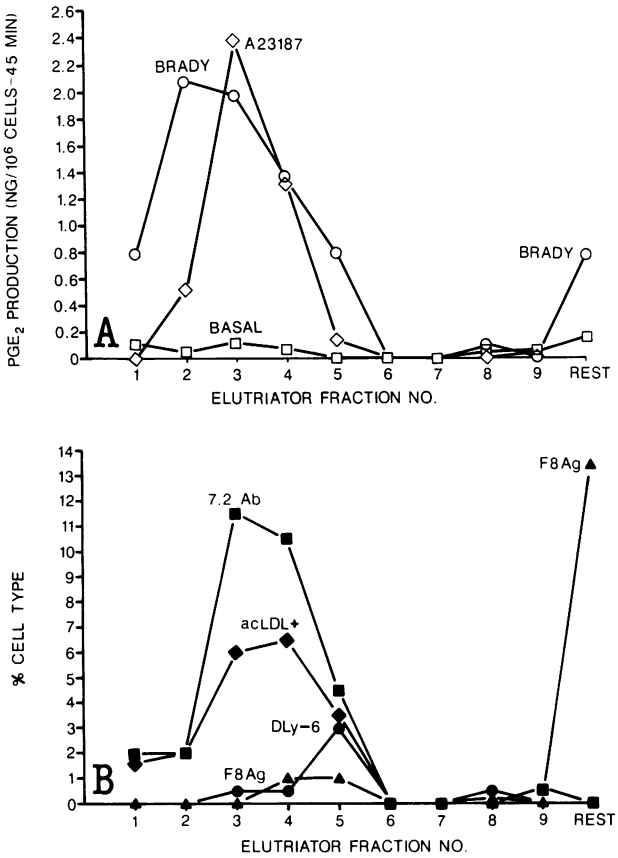

Figure 1. PGE_2 production by canine fundic cell separated by elutriation. Canine fundic mucosal cells were separated by elutriation into nine fractions plus a rest fraction using the large Beckman elutriator rotor (JE-10X) and the protocol described in Methods and Table I. (*A*) Data for PGE_2 release have been expressed as the increment in medium PGE_2 concentration (nanograms per 10^6 cells) between t_0 and t_{45}. Cells were treated with 1 μM bradykinin (○), 1 μM ionophore A23187 (◇), or Hanks' balanced solution (□). (*B*) The distribution of various cell markers was examined as described in Methods and Table II, and data expressed as percentage of a given cell type. Macrophage markers were identified by immunohistochemistry with antibody to the human Ia antigen (*7.2 Ab*, ■) and by the uptake of DiL labeled-acetylated-LDL (*acLDL*, ♦). Lymphocytes were examined with immunoreactivity against DLy-6 antibody (*DLy-6*, ●). The capillary endothelial cells were identified by the presence of factor VIII-related antigen (*F8 Ag*, ▲). These data are from a single preparation, representative of four other similar studies.

enriched in fractions 4–5, 6–8, and 8–9, respectively (6), therefore indicating that these three mucosal cell types did not account for the major peak of PG production in the SCEF. There are several cell types present in the SCEF, including mast cells (5), endocrine cells containing somatostatin and glucagon (20), and endocrine-like cells containing serotonin and DOPA-decarboxylase activity (20, 21).

Macrophage markers. Several potential macrophage markers were evaluated, including the uptake of acLDL (11, 12),[3] the presence of the Ia antigen, and phagocytosis of latex beads. A proportion of fundic cells present in the SCEF avidly accumulated acLDL (Figs. 1 *B* and 2 *A*). Immunoreactivity with monoclonal antibodies 7.2 (Fig. 1 *B*) and B1F6-16 was present in both a rimmed and speckled pattern on cells in the SCEF; both patterns were considered positive (Figs. 2 *B* and 2 *C*). In our hands B1F6-16 detected more cells in the SCEF (18.3±4.1%, mean±SE, n = 9) than did antibody 7.2 (6.5±2.6%, mean±SE, n = 4).[4] In colocalization studies 29±7% (mean±SE, n = 3) of the cells positive for B1F6-16 also accumulated acLDL. All of the cells examined in these three cell preparations that had accumulated acLDL were immunoreactive with antibody B1F6-16. We also examined accumulation into cells of lucifer yellow (13). This marker was positive in both large- and small-cell fractions (data not shown), indicating that this was not selective marker for a specific cell population in the canine fundus.

Phagocytosis of fluorescent latex particles was also assessed and 3.2±1.3% (mean±SE, n = 4) of the cells in the SCEF ingested latex particles, with an average of 6.6±2.1 (mean±SE, n = 4) latex particles per phagocytic cell (Fig. 2 *D*). Of the cells that had ingested latex particles, 93.4±4.4% (n = 3) were immunoreactive with B1F6-16. Of the cells reactive with antibody B1F6-16, 45.2±13.2% (mean±SE, n = 3) demonstrated phagocytosis of latex particles.[5] Furthermore, both speckled and rimmed patterns were present on cells demonstrating phagocytosis of latex particles or accumulation of acLDL.

These markers were examined in the various elutriator fractions to further establish specificity. Immunoreactivity with antibody 7.2 and uptake of acLDL were consistently enriched in the SCEF and absent from the larger cell fractions 6–9 (Fig. 1 *B;* Table III). The distribution pattern shifted slightly among preparations and macrophage markers were occasionally identified in fraction 5. The distribution of the Ia markers and the uptake of acLDL were present in fractions 2–4 of three complete elutriation profiles. Furthermore, cells phagocytic for latex particles were consistently detected in the small cell fractions 2–4, but were absent in larger cell fractions (n = 3, data not illustrated).

A panlymphocytic antibody (DLy-6), that detects both T and B cells, but not macrophages (10), was positive on cells in elutriator fractions 3–5. The number of cells positive in the SCEF for this marker varied from 0 to 8.5% (n = 5). In the same preparations, the percentage of cells immunoreactive with DLy-6 (3.0±1.4%, mean±SE, n = 3) was considerably less than for the Ia antigen (12.1±5.0%, n = 3). A different pattern of distribution between 7.2 Ab and DLy-6 was found in the three elutriator gradients examined and cells accumulating acLDL were found to be negative for DLy-6 (Fig. 1 *B*).

3. acLDL is taken up into macrophages and endothelial cells by a pathway utilizing receptors that are distinct from those for LDL. We observed that endothelial cells present in intact capillary fragments also took up acLDL; a fine granular pattern distinguished this uptake from that found for macrophages in the SCEF. The single endothelial cells in the SCEF occasionally accumulated acLDL, but in contrast to macrophages, endothelial cells were much smaller, lacked the intense whole cell staining, and were negative for Ia antigen when stained simultaneously with acLDL and antibody 7.2.

4. Because these two antibodies were available to us at different times during these studies, direct comparison was only performed for a few times. In addition, studies with B1F6-16 were done with a microscope with improved epifluorescence and optics, increasing our ability to detect positive cells.

5. Our studies evaluating the coincident occurrence of reactivity with B1F6-16 and phagocytosis of latex beads were done on cell preparations demonstrating a high number of phagocytic cells.

