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FACTORS INFLUENCING THE RESTITUTION OF THE DUODENAL AND COLONIC MUCOSA AFTER DAMAGE

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Rapid epithelial restitution is an important protective mechanism which enables the gastrointestinal mucosa to reestablish epithelial integrity following superficial injury within hours. In this study we examined the influence of an acidic luminal pH, removal of the necrotic layer, nutrient bicarbonate, calcium and sodium desoxycholate (Na-DOC) on restitution in the rabbit duodenum in vitro and the role of Na-DOC and calcium for rapid restitution of the human colon in vitro. Transmucosal potential difference (PD), short-circuit current (Isc) were measured and resistance against passive ion flux (R) was calculated. Electrophysiological changes paralleled morphological injury but did not necessarily reflect restitution in all experiments. The extent of mucosal injury was assessed by computerized real-time morphometry. 5 hrs after luminal exposure to 10 mH HCl for 10 min residual damage (RD) was 14% in the duodenum. Luminal pH of 3.0 (RD of 30%), removal of necrotic layer at acidic luminal pH (RD of 66%), absence of bicarbonate from the serosal solution (RD of 35% at neutral luminal pH; RD of 96% at acidic luminal pH) and removal of calcium from the serosal solution (RD of 58%) impaired restitution in the duodenum. Continuous postinjury luminal Na-DOC exposure did not influence restitution in the duodenum (RD of 19%). 5 hrs after luminal exposure to 0.5 mM Na-DOC for 10 min RD was 26% in the human colon. Continuous postinjury luminal Na-DOC exposure (RD of 51%) and removal of calcium from the nutrient solution (RD of 65%) impaired restitution in the human colon. Thus we conclude that restitution of the rabbit duodenum in vitro requires a necrotic layer and bicarbonate flux to withstand acidic luminal pH, while restitution is not affected by Na-DOC. In the human colon Na-DOC inhibits restitution. Both the duodenum and colon require calcium for rapid restitution.

Key words: rapid epithelial restitution, residual damage, sodium — deoxycholate, calcium, morphometry.

INTRODUCTION

As a part of the digestive system both the duodenal and the colonic mucosa represent an important barrier between the lumen of the gut and the vascular system. The morphological (mechanical protection) and physiological (resorption, secretion, immune — homeostasis) functions of this barier
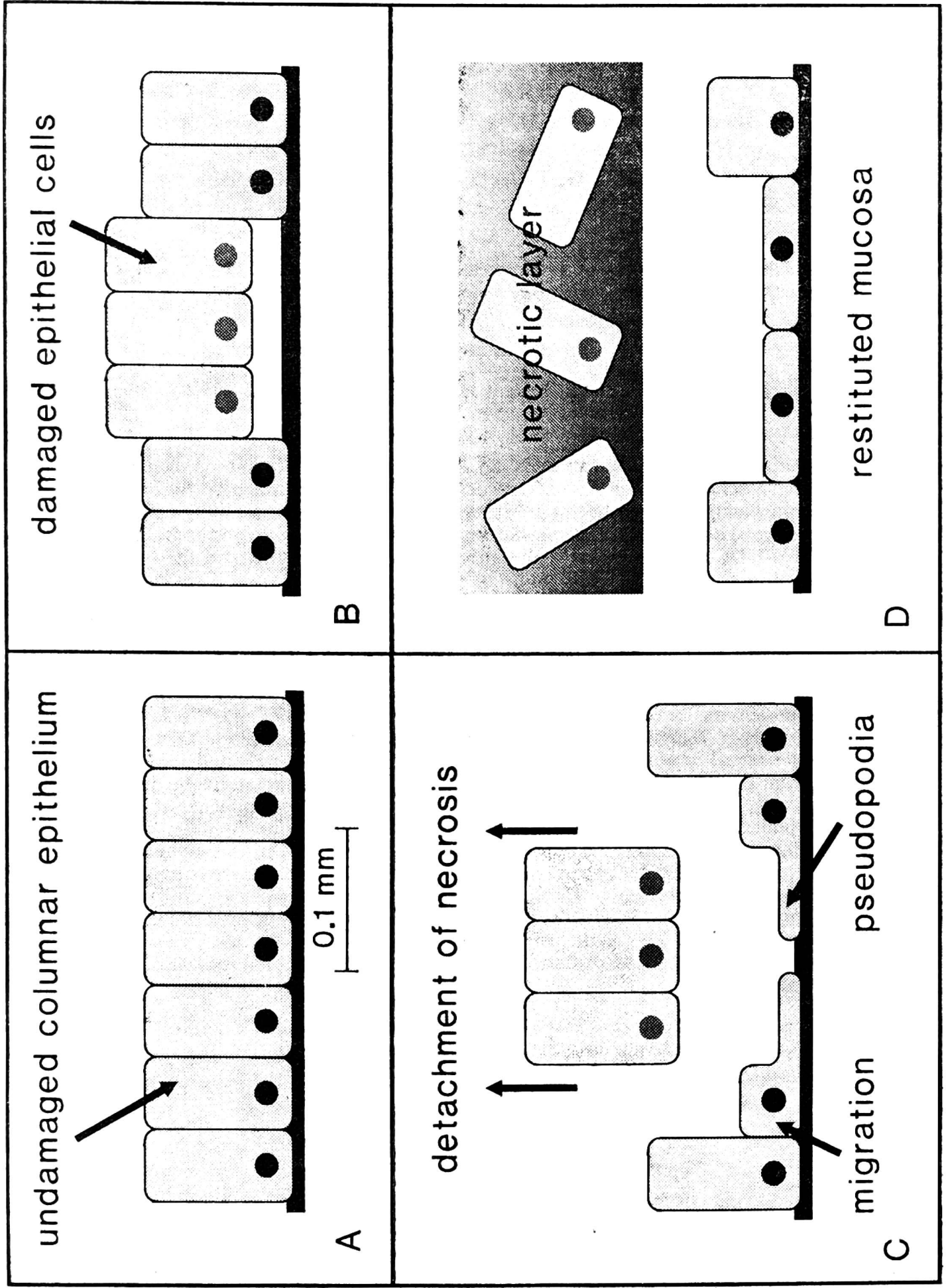
Fig. 1. Time course of rapid epithelial restitution.