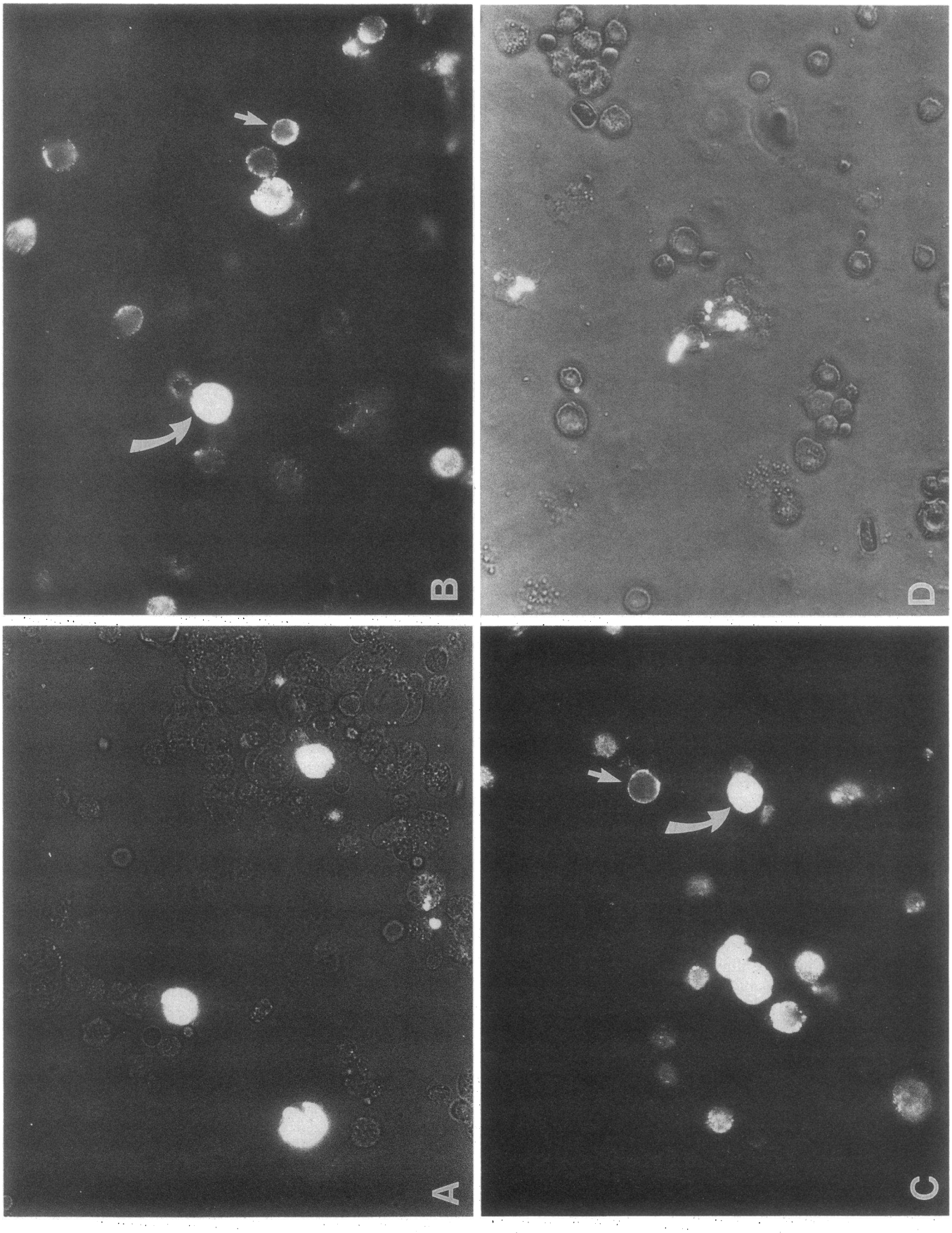
B
D
A
C

Table III. Comparison of Macrophage Markers

	SCEF*	6, 7	MV
	%	%	%
7.2	6.08±1.46	0	—‡
B1F6-16	17.93±3.58	1.13±0.31	1.67±0.98
acLDL	5.20±1.14	0	—‡

* SCEF, fractions 6 and 7 and crude microvessels (MV) were prepared from fundic mucosa. Three macrophage markers were examined (see Table II for detail method). Data are expressed as percentage of positive cells and are means±SE from four preparations.
‡ Data are not available.

Endothelial cell markers. Endothelial cells were identified using an antibody to factor VIII-related antigen (17, 18, 22). On cryostat sections of whole mucosa, positive staining was only present over capillary structures in the lamina propria (data not illustrated). Using this antibody, intact capillary fragments were identified in the dispersed mucosal cell population (Fig. 3, *left*). The FITC-lectin GS-1 also selectively bound to intact capillary fragments (Fig. 3, *right*). In the SCEF a small number of single cells were positive for both factor VIII-related antigen (Fig. 1 *B*) and GS-1. Fragments of microvessel chains positive for these markers were present occasionally in the large cell elutriator fractions (fraction 9) and in the material remaining in the rotor at the end of the separation ("rest" fraction, Fig. 1 *B*). Intact capillary fragments were enriched by selective filtration as described in Methods.

Sequential density gradient separation of the SCEF: PG production and cell markers. We performed a linear density gradient (Table IV) to further separate cells in the SCEF. In five separate density gradients, basal and bradykinin-stimulated PGE_2 production were maximal in fractions of relatively light density (1.050–1.058 g/ml, Fig. 4 *A*). The density of cells producing PGE_2 production fell between those found for cells containing SLI and 5-HT-LI (n = 3, Fig. 4 *B*). Mast cells (23) and a small number of endothelial cells were present in the dense portion of the gradient (n = 2, Fig. 4 *C*), clearly distinct from fractions producing PGE_2. However, antibody 7.2 to the Ia antigen was present in the same portion of the density gradient as was maximal PGE_2 production, and correlated with PG production with a correlation coefficient (r) of 0.78 and 0.73 (n = 2 gradients; Fig. 4 *D*).[6] Cells positive for B1F6-16 also correlated with the distribution for bradykinin-enhanced PGE_2 production (Fig. 4 *E*) (n = 2 gradients, r = 0.87 and 0.75). Cells taking up acLDL were also present in the same fractions as bradykinin-stimulated PGE_2 production (Fig. 4 *D*); the correlation coefficients in the two gradients examined were 0.85 and 0.92. Zymosan, a sulfated polysaccharide known to stimulate macrophage function (24), increased PGE_2 production in the same density gradient fractions that responded to bradykinin (n = 2, Fig. 4 *A*). In the two gradients studied, EGF also increased PGE_2 production in the fractions that contained the macrophage markers (data not illustrated).

Phagocytosis of latex particles. Phagocytosis of latex particles was found in cells of light density in the SCEF. These cells were recovered between 100% Lt layer and 40% Hv/60% Lt layer (a density of 1.053 g/ml) by step density gradient. In this cell fraction, 6.2±1.4% (n = 3) of the cells ingested latex particles, compared with < 1% of cells pelleting through the 40% Hv/60% Lt density step.

Iron separation of putative macrophages. Iron particles covalently coupled to goat anti-mouse antibody (BioMag M4400) undergo phagocytosis. The BioMag apparatus separates cells that had taken up or bound these iron particles (attracted cells) from the remaining cells (nonattracted cells). PGE_2 production was considerably greater in the attracted than in the nonattracted cells. The difference between attracted and nonattracted cells was greater when cells were incubated with iron particles at 37°C, compared with 4°C (Fig. 5). This temperature effect suggests that phagocytosis of iron particles accounted for the enhanced PGE_2 production in the attracted cell fraction. As a control for possible nonspecific effects of the iron particle separation on PGE_2 production, SCEF cells were incubated with iron, exposed to a magnetic field, but resuspended without separating the attracted and nonattracted cells. These cells produced PGE_2 at a rate indistinguishable from control SCEF cells (n = 3, data not shown). PGE_2 production from fraction 6 or 7 cells incubated with iron particles was not significantly enhanced in the population attracted or trapped by magnetic field (Fig. 5).

Iron separation was also done on SCEF cells following separation by step density gradient (Fig. 6). PGE_2 production was considerably increased in the iron-attracted cells in the lightest density fraction, whereas iron-attracted cells in the intermediate and dense fractions produced little or no PGE_2.

Glass adherence. Adherence to glass by the cells in the SCEF and by blood monocytes was evaluated. Canine blood monocytes readily adhered to glass and these adherent cells ingested latex particles; β-TPA increased the number of phagocytotic cells (n = 3, data not illustrated). In contrast, only a small number of phagocytotic cells from the fundic SCEF adhered to glass under the same conditions.

Endothelial cells. PGE_2 production was enhanced in cell fraction remaining in the rotor at the end of a separation (the "rest" fraction, Fig. 1 *B*). PGE_2 production in this rest fraction was not related to the presence either of macrophages (none were found) (Table III) or of mucous, chief and parietal cells, which were present in greater enrichment in the medium and

6. The number of individual points examined in performing these correlation coefficients with PG production ranged between 10 and 12. The P values for these correlation coefficients were all < 0.05.

Figure 2. Photomicrographs of macrophage markers. Cells in the SCEF were stained with various macrophage markers (see Table II and Methods). (*A*) Cells accumulating acLDL (×390), viewed with rhodamine excitation. (*B* and *C*) Cells showing immunoreactivity with antibody 7.2 (*B*) and B1F6-16 (*C*) to human Ia antigen. These pictures were from two different preparations (×390). The rimmed pattern (*small white arrow*) and the solid pattern (*large white arrow*) are indicated. (*D*) Cells were incubated with fluorescent latex beads at 37° for 60 min. After separating the cells from free beads, cytospins were examined for phagocytosis (×500).

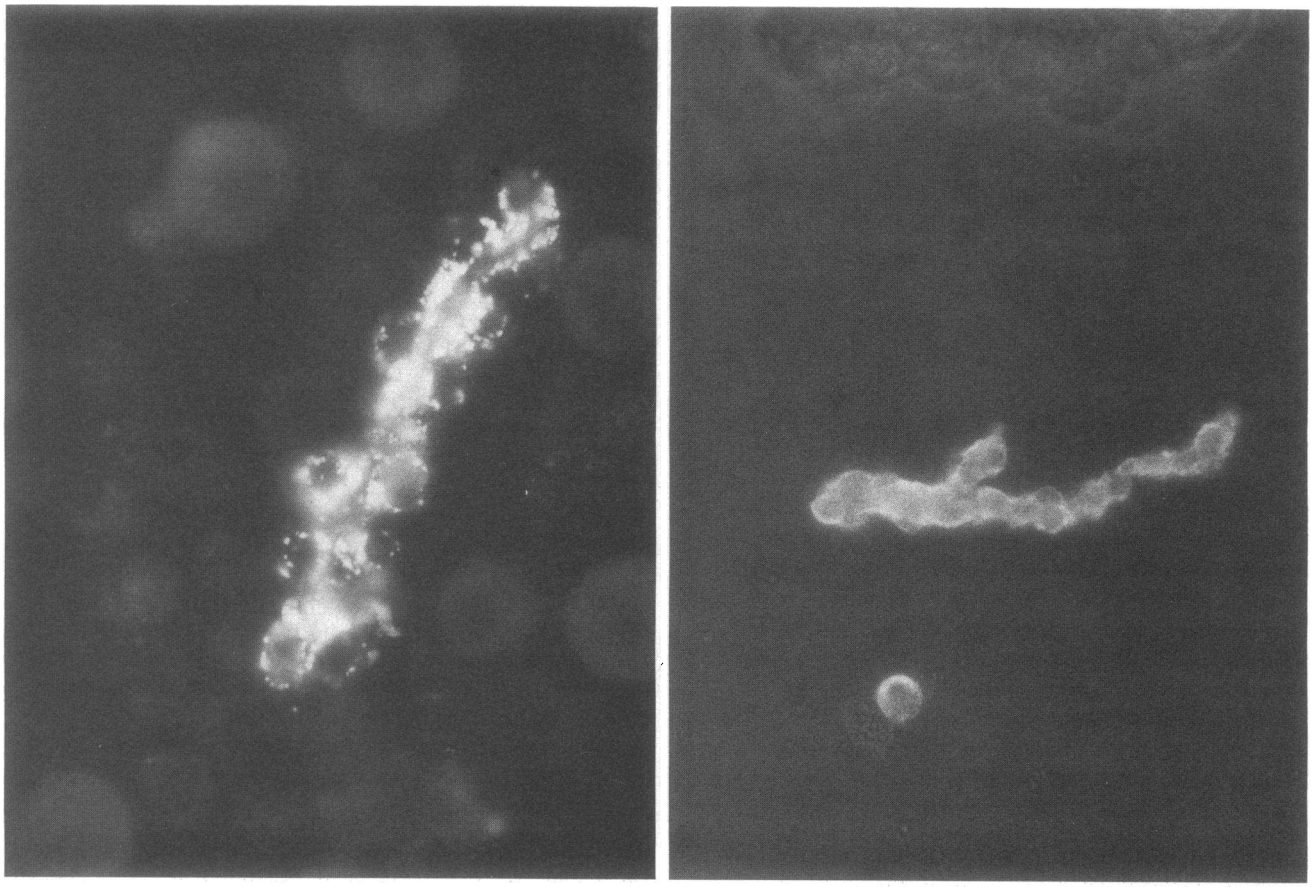

Figure 3. Photomicrographs of endothelial cells. Microvessel fractions were partially enriched by selective filtration and used for marker identification. (*Left*) Cytospins of the microvessels were stained with antibody to factor VIII-related antigen and then with FITC-labeled second antibody. Illustrated is the capillary fraction with positive staining. (×625). (*Right*) Microvessel fractions were incubated with FITC-lectin GS-1. Cytospins were made after washing (×500).

larger cell fractions, respectively. Since intact capillary fragments were present in this rest fraction, we attempted to prepare fractions enriched in these capillary fragments using selective filtration over nylon mesh and elutriation. Microvessel fragments were retained on no. 195 nylon mesh. Although some enrichment was obtained with elutriation, recovery was low and separation from residual gastric gland fragments was poor. The fraction retained on the no. 195 filter was enriched to about 35±9% endothelial cells (n = 7) and provided an opportunity to evaluate the role of endothelial cells in PG production. This microvessel-enriched fraction did not contain cells with macrophage markers; specifically, cells did not react with antibody B1F6-16 (Table III, n = 4). Gastric gland fragments were the predominant contaminating cell type, and from our elutriator studies, the parietal, chief, and mucous cells that comprise these glands are not likely to be major PGE_2 producers (Fig. 1 *A*).

Comparison between PGE_2 production by the SCEF, chief cells, and microvessels. We have compared PGE_2 production by the SCEF, by an elutriator fraction enriched in chief cells, but containing mucous cells and parietal cells (fractions 5–7), and by the preparation enriched by filtration in intact capillary fragments. We have expressed PGE_2 production data as picograms per microgram DNA (Fig. 7). The SCEF had a basal PGE_2 production rate that was four times that of fractions 5–7 and twice that of the microvessel fraction. Several agents differentially stimulated PGE_2 production by these three populations. Bradykinin and the calcium ionophore A23187 stimulated PGE_2 production in each fraction. In contrast, zymosan had a differential effect stimulating PGE_2 by macrophage-containing SCEF, but having no effect on endothelial cells, and only a minimal effect in fractions 5–7. Occasionally, a small number of cells with macrophage markers may be present in fraction 5, possibly accounting for the response to zymosan. Supporting this interpretation, in four recent experiments using only fractions 6 and 7, zymosan failed to stimulate PGE_2 production ($P > 0.2$). Carbachol stimulated PGE_2 production in both fractions 5–7 and in the endothelial cell fraction, but not in the SCEF. EGF stimulated PGE_2 production in the SCEF and the endothelial cell fractions, but not in the chief cell-enriched fraction (Fig. 7).

Fibroblasts. Since fibroblasts produce PGs (25), we sought to determine if fibroblasts were present in our elutriator fractions. For a positive control, we dispersed cells from the fundic submucosa; fibroblastic cells were easily cultured from elutriator fractions of these submucosal cells. During a 10-d culture period, T flasks that were sparsely seeded with submucosal cells became nearly confluent with fibroblastic cells. In contrast to the ease with which fibroblasts were obtained from these submucosal preparations, the same methods did not yield fibroblastic cultures from any of the elutriator-separated fundic mucosal fractions (2–4, 5–7, or 8–9; n = 4).

Table IV. Specific Densities for Linear Density Gradient

Fraction no.	Density experiment 1 (Fig. 4 *B*)‡	Density experiment 2§ (Fig. 4 *E*)‡
	g/ml	
Cushion*	1.088	1.08
1	1.085	1.082
5	1.087	1.081
9	1.078	1.079
13	1.078	1.071
17	1.075	1.066
21	1.068	1.057
25	1.067	1.051
29	1.065	1.050
33	1.054	1.040
37	1.050	1.030
41	1.041	1.030
45	1.031	1.030

* Cushion was taken from the bottom of gradient, i.e., the very first fraction recovered after gradient centrifugation.
‡ Experiment 1 is shown in Fig. 4 *B*. Experiment 2 is shown in Fig. 4 *E*.
§ 100 ml excess of light BSA solution was used in experiment 2 to form linear gradient to intentionally move the PG production peak to the center portion of the gradient.

Discussion

PGE_2 production was rapid by dispersed canine fundic mucosal cells. Cell separation using the elutriator rotor indicated that the small cell fraction produced more PGE_2 on a per cell basis than did the medium- or large-cell fractions. Since the latter fractions contained the mucous, chief, and parietal cells, it appears that nonepithelial cells are major contributors to PG production under these conditions. Enhanced PG production by the SCEF was found for basal PG production and PG production stimulated by the calcium ionophore A23187, bradykinin, and epidermal growth factor. Since the calcium ionophore activates PG production by postreceptor mechanisms (26), the distribution pattern we observed reflects not only the distribution of receptors, but also the capacity to produce PGs. The PGE_2 produced by this fraction reached 10 ng/10^6 cells per h and at this rate PGE_2 concentrations in the medium would exceed 0.1 μM after a 1-h incubation, a concentration sufficient to modulate cell responses, such as inhibition of parietal cell function (27).