are based on the integrity of the epithelial cell layer (1, 2). Therefore the epithelium possesses protective mechanisms against disruption (acid overload in the duodenum, bacterial overgrowth in the colon). Besides the physiological cell turnover (1), bicarbonate secretion (3, 4) and mucus production (5), rapid epithelial restitution represents an important mechanism against disruption of an epithelial barrier in case of superficial damage. The mechanism of epithelial restitution has been described in the stomach (6, 7), the duodenum (8), gallbladder (9), small intestine (10) and colon (11) It involves two main processes: viable intact epithelial cells extend pseudopodia and migrate over the denuded basal lamina to reseal the epithelial defect (11) (*Fig. 1*).

This mechanism does not involve mitosis. The aim of this study was to investigate the factors influencing epithelial restitution.

METHOD

Rabbit duodenum in vitro. Tissues were obtained from anesthetized New Zealand white rabbits with an average weight of 3 kg (5,6). Mucosal sheets were mounted in incubation chambers bathed on the serosal side with a standard nutrient solution containing (mM/l): NaCl 122.0, KCL 5.0, $CaCl_2$ 2.0, $MgSO_4$ 1.3, glucose 20.0. It was buffered with 25.0 mM $NaHCO_3$ (pH = 7.4) and gassed with 95% O_2/5% CO_2). Luminal pH was kept constant by autotitration (4—6). The mucosal side was perfused with with isotonic NaCl — solution (gassed with 100% O_2 prewashed in 1M KOH; pH = 7.4).

Human colon in vitro. Tissues were obtained from patients who underwent surgery for colorectal cancer (11). Mucosal sheets were incubated as described above.

Transmucosal potential difference (PD) and short curcuit curent (Isc) were measured using a voltage/current clamp (DVC — 1000, World Precision Instruments Inc., Newhaven, CT; USA). Resistance was calculated using Ohm's law Values for PD were corrected for junction potentials between the nutrient and luminal solutions which were determined after all experiments.

EXPERIMENTAL GROUPS

Duodenum in vitro. controls: tissues were incubated under baseline conditions for 5hrs (n = 6).

Undisturbed restitution: Following exposure to 10 mM HCl for 10 min and acid washout tissues remained in the chambers for 5hrs (n= 6).

Influence of luminal pH: throughout the experiments luminal pH was kept at 3 by automatic titration with 0.1 N HCl (n = 6).