The SCEF contained a variety of cell types and we utilized sequential density gradient to further discriminate cells. In these linear-density gradients, basal and bradykinin-stimulated PGE_2 production occurred in a unimodal peak in fractions of relatively light density. No correlation was found between PGE_2 production and markers for mast cells, endocrine and endocrine-like cells, or lymphocytes. However, close correlation was found with several macrophage markers: antibodies 7.2 and B1F6-16 to the Ia antigen and acLDL uptake. Furthermore, zymosan, a sulfated polysaccharide that stimulates phagocytosis, also stimulated PGE_2 production in the same fractions, supporting the conclusion that macrophages accounted for this distribution of PGE_2 production.

The presence of macrophages in the SCEF was confirmed by several approaches. Cells ingested latex particles and accumulated acLDL and these cells were immunoreactive with the antibodies to the human Ia antigen. Cells in the SCEF exposed to iron particles and separated by magnetic field displayed markedly enhanced PG production; macrophages are well known to be separated by binding and/or phagocytosis of iron particles (16). Furthermore, we separated the SCEF by step density gradient and found that iron separation enhanced PGE_2 production only in the fraction of light density, the fraction enriched in macrophage markers. Furthermore, the chief cell-enriched fractions, which lacked macrophage markers, did not display any increase in PG production with iron separation. Taken together, these data indicate that macrophages account for the major proportion of PGE_2 production in the small cell fraction. Macrophages and monocytes are known to produce PGE_2 (28–31).

Identification of macrophages is complicated by species differences and macrophage heterogeneity. Species differences limit the utility of the immunological markers developed in rat, mouse or human for studies with canine macrophages. Most macrophage markers have been characterized on adherent blood monocytes or peritoneal or alveolar macrophages, and the characteristics of these cells may not be typical of macrophages in other tissues or microenvironments. The Ia antibodies that we utilized potentially interact both with macrophages and B lymphocytes (8, 9). Our studies with a B lymphocyte specific marker make it unlikely that these lymphocytes accounted for the distribution of Ia marker we observed. Adherence to glass is frequently a useful property of macrophages that allows separation and identification. Although we found that canine monocytes readily adhered to glass, adherence was not useful in our studies with fundic macrophages. Whether this loss of adherence represents a characteristic of fundic macrophages or a consequence of the methodology we used remains unclear. Macrophages isolated from human intestine by Ficoll-Hypaque density gradient failed to adhere to glass or plastic (32).

Macrophages may display heterogeneity, with discrete subpopulations responsible for functions such as phagocytosis, cytotoxicity, or PG production (30, 33, 34). Exposure to specific signals or tissue environment may induce macrophages to differentially express a set of functions (35, 36). Distinct heterogeneous subsets were clearly evident when peritoneal macrophages induced by *Staphylococcus enteritidis* were separated by sedimentation velocity; larger (high sedimentation velocity) macrophages were found to produce PGs, while smaller macrophages displayed enhanced cytolytic activity (33). Since iron exposure and magnetic adherence enriches PG production in our studies, we conclude that phagocytic fundic macrophages produce PGE_2. Whether the residual PGE_2 production in the nonadherent population represents nonphagocytic macrophages or other cell populations remains uncertain.

Since macrophages possess the capacity to secrete factors amplifying tissue injury (e.g., lysosomal enzymes and superoxide) or factors potentially beneficial to mucosal defense (e.g., PGs) or mucosal repair and healing (fibroblast-stimulating factor), their number, state of activation, and expression of heterogenous functions may influence gastric mucosal sensitivity to injury and healing. Our data suggest that macrophages are an important cell to consider in assessing mucosal PG production. It is possible that the variable number and state of

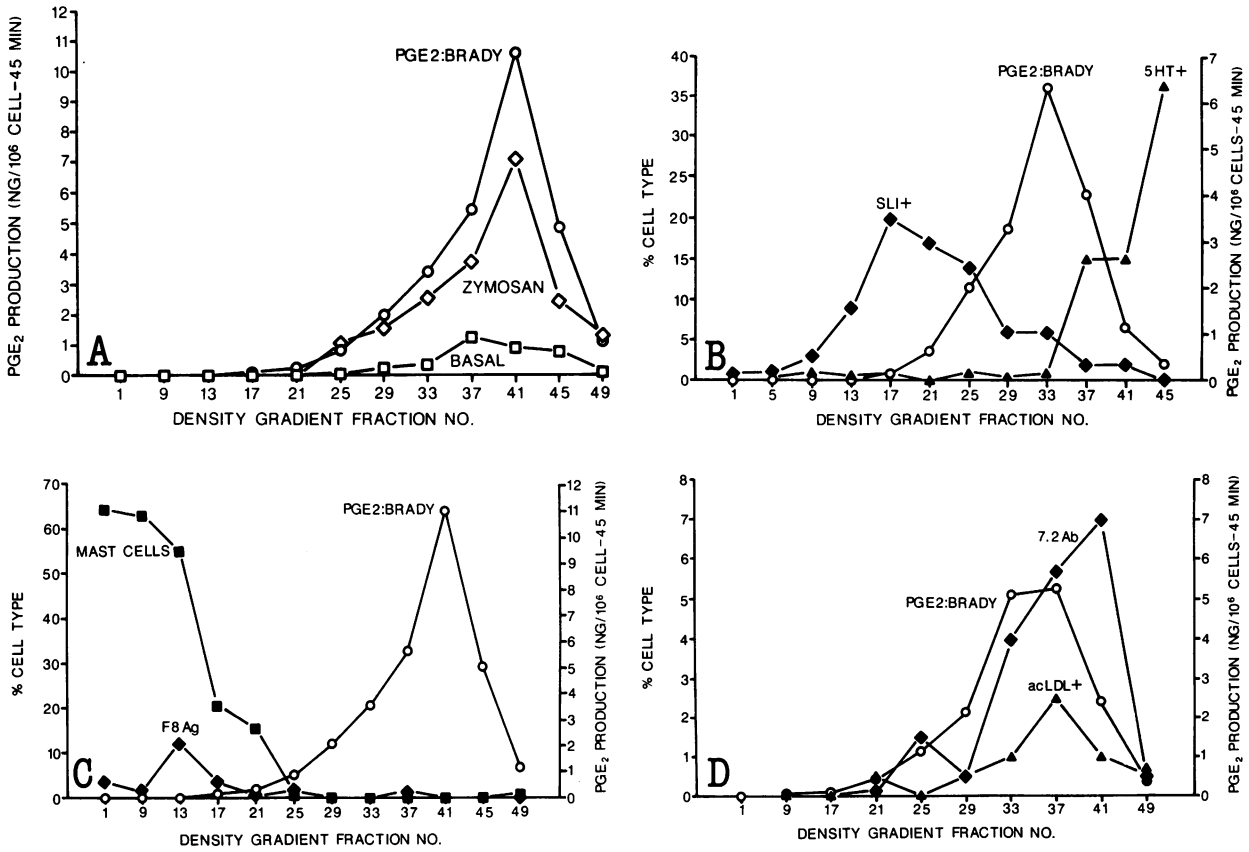

Figure 4.

activation of macrophages underlie some of the variability of mucosal PG production among individuals.

Our present findings may have relevance to the capacity of the mucosa to enhance its resistance to injury following an initial minor insult, a phenomenon termed "adaptive cytoprotection" (37). As noted above, macrophages respond to inflammatory stimuli with enhanced release of prostaglandins (30, 31). The increase in resistance to subsequent injury following treatment with mild injurious agents requires endogenous PG production (1, 37). It is possible that macrophages are the cell responding to signals produced by mild tissue injury with an increased rate of PG production and, therefore, may participate in this adaptive response.

Endothelial cells also produce PGs (38); although prostacyclin (PGI_2) is the major product of aortic endothelial cells, the type of PG and capacity for prostanoid production varies between major vessel and capillary endothelium (38, 39). The relative quantitative importance of PG production by capillary endothelial cells in tissues remains uncertain. Utilizing antibodies to factor VIII-related antigen (17, 18, 22) and a selective interaction with the plant lectin GS-1, we found that our dispersed canine fundic mucosal population contained both intact capillary fragments and a few single endothelial cells. We enriched intact capillary fragments by selective filtration. This endothelial cell enriched fraction contained gastric gland fragments as the main contaminants and was devoid of macrophage markers. Significant PG production was found, with the pattern of stimulation different from that found in the small cell fraction, in that carbachol, bradykinin, and EGF, but not zymosan, stimulated PG production.

Fibroblasts are another well recognized source of prostanoid production (25). We cultured fibroblasts from dispersed fundic submucosal cells, but not from any of the fundic elutriator fractions using similar conditions. These findings suggest that fibroblasts were, at most, present in small numbers in the dispersed canine fundic cell preparation and, therefore, were not major contributors to PG production in our small cell elutriator fraction.

The contribution of specific cell types in complex tissues to the overall rates of PG production remains uncertain. However, several lines of evidence obtained in studies with uterus, intestine, and kidney indicate that stromal or lamina propria elements, rather than epithelial cells, may be major PG producing cells (40–42) and that inflammatory cells specifically may be important contributors to tissue PG production. After the induction of hydronephrosis, renal PG production is markedly increased. This increase has been correlated with the migration of macrophages into the hydronephrotic kidneys; furthermore, the macrophage appear to induce fibroblast PG production (43, 44). Additional evidence that inflammatory cells may be important PG producers comes from studies in heart tissue after ischemic injury (45). Among inflammatory cells, macrophages are very important PG producers. Polymorphonuclear cells produce leukotrienes, but appear to be only a minor source of prostanoid production (46). Lymphocytes probably do not produce prostanoids in significant quantities (47). We found a small, but variable, number of lymphocytes in our preparation, and lymphocyte markers did not correlate with the distribution of PGE_2 production.

Previous studies have indicated that gastric mucosa pro-

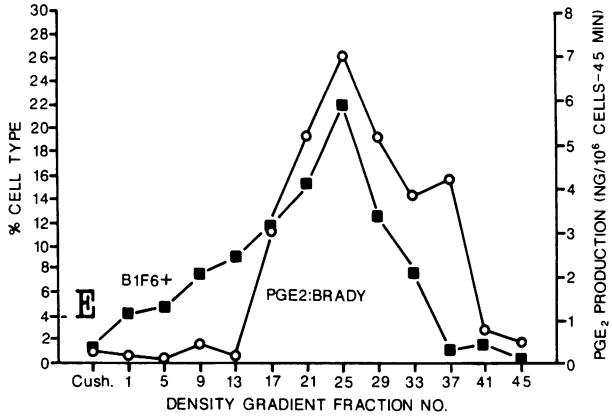

Figure 4. PGE_2 production by the SCEF subsequently separated by linear density gradient. The rotor was loaded dynamically with ~ 3.5×10^8 cells from the SCEF and then centrifuged at 6,000 rpm for 30 min (see Methods). Dense fractions are on the left, and light fractions on the right. A total of five gradients were performed, with the distribution of bradykinin-stimulated PGE_2 production determined in each one. The other markers were also studied. The slight change in position of the bradykinin peak reflects small changes in the procedure of establishing the gradient between preparations (as shown in Table IV). Owing to the limit of cell number present in fractions enriched with macrophages, only two or three markers can be studied with PGE_2 production in each gradient preparation. Lines with open symbols represent PGE_2 production, and lines with filled symbols represent cell markers. (*A*) PGE_2 release was expressed as nanograms of $PGE_2/10^6$ cells per 45 min. Cells were treated with 1 μM bradykinin (○), 500 μg/ml Zymosan (◇), or Hanks' balanced solution (□). (*B*) Cell containing somatostatin-like (*SLI*, ♦) and serotonin-like immunoreactivity (*5HT*, ▲) were identified by immunohistochemistry and the data expressed as percentage of total cells present in the fraction. (*C*) Mast cells were identified with toluidine blue staining (■) and the capillary endothelial cells were identified by immunofluorescence using antibodies to factor VIII-related antigen (♦). (*D*) Illustrated is the distribution of cells showing immunofluorescence with antibody 7.2 (♦) and cells accumulating acLDL (▲). (*E*) Macrophages were identified by the immunoreactivity against the Ia antigen B1F6-16 (■). *A* and *C* are from the same gradient and *B*, *D*, and *E* are from three separate preparations.

duces considerable quantities of PGs (48–50). The different patterns of PG production found in these studies possibly reflect the different methodology and species employed. A few studies have been performed with dispersed or cultured mucosal cells. Both parietal cell enriched and depleted fractions generated prostaglandins (50); however, these studies did not identify other cell types, such as macrophages and endothelial cells. Contamination of the parietal cell fractions by these latter cell types requires consideration before these findings can be interpreted as reflecting parietal cell production. Cultured neonatal rabbit gastric cells were also found to produce PGs, but the rates of production were low and required addition of arachidonic acid to allow detection (51).

It is likely that all cells produce some prostanoids, but our present studies indicate that certain cell types may be major producers of prostanoids in the fundic mucosa. Our data do not exclude PG production by epithelial cells, but rather suggest that cells may vary considerably in their relative contribution to tissue PG production and that cells present in the lamina propria are important contributors to PG production. Our data indicate a correlation between stimulatable PGE_2 and macrophage numbers present in SCEF. However, our data do not exclude the possibility that stimulatable PGE_2 production may be the result of cell–cell interaction from macrophages and other epithelial cells.

We studied bradykinin stimulation of PG production in our isolated cell system because bradykinin is a well-described inducer of PG synthesis by fibroblasts (52), endothelial cells (53, 54), and ileal lamina propria (55, 56). We found that the distribution of bradykinin-stimulated PGE_2 production both in density gradient and iron-separated fractions of SCEF corresponded to zymosan-stimulated PGE_2 production and with macrophage markers. Canine fundic macrophages may thus account for a component of the bradykinin-stimulated PGE_2 production in the small cell fraction. Bradykinin has not been reported as a stimulant of macrophage PG production (25, 57), although bradykinin does induce spreading of macrophages (58). It is possible that bradykinin only stimulates PG production in tissue macrophages under selected conditions. Bradykinin also stimulated PGE_2 production in the endothelial cell-enriched fractions, but had no significant effect in the chief cell-enriched fraction.

EGF inhibits acid secretion and enhances gastric mucosal resistance to injury. EGF has also been reported to increase PG production by Madin-Darby canine kidney (MDCK) cells and by cultured mouse calvaria (59, 60), however, direct effects of EGF on PG production in fundic mucosal cells have not been elucidated. In our studies, EGF increased PG production in the SCEF, presumably by a direct effect on macrophages, but data are insufficient to firmly establish this point. EGF also increased PG production by the endothelial cell enriched fraction, but not by the chief cell-enriched fraction. The relative importance of these EGF effects on PG production to the antisecretory and cytoprotective actions of EGF remains unclear. EGF inhibition of isolated canine parietal cell function is not blocked by indomethacin treatment, suggesting that PG formation does not play a critical role at least in its antisecretory actions (M. C.-Y. Chen and A. H. Soll, unpublished observation). This result agrees with other studies by Konturek et al. (61).

The cholinergic agonist carbachol stimulated PG production in the parietal and chief cell-enriched fraction, consistent with previous observations by others (62, 63). Carbachol also increased PG production by the endothelial cell fraction, while stimulatory effects were not of statistical significance in the macrophage-containing SCEF. The differences were observed in the effects of these various stimuli on PG production among cell types probably reflects differences in distribution of receptors among these cell types. In contrast, the calcium ionophore A23187 stimulated PG production in all of the cell fractions, although differences in the magnitude of response were observed.

We conclude that macrophages and endothelial cells are important prostanoid producers in the canine fundic mucosa. The factors that alter the number of these cells in the mucosa and that influence their rate of PG production may have a significant bearing upon mucosal resistance to injury. It is also possible that macrophages in gastric tissues under some circumstances exert deleterious effects by releasing mediators of injury, such as lysosomal enzymes and oxygen free radicals.