Influence of the necrotic layer: One hour after the end of luminal acid exposure the necrotic layer was removed by scraping smoothly with a glass slide. After removal of necrotic layer the tissues were washed out with isotonic NaCl — solution and brought into the incubation chambers. The procedure itself did not have any effect on PD.

Importance of nutrient bicarbonate: in this experimental group buffering bicarbonate was removed from the nutries solution and replaced by a bicarbonate — free HEPES solution. Following luminal acid exposure experiments were performed keeping luminal pH at 7.4 and 3.0, as described above (n = 6 in each group).

Role of calcium: Calcium was removed from the nutrient solution and replaced by NaCl.

Following luminal acid exposure for 10 min tissues remained in the chambers for 5 hrs (n = 6) in calcium free solutions.

Influence of luminal sodium — deoxycholate: Following luminal acid exposure the tissues remained in the chambers for 5 hrs luminally perfused with 0.05 mM Na — DOC solution.

Human colon in vitro. controls: tissues remained in the chambers under baseline conditions for 5 hrs (n = 6).

Undisturbed restitution: Following luminal exposure to 0.5 mM Na — DOC for 10 min tissues were incubated for 30 min
(n = 6) and 5 hrs (n = 6).

Influence of luminal Na — DOC: In this experimental group tissues were exposed to 0.05 m Na — DOC solution after an initial 10 min exposure to 0.5 mM Na — DOC (n = 6).

Role of calcium: After Na — DOC exposure tissues were bathed in calcium free nutrient solution as described above for 5 hrs (n = 6).

Histology. All experimental and control tissues were prepared for light microscopical evaluation by an independent observer. In selected specimens transmission and/or electron microscopy was performed (5, 6).