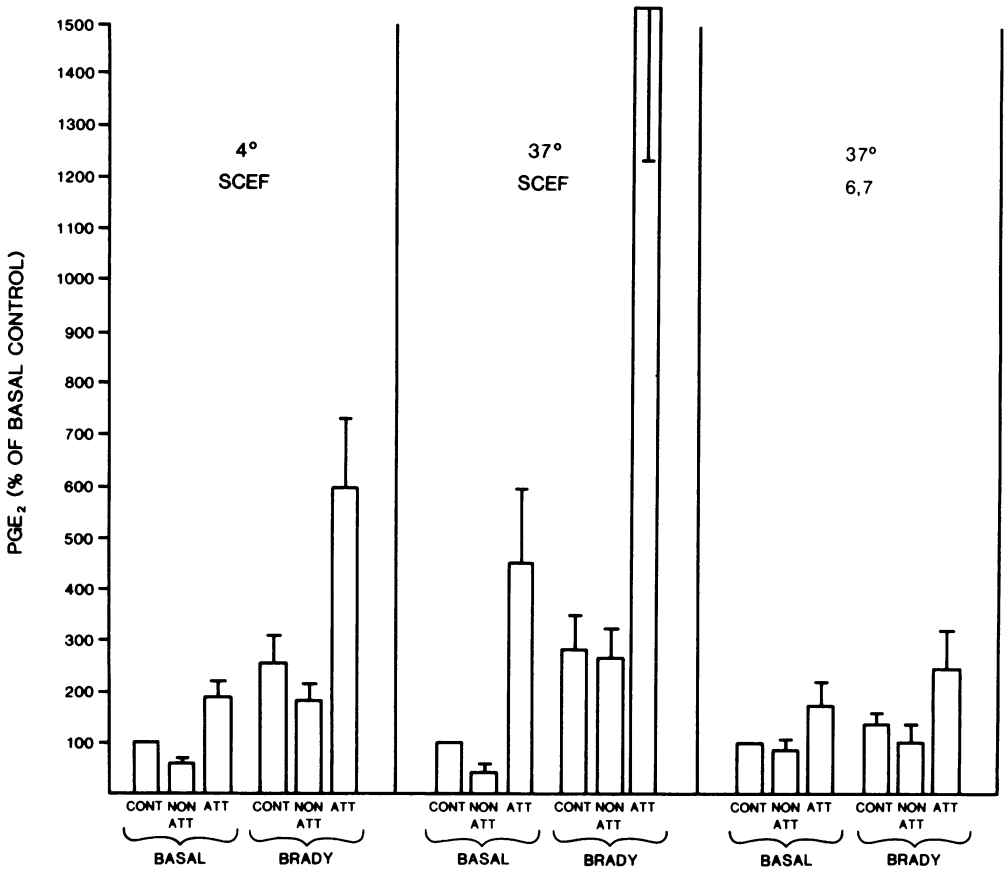

Figure 5. PGE_2 production by the SCEF after iron separation. Fundic mucosal cell fractions 2–4 (*SCEF*) and 6 and 7 were collected from elutriator run and incubated with antibody-coated iron particles for 30 min. Cells were then separated by magnetic field. Both attracted and nonattracted cells were examined for PGE_2 release basally and following treatment with 1 μM bradykinin. Control cells have not been treated with iron. Data represent the mean±SE from $n = 6$ (*SCEF*) and $n = 4$ (*6, 7*). Data are expressed in percentage of basal control calculated from values of nanograms of $PGE_2/10^6$ cells per 45 min.

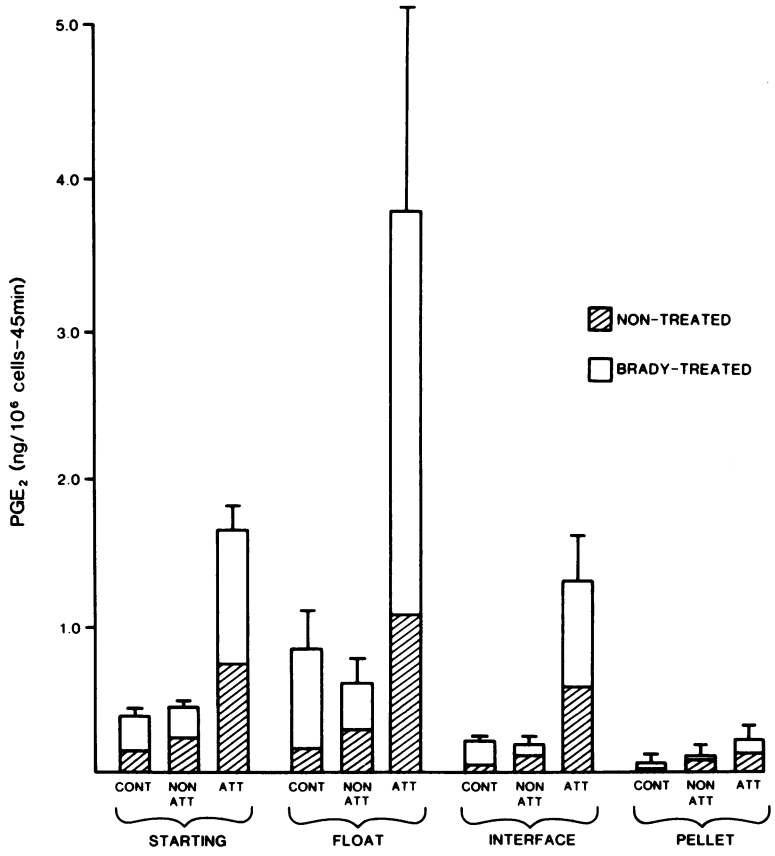

Figure 6. Iron separation of canine fundic SCEF after separation by step density gradient. The SCEF was separated by a two-step density gradient (50% Hv/50% Lt and 70% Hv/30% Lt, 60×10^6 cells per gradient tube) as described in Methods. The two interface fractions and pellet from the gradient and the control SCEF were incubated with antibody-coated iron particles at 37°C for 30 min, and then separated by magnetic field. Control cells were not exposed to iron. Control, attracted, and nonattracted cells were studied for PGE_2 release during a 45 min incubation in the presence (*open bars*) or absence (*hatched bars*) of 1 μM bradykinin. Data are the mean±SE from four cell preparations and are expressed in nanograms/10^6 cells per 45 min.

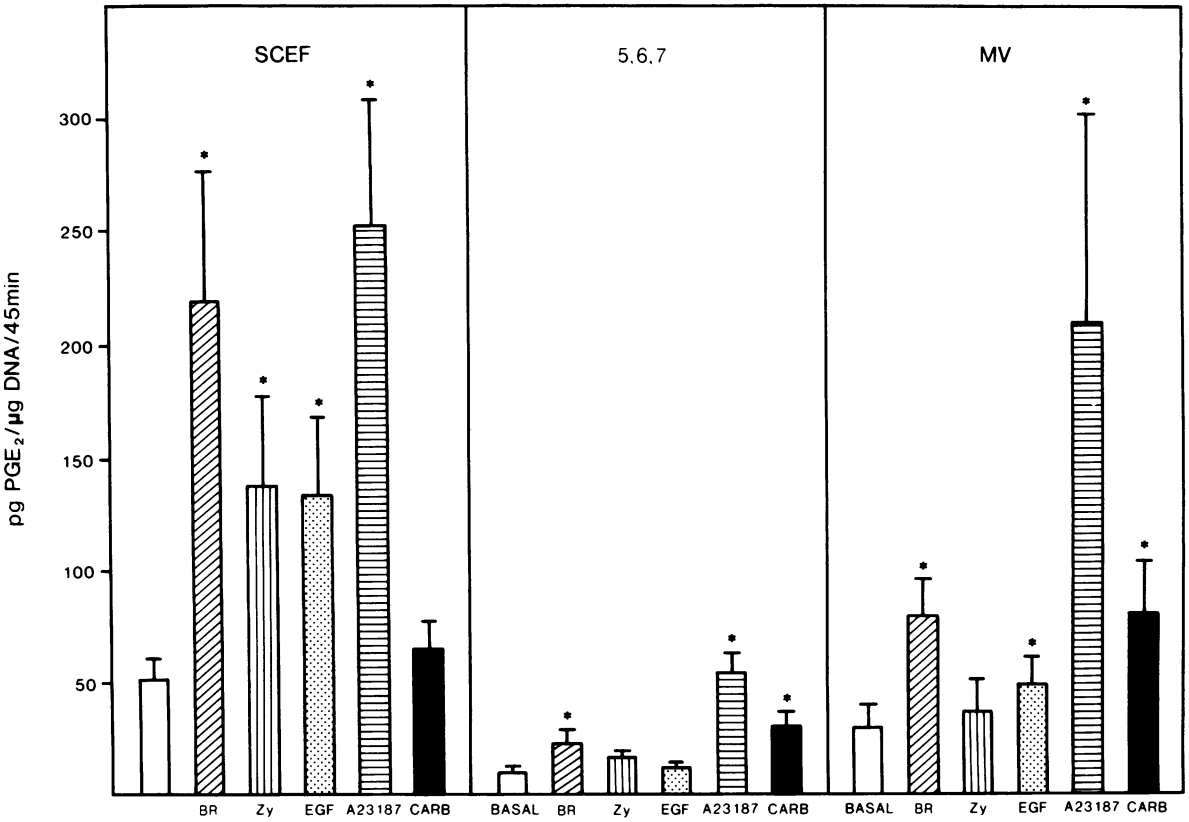

Figure 7. Comparison of PGE_2 production by the SCEF, elutriator-enriched chief cell fraction and microvessel-enriched fraction. The SCEF (elutriator fractions 2–4) and the chief cell enriched elutriator fraction (*5, 6, 7*) were compared to the crude microvessel fraction collected by filtration. In three of these seven preparations, fractions 6 and 7 were collected instead of fractions 5, 6, and 7. All cells were preincubated for 30 min and then incubated for 45 min untreated or after treatment with stimulant (*BR* [Bradykinin, 1 µM], *Zy* [zymosan, 500 µg/ml], *A23187* [ionophore A23187, 1 µM], *EGF* [0.1 µM] or *CARB* [carbachol, 100 µM]). Data represent the mean±SE from seven preparations and are expressed as picograms of PGE_2 per microgram of DNA · 45 min. Because of the wide variation between preparations, paired *t* tests were done on log-transformed data. $*P < 0.05$.

These cells may also play a key role in repair and healing, as they produce factors that induce fibroblast proliferation and collagen formation.

Acknowledgments

The authors thank Mary Toomey for technical assistance, and Therese Sonnenfeld and Rebecca Fox for help in preparation of the manuscript.

This work was supported by grants from the NIDDK DK 30444 and DK 17328 and by the Medical Research Service of the Veterans Administration. Dr. Soll is a recipient of a Medical Investigatorship, VA Wadsworth Hospital Center.

References

1. Wallace, J. L., and M. M. Cohen. 1984. Gastric mucosal protection with chronic mild restraint: role of endogenous prostaglandins. *Am. J. Physiol.* 247(Gastrointest. Liver Physiol. 10):G127–G132.

2. Soll, A. H., J. Kurata, and J. E. McGuigan. 1989. Ulcers, nonsteroidal antiflammatory drugs and related matters. *Gastroenterology.* 96:561–568.

3. Soll, A. H. 1978. The actions of secretatagogues on oxygen uptake by isolated mammalian parietal cells. *J. Clin. Invest.* 61:370–380.

4. Ayalon, A., M. J. Sanders, L. P. Thomas, D. A. Amirian, and A. H. Soll. 1982. Electrical effects of histamine on monolayers formed in culture from enriched canine gastric chief cells. *Proc. Natl. Acad. Sci. USA.* 79:7009–7013.

5. Soll, A. H., K. Lewin, and M. A. Beaven. 1979. Isolation of histamine containing cells from canine fundic mucosa. *Gastroenterology.* 77:1283–1290.

6. Soll, A. H., D. A. Amirian, L. P. Thomas, T. J. Reedy, and J. D. Elashoff. 1984. Gastrin receptors on isolated canine parietal cells. *J. Clin. Invest.* 73:1434–1447.

7. Levine, R. A., K. R. Kohen, E. H. Schwartzel, Jr., and C. E. Ramsay. 1982. Prostaglandin E_2-histamine interactions on cAMP, cGMP, and acid production in isolated fundic glands. *Am. J. Physiol.* 242(Gastrointest. Liver Physiol. 5):G21–G26.

8. Alejandro, R., F. L. Shienvold, L. Zuhair, V. Esquenazi, J. Miller, and D. H. Mintz. 1984. Monoclonal antibodies recognizing canine and human Ia-Like antigens. *Transplantation (Baltimore).* 38:542–544.

9. Deeg, H. J., J. C. Wulff, S. DeRose, G. E. Sale, M. Braun, M. A. Brown, S. C. Springmeyer, P. J. Martin, and R. Storb. 1982. Unusual distribution of Ia-like antigens on canine lymphocytes. *Immunogenetics.* 16:445–457.

10. Aprile, J. A., H. J. Deeg, and R. F. Raff. 1984. Purified canine monocytes fail to confer concanavalin-A responsiveness to accessory-cell-depleted lymphocytes. *Exp. Hematol.* 12:45–51.

11. Hass, A. J., H. R. Davis, V. M. Elner, and S. Glagov. 1983. Identification of macrophages in sections of rabbit lung using acetoacetylated lipoproteins. *J. Histochem. Cytochem.* 31:1136–1138.

12. Pitas, R. E., T. L. Innerarity, J. N. Weinstein, and R. W. Mahley. 1981. Acetoacetylated lipoproteins used to distinguish fibro-

blasts from macrophages in vitro by fluorescence microscopy. *Arteriosclerosis.* 1:177–185.

13. Swanson, J. A., B. D. Yirinec, and S. C. Silverstein. 1985. Phorbol esters and horseradish peroxidase stimulate pinocytosis and redirect the flow of pinocytosed fluid in macrophages. *J. Cell Biol.* 100:851–859.

14. Shaw, S. E., and N. V. Anderson. 1984. Isolation and functional analysis of normal canine blood monocytes and resident alveolar macrophages. *Am. J. Vet. Res.* 45:87–90.

15. English, D., and B. R. Andersen. 1974. Single-step separation of red blood cells, granulocytes, and mononuclear leukocytes on discontinuous density gradients of Ficoll-Hypague. *J. Immunol. Methods.* 5:249–252.

16. Lafranconi, W. M., R. D. Spall, G. Sipes, R. C. Duhamel, E. Meezan, and K. Brendel. 1983. Rapid isolation of type II pneumocytes with magnetic removal of macrophages. *Exp. Lung Res.* 4:191–204.

17. Dilley, R., M. Herring, L. Boxer, A. Gardner, and J. Glover. 1979. Immunofluorescent staining for factor VIII-related antigen: a tool for study of healing in vascular prostheses. *J. Surg. Res.* 27:149–155.

18. Irving, M. G., F. J. Roll, S. Huang, and B. D. Montgomery. 1984. Characterization and culture of sinusoidal endothelium from normal rat liver: lipoprotein uptake and collagen phenotype. *Gastroenterology.* 87:1233–47.

19. Eanes, R. Z. 1985. Effects of fructose on human fibroblast metabolism: the application of DNA measurements as a basis for interpretation. *In Vitro Cell. Dev. Biol.* 21:328–332.

20. Soll, A. H., T. Yamada, J. Park, and L. P. Thomas. 1984. Release of somatostatin-like immunoreactivity from canine fundic mucosal cells in primary culture. *Am. J. Physiol.* 247(Gastrointest. Liver Physiol. 10):G567–G573.

21. Beaven, M. A., A. H. Soll, and K. J. Lewin. 1982. Histamine synthesis by intact mast cells from canine fundic mucosa and liver. *Gastroenterology.* 82:254–62.

22. Jaffe, E. A., R. L. Nachman, C. G. Becker, and R. E. Minick. 1973. Culture of human endothelial cells derived from umbilical veins: identification by morphologic and immunologic criteria. *J. Clin. Invest.* 52:2745–2756.

23. Soll, A. H., and A. Wollin. 1979. Histamine and cyclic AMP in isolated canine parietal cells. *Am. J. Physiol.* 237:E444–E450.

24. Hsueh, W., C. Kuhn, and P. Needleman. 1979. Relationship of prostaglandin secretion by rabbit alveolar macrophages to phagocytosis and lysosomal enzyme release. *Biochem. J.* 184:345–354.

25. Goldstein, R. H., K. Miller, J. Glassroth, R. Linscott, G. L. Snider, C. Franzblau, and P. Polgar. 1982. Influence of asbestos fibers on collagen and prostaglandin production in fibroblast and macrophage co-cultures. *J. Lab. Clin. Med.* 100:778–785.

26. Hassid, A. 1982. Regulation of prostaglandin biosynthesis in cultured cells. *Am. J. Physiol.* 243:205–211.

27. Chen, M. C. Y., A. H. Soll, D. A. Amirian, M. Toomey, and M. J. Sanders. 1988. Prostanoid inhibition of canine parietal cells: mediation by the inhibitory GTP-binding protein of adenylate cyclase. *Gastroenterology.* 94:1121–1129.

28. Rouzer, C. A., W. A. Scott, J. Kempe, and Z. A. Cohn. 1980. Prostaglandin synthesis by macrophages requires a specific receptor-ligand interaction. *Proc. Natl. Acad. Sci. USA.* 77:4279–4282.

29. Stenson, W. F., and C. W. Parker. 1980. Prostaglandins, macrophages, and immunity. *J. Immunol.* 125:1–5.

30. Takemura, R., and Z. Werb. 1984. Secretory products of macrophages and their physiological functions. *Am. J. Physiol.* 246(Cell Physiol. 15):C1–C9.