Morphometry: To assess the extent of mucosal damage and to follow the time course of epithelial restitution morphometry was performed on coded paraffin — embedded and H & E stained slides from all experiments by an independent observer. We used a Leitz Diaplan research microscope (Wild Leitz Ltd, Heerbrugg, Switzerland; objective magnification, ×4, ×6.3, ×16, ×25, ×40; ocular magnification, ×12.5). Pictures were transmitted via a Panasonic color CCD videocamera (model WV-CD 130/G, Matshushita Com. Ind. Co. Ltd; Japan) to an analog/digital monitor screen (model PVM—1271Q, superfine pitch, Sony; Japan) for live imaging. In addition we used a personal computer (IBM — PS/2, moedl 30286; IBM, Armonk, N.Y.; USA). A new custom-made morphometry program, which was specially developed for this purpose (MIPSY: The Micro — based Image Processing System) was botted from the hard disk and followed on a second monitor screen (IBM Color Display 8512, IBM, USA). The program was controlled both via keyboard and a graphic table (Summasketch plus 12″×12″ MM 1201; Summagraphics Corp., Fairfield, CT; USA). The PC was extended by insertion of a frame grabber (ITI PCVision plus board; Imaging Technology Corp., Woburn, MA; USA). The videocamera was not only directly connected to the monitor to obtain a live color image, but the video signal was also guided through the frame grabber to achieve analog-digital conversion of the picture. By switching the Line A (analog) mode on the monitor to RGB the screen picture changed to a digital frame. Additionally the cursor signal generated with the graphic table controled by MIPSY could be overaid to this monitor screen. Thus it became possible to follow the contours of a real-time digitized microscopic picture (e.g. mucosa) with the cursor of the computer. In order to obtain absolute measurements it became necessary to calibrate the system to each objective magnification. This procedure was performed using a micrometer slide (2 mm in 200 equal parts; Wild Leitz, Heerbugg, Switzerland). Calibration was performed both in the X and Y axis because of a possible frame distortion on the monitor. The calibration values were stored as a separate file ion the hard disk in the computer and downloaded objective magnification electively. With the "puck" of the graphic tablet the invesitigator was now allowed to draw the contours of the mucosa on the monitor. The lenght of the undamaged mucosal lining was randomly added to the variable "0", the damaged areas in between to the variable "1". Thereafter the overlay signal was discarded and the microscopic specimen moved to the next consecutive area of investigation and the measurements reperformed. Values were automatically added to the corresponding variables. After finishing a slide the length (given in microns) and the percentage (%) of the damaged (variable "1") and undamaged (variable "0") areas were stored as a separate ASCII-files on the hard disk. The data from these ASCII-files (*.PRN) were later exported to a combined spread-sheet and graphic program (Micrografx Graph Plus Vs. 1.3; Micrografxs Inc., Richardson, TX; USA) for statistical evaluation and graphics (*Fig. 2*).
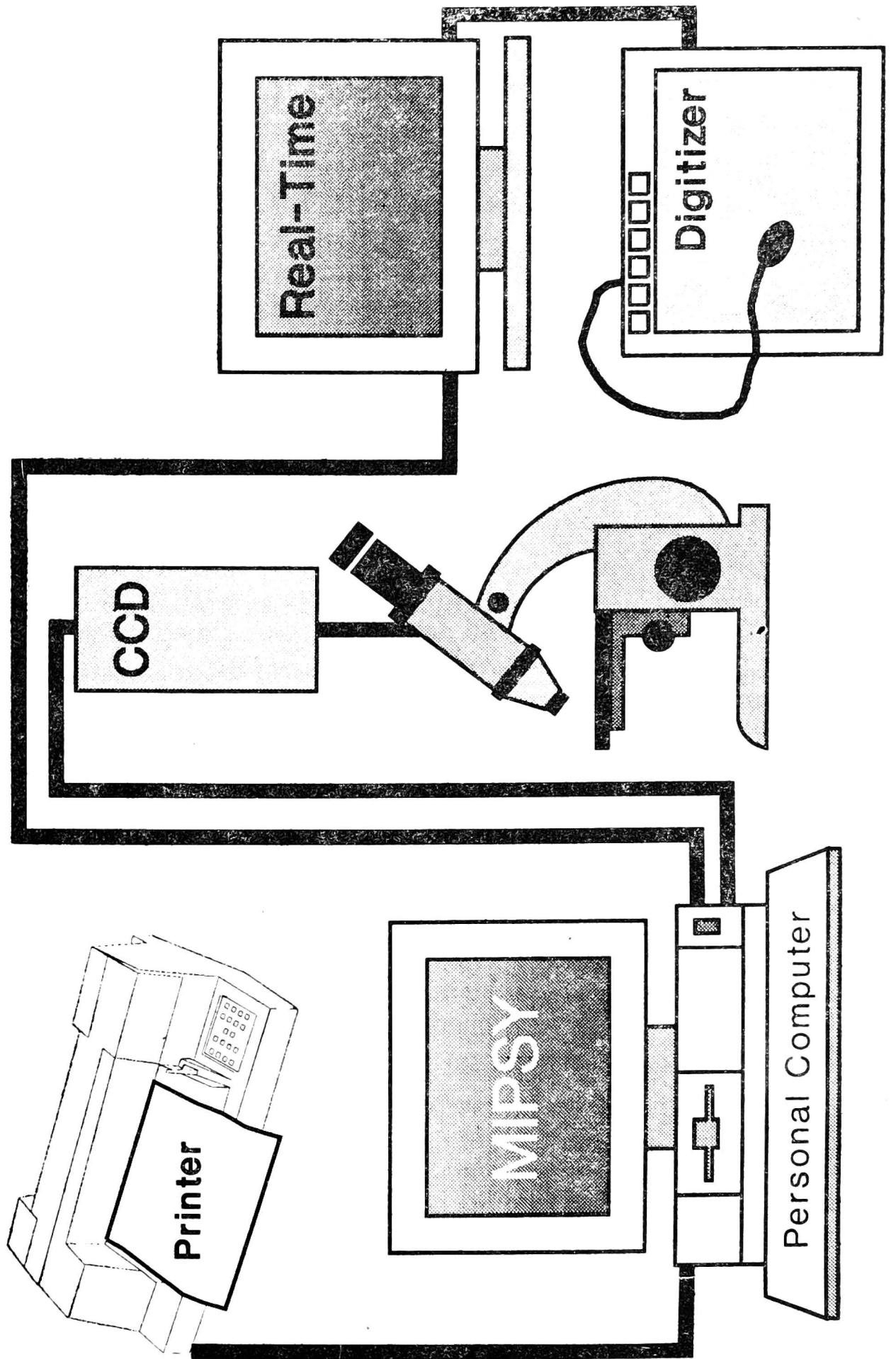
MIPSY - Real-Time Morphometry

Fig. 2. Hardware configuration for real-time morphometry with MIPSY.