31. Humes, J. L., R. J. Bonney, L. Pelus, M. E. Dahlgren, S. J. Sadowski, F. A. Kuehl, Jr., and P. Davies. 1977. Macrophages synthesis and release prostaglandins in response to inflammatory stimuli. *Nature (Lond.).* 269:149–161.

32. Golder, J. P., and W. F. Doe. 1983. Isolation and preliminary characterization of human intestinal macrophages. *Gastroenterology.* 84:795–802.

33. Hopper, K. E., and J. M. Cahill. 1983. Immunoregulation by macrophages. II. Separation of mouse peritoneal macrophages having humoricidal and bactericidal activities and those secreting PGE and interleukin I. *J. Reticuloendothel. Soc.* 33:443–456.

34. Beelen, R. H. J., and W. S. Walker. 1983. Dynamics of cytochemically distinct subpopulations of macrophages in elicited rat peritoneal exudates. *Cell. Immunol.* 82:246–257.

35. Scott, W. A., N. A. Pawlowski, H. W. Murray, M. Andreach, J. Zrike, and Z. A. Cohn. 1982. Regulation of arachidonic acid metabolism by macrophage activation. *J. Exp. Med.* 155:1148–1160.

36. Adams, D. O., and T. A. Hamilton. 1984. The cell biology of macrophage activation. *Annu. Rev. Immunol.* 2:283–318.

37. Robert, A., J. E. Nezamis, C. Lancaster, J. P. Davis, S. O. Field, and A. J. Hanchar. 1983. Mild irritants prevent gastric necrosis through "adaptive cytoprotection" mediated by prostaglandins. *Am. J. Physiol.* 245(Gastrointest. Liver Physiol. 8):G113–G121.

38. Brotherton, A. F., and J. C. Hoak. 1982. Role of Ca^{2+} and cyclic AMP in the regulation of the production of prostacyclin by the vascular endothelium. *Proc. Natl. Acad. Sci. USA.* 79:495–499.

39. Gerritsen, M. E., and C. D. Cheli. 1973. Arachidonic acid and prostaglandin endoperoxide metabolism in isolated rabbit and coronary microvessels and isolated and cultivated coronary microvessel endothelial cells. *J. Clin. Invest.* 72:1658–1671.

40. Smith, G. S., G. Warhurst, and L. A. Turnberg. 1982. Synthesis and degradation of prostaglandin E_2 in the epithelial and sub-epithelial layers of the rat intestine. *Biochim. Biophys. Acta.* 713:684–687.

41. Gal, D., M. L. Casey, J. M. Johnston, and P. C. MacDonald. 1982. Mesenchyme-epithelial interactions in human endometrium: prostaglandin synthesis in separated cell types. *J. Clin. Invest.* 70:798–805.

42. Davis, B. B., D. Thomasson, and T. V. Zenser. 1983. Renal disease profoundly alters cortical interstitial cell function. *Kidney Intl.* 23:458–464.

43. Jonas, P. E., K. DeSchryver, T. Okegawa, K. Leahy, and P. Needleman. 1983. Presence of inflammatory cells associated with exaggerated arachidonic acid metabolism in renal injury. *Adv. Prostaglandin Thromboxane Leukotriene Res.* 12:65–73.

44. Jonas, P. E., K. M. Leahy, K. DeSchryver-Kecskemeti, and P. Needleman. 1984. Cellular interactions and exaggerated arachidonic acid metabolism in rabbit renal injury. *J. Leukocyte Biol.* 35:55–64.

45. Goldyne, M. E., G. F. Burrish, P. Poubelle, and P. Borgeat. 1984. Arachidonic acid metabolism among human mononuclear leukocytes: lipoxygenase-related pathways. *J. Biol. Chem.* 259:8815–8819.

46. Evers, A. S., S. Murphree, J. E. Saffitz, B. A. Jakschik, and P. Needleman. 1985. Effects of endogenously produced leukotrienes, thromboxane, and prostaglandins on coronary vascular resistance in rabbit myocardial infarction. *J. Clin. Invest.* 75:992–999.

47. Bankhurst, A. D., E. Hastain, J. S. Goodwin, and G. T. Peake. 1981. The nature of the prostaglandin-producing mononuclear cell in human peripheral blood. *J. Lab. Clin. Med.* 97:179–186.

48. Whittle, B. J. R., and J. A. Salmon. 1983. Biosynthesis of prostacyclin and prostaglandin E_2 in gastrointestinal tissue. *In* Intestinal Secretion: Proceedings of the third BSG-SK&F International Workshop. L. A. Turnberg, editor. Knapp Drewett & Sons Ltd., Welwyn Garden City, Great Britain. 69–73.

49. LeDuc, L. E., and P. Needleman. 1979. Regional localization of prostacyclin and thromboxane synthesis in dog stomach and intestinal tract. *J. Pharmacol. Exp. Ther.* 211:181–188.

50. Skoglund, M. L., J. G. Gerber, R. C. Murphy, and A. S. Nies. 1980. Prostaglandin production by intact isolated gastric parietal cells. *Eur. J. Pharmacol.* 66:145–148.

51. Matuoka, K., M. Tanaka, Y. Mitsui, and S.-I. Murota. 1983. Cultured rabbit gastric epithelial cells producing prostaglandin I_2. *Gastroenterology.* 84:498–505.

52. Murayama, T., and M. Ui. 1985. Receptor-mediated inhibition of adenylate cyclase and stimulation of arachidonic acid release in 3T3 fibroblasts. *J. Biol. Chem.* 260:7226–7233.

53. Clark, M. A., D. Littlejohn, T. M. Conway, S. Mong, S. Steiner, and S. T. Crooke. 1986. Leukotriene D_4 treatment of bovine aortic endothelial cells and murine smooth muscle cells in culture results in an increase in phospholipase A_2 activity. *J. Biol. Chem.* 261:10713–10718.

54. Clark, M. A., T. M. Conway, C. F. Bennett, S. T. Crooke, and J. M. Stadel. 1986. Islet-activating protein inhibits leukotriene D4- and leukotriene C4- but not bradykinin- or calcium ionophore-induced prostacyclin synthesis in bovine endothelial cells. *Proc. Natl. Acad. Sci. USA.* 83:7320–7324.

55. Hojvat, S. A., M. W. Musch, and R. J. Miller. 1983. Stimulation of prostaglandin production in rabbit ileal mucosa by bradykinin. *J. Pharmacol. Exp. Ther.* 226:749–755.

56. Lawson, L. D., and D. W. Powell. 1987. Bradykinin-stimulated eicosanoid synthesis and secretion by rabbit ileal components. *Am. J. Physiol.* 252:G783–G790.

57. Bonney, R. J., P. D. Wightman, P. Davies, S. J. Sadowski, F. A. Kuehl, and J. L. Humes. 1987. Regulation of prostaglandin synthesis and of the selective release of lysosomal hydrolases by mouse peritoneal macrophages. *Biochem. J.* 176:433–442.

58. Stahl, K.-W., M. Roch-Arveiller, D. Regoli, and J. P. Giroud. 1981. Receptors for bradykinin in murine periotoneal macrophages: modulation of short-term spreading. *Agents Actions.* 11:624–627.

59. Levine, L., and A. Hassid. 1977. Epidermal growth factor stimulates prostaglandin biosynthesis by canine kidney (MDCK) cells. *Biochem. Biophys. Res. Commun.* 76:1181–1187.

60. Tashjian, A. H. Jr., and L. Levine. 1978. Epidermal growth factor stimulates prostaglandin production and bone resorption in cultured mouse calvaria. *Biochem. Biophys. Res. Commun.* 85:966–975.

61. Konturek, S. K., T. Brzozowski, I. Piastucki, A. Dembinski, T. Radecki, A. Dembinski-Kiec, A. Zimudu, and H. Gregory. 1981. Role of mucosal prostaglandins and DNA synthesis in gastric cytoprotection of luminal epidermal growth factor. *Gut.* 22:927–932.

62. Schepp, W., B. Steffen, V. Schusdziarra, and M. Classen. 1986. Calcium, calmodulin, and cyclic adenosine monophosphate modulate prostaglandin E2 release from isolated human gastric mucosal cells. *J. Clin. Endocrinol. Metab.* 63:886–91.

63. Payne, N. A., and J. G. Gerber. 1987. Prostaglandin E_2 and [^{14}C] arachidonic acid release by carbachol in the isolated canine parietal cell. *J. Pharmacol. Exp. Ther.* 243:511–516.