RESULTS

Duodenum in vitro. Controls: Baseline PD was -1.90 mV± 0.19 mV and remained stable till the end of the experiment (-2.28 mV± 0.18 mV) ($n = 6$). After 5 hrs of baseline incubation $1.09\% \pm 0.24\%$ of the mucosal surface were damaged.

Undisturbed restitution: Exposure to 10 mM HCl for 10 min resulted in a significant PD drop from -2.41 mV± 0.18 mV to -0.55 mV± 0.08 mV; there was a slight recovery within 5 hrs to -1.35 mV± 0.13 mV. Histology showed uniform mucosal damage confined to the villi. Morphometry revealed $68.30\% \pm 0.66\%$ damage of the mucosal surface after 30 min and $64.42\% \pm 15.10\%$ after one hour. Thereafter we observed migration of the intact cells over the denuded surface along the basal lamina. 5 hours after the end of acid exposure $14.01\% \pm 1.41\%$ of the mucosal surface showed residual damage (RD).

Influence of luminal pH: In the pHL $= 3.0$ series luminal exposure to 10 mM HCl for 10 min caused a drop of PD from -2.15 mV± 0.21 mV to -0.61 mV± 0.08 mV; after 5 hrs. PD was -1.5 mV± 0.12 mV. There was no significant difference to the pH $= 7.4$ group. After 5 hrs $29.71\% \pm 0.73\%$ of the mucosal surface remained damaged, which shows a delay in restitution compared to the pHL $= 7.4$ series, where only $14.01\% \pm 1.41\%$ of the mucosal surface were residually damaged.

Influence of the necrotic layer: The removal of the necrotic layer (RNL) was studied at pHL $= 7.4$ and pHL $= 3.0$. Under both conditions damage caused a significant PD drop: in the pHL $= 7.4$ group PD fell from -2.82 mV± 0.08 mV to -0.8 mV± 0.04 mV; at pHL $= 3.0$ PD decreased from -2.85 mV± 0.07 mV to -0.86 mV± 0.03 mV after damage. 5 hrs after the end of luminal acid exposure (4 hrs after RNL) PD was -1.86 mV± 0.16 mV at pHL $= 7.4$ and -0.95 mV± 0.05 mV at pHL $= 3.0$. RNL did not influence PD recovery at pHL $= 7.4$ but caused inhibition of PD restoration at PHL $= 3.0$. Morphometry revealed no additional damage by RNL ($70.50\% \pm 1.02\%$ of the mucosal surface were damaged immediately after RNL).

When luminal pH was kept at 7.4 during post damage incubation $14.72\% \pm 0.7\%$ of the mucosal surface were residually damaged after 5 hrs. In contrast RNL at pHL $= 3.0$ resulted in a delay of restitution ($65.52\% \pm 1.04M$ of RD after 5 hrs).

Influence of nutrient bicarbonate: When experiments were performed in bicarbonate free HEPES solutions PD fell from -3.00 mV± 0.08 mV to -0.66 mV± 0.06 mV in the pHL $= 7.4$ series after damage; in the pHL $= 3.0$ group PD dropped from -2.81 mV± 0.11 mV to -0.60 mV± 0.07 mV. In both groups electrophysiological restoration was inhibited by removal

of bicarbonate in the nutrient solution. After 5 hrs PD was -0.62 mV$\pm$ 0.15 mV in the pHL $= 7.4$ group and -0.73 mV±0.09 mV in the pHL $= 3.0$ group. Morphometry revealed impairment of restitution at pHL $= 7.4$ (RD of the mucosa was 34.72%$\pm$1.31% after 5 hrs). When luminal pH was kept at 3.0 in the post damage period additional damage occured (96.17%$\pm$ 0.38% of the mucosal surface were injured).

Role of calcium: Incubation with serosal calcium — free nutrient solution resulted in a continuos fall of PD (baseline PD was -1.95 mV±0.10 mV; after 5 hrs. PD was -0.80 mV±0.21 mV). Baseline incubation with calcium — free nutrient solution caused 12.31%±0.46% damage of mucosal surface (n $= 3$). In the injured areas histology revealed increased depletion of epithelial cells while the remaining mucosa showed normal morphology.

Following exposure to 10 mM HCl PD dropped from -2.10 mV±0.11 mV to -0.43 mV±0.09 mV and did not recover till the end of the experiment (-0.73 mV±0.19 mV after 5 hrs). Morphometry revealed lack of rapid restitution and additional damage due to depletion of epithelial cells and inhibition of migration: RD was 55.74%±3.85% after 5 hrs (n $= 6$).

Influence of luminal sodium — deoxycholate: Luminal exposure to 0.05 mM Na — DOC for 5 hrs did not have any effect on electrophysiology (baseline PD was -1.92 mV±0.14 mV and reached -2.05 mV±0.20 mV after 5 hrs) and mucosal morphology (1.35%±0.24% of mucosal surface were injured after 5 hrs).

Following exposure to 10 mM HCl for 10 min both electrophysiology and morphometry did not reveal a significant effect compared to undistirbed restitution. PD fell from -2.45 mV±0.21 mV to -0.88 mV±0.23 mV and was -1.53 mV±0.20 mV after 5 hrs. RD was 18.80%±1.76% after 5 hrs.

Human colon in vitro. Controls: In the control group PD and R remained stable for the whole incubation time (baseline — PD was -19.07 ± 2.90 mV, after 5 hrs. -15.77 ± 2.34 mV; baseline — R was 174.33 ± 18.13 Ohm.cm², after 5 hrs. R 185.50 ± 13.78 Ohm.cm²).

Isc remained stable for 2 hrs. (baseline — Isc was 117.17 ± 20.65 μA/cm²; 114.67 ± 10.96 μA/cm² after 2 hrs.); thereafter values showed a slight drop to the end of the experiments and reached 87.17 ± 14.04 μA/cm² after 5 hrs. Histological examination did not reveal any signs of mucosal damage in the control group (0.27%±0.09% of the mucosal surface were injured after 5 hrs. baseline incubation, n $= 8$).

Undisturbed restitution: Luminal exposure to 0.5 mM Na — DOC for 10 min resulted in a significant drop of all electrophysiological parameters: PD from -26.53 ± 3.23 mV (baseline) to -12.22 ± 1.50 mV (10 min postinjury), Isc from 121.83 ± 11.62 μA/cm² (baseline) to 79.17 ± 8.81 μA/cm² 10 min postinjury and transmucosal R dropped from 215.33 ± 11.00 Ohm.cm² (base-

line) to 154.67 ± 7.08 Ohm.cm² (10 min postinjury). The lowest values were reached 30 min after the end of Na — DOC exposure (PD -9.50 ± 1.45 mV, Isc 65.67 ± 9.21 μA/cm² and R 143.67 ± 3.33 Ohm.cm²). Then PD and Isc icreased to stable values till the end of the experiments (PD -14.50 ± 2.34 mV and Isc 99.50 ± 15.58 μA/cm² 5 hrs. after damage) (*Fig. 3*). Time course of R remained constant to reach 146.83 ± 4.10 Ohm.cm² after 5 hrs. Following
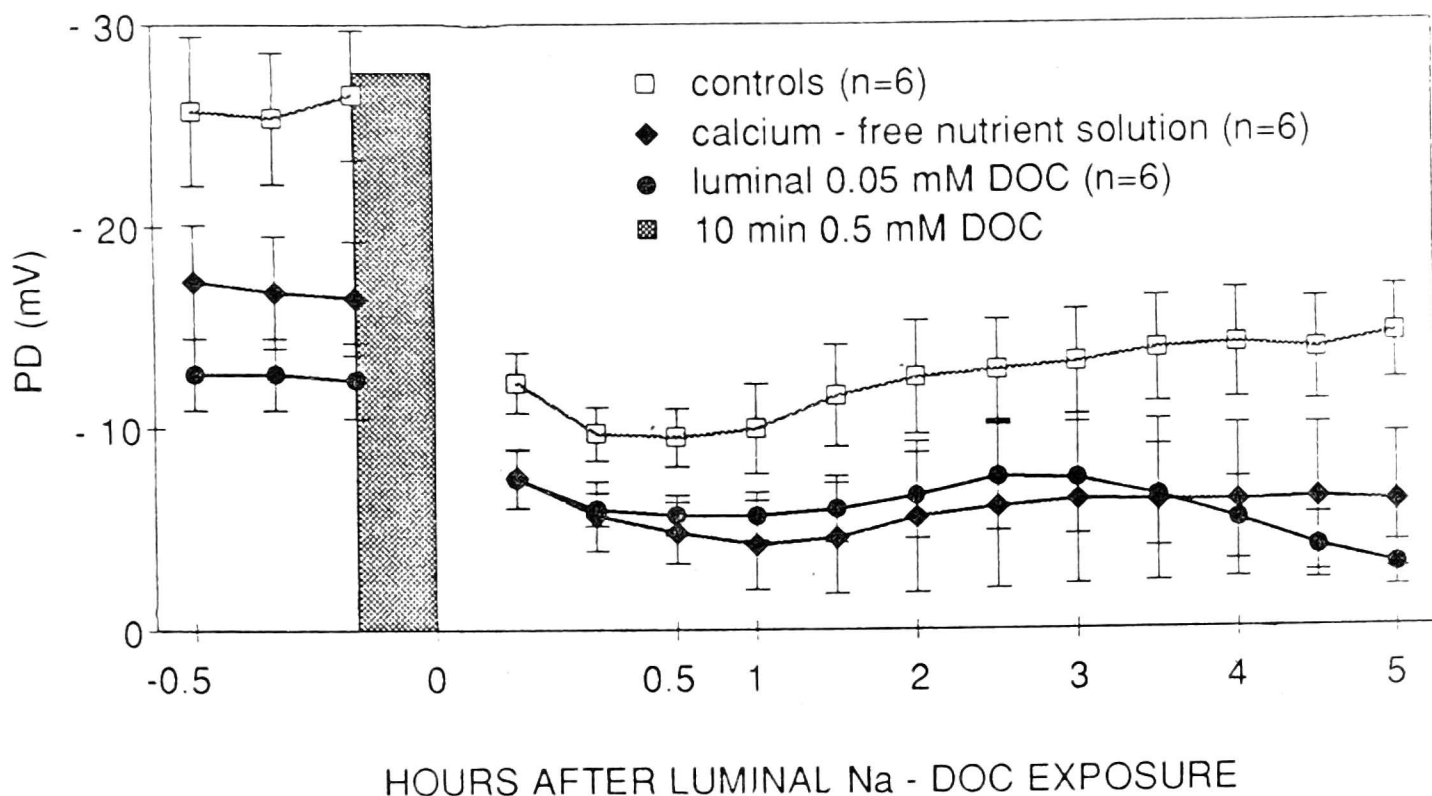

Fig. 3. Transmucosal potential difference of the human colon after damage with 0.5 mM Na — DOC with a Ca_{++}-free nutrient solution or luminal exposure to 0.05 mM Na — DOC.

exposure to 0.5 mM Na — DOC for 10 min $50.63\pm3.61\%$ of the mucosal surface were damaged. 5 hrs after damage RD was $26.15\pm2.91\%$ of the mucosal surface (n = 14; paired data).

Influence of Na — DOC: In the control group the addition of 0.05 mM Na — DOC to the luminal solution for 5 hrs resulted in a constant fall of PD up to the end of incubation (baseline — PD was 15.33 ± 1.76 mV; 9.67 ± 2.60 mV after 5 hrs) (n = 3). Luminal exposure to 0.05 mM DOC for 5 hrs. caused $2.25\%\pm0.15\%$ (n = 3) damage. When tissues were exposed to 0.5 mM Na — DOC for 10 min additionally PD dropped from -15.15 ± 1.65 mV to -7.35 ± 1.00 mV and did not recover until the end of the experiments (-4.08 ± 0.95 mV after 5 hrs) (n = 6) Figure 3.

Continous Na — DOC exposure during the restitution period resulted in a RD of $51.88\pm2.79\%$ of the mucosal surface after 5 hrs.

Influence of calcium (n = 6); Incubation with calcium — free nutrient solutions did not show a significant effect on transmucosal PD (baseline —

PD was -15.88 ± 3.60 mV, -10.42 ± 3.30 mV after 5 hrs. in a controls group) and resulted in slight epithelial depletion ($3.14\%\pm2.18\%$ of the mucosal surface were unjured). When tissues were additionally exposed to 0.5 mM Na — DOC for 10 min PD dropped from -16.43 ± 7.50 mV to -7.50 ± 1.48 mV and did not recover thereafter (-6.20 ± 3.3 mV after 5 hrs). Figure 3. In a calcium free solution RD was $64.61\pm2.35\%$ of the mucosal surface.

DISCUSSION

In our study we used physiological models to damage the duodenal and colonic mucosa. Supporting the results of recent studies (8, 11) we could demonstrate that both mucosae are restituted by the same mechanism. However the restituted barrier does not have the same electrophysiological properties as the original epithelium.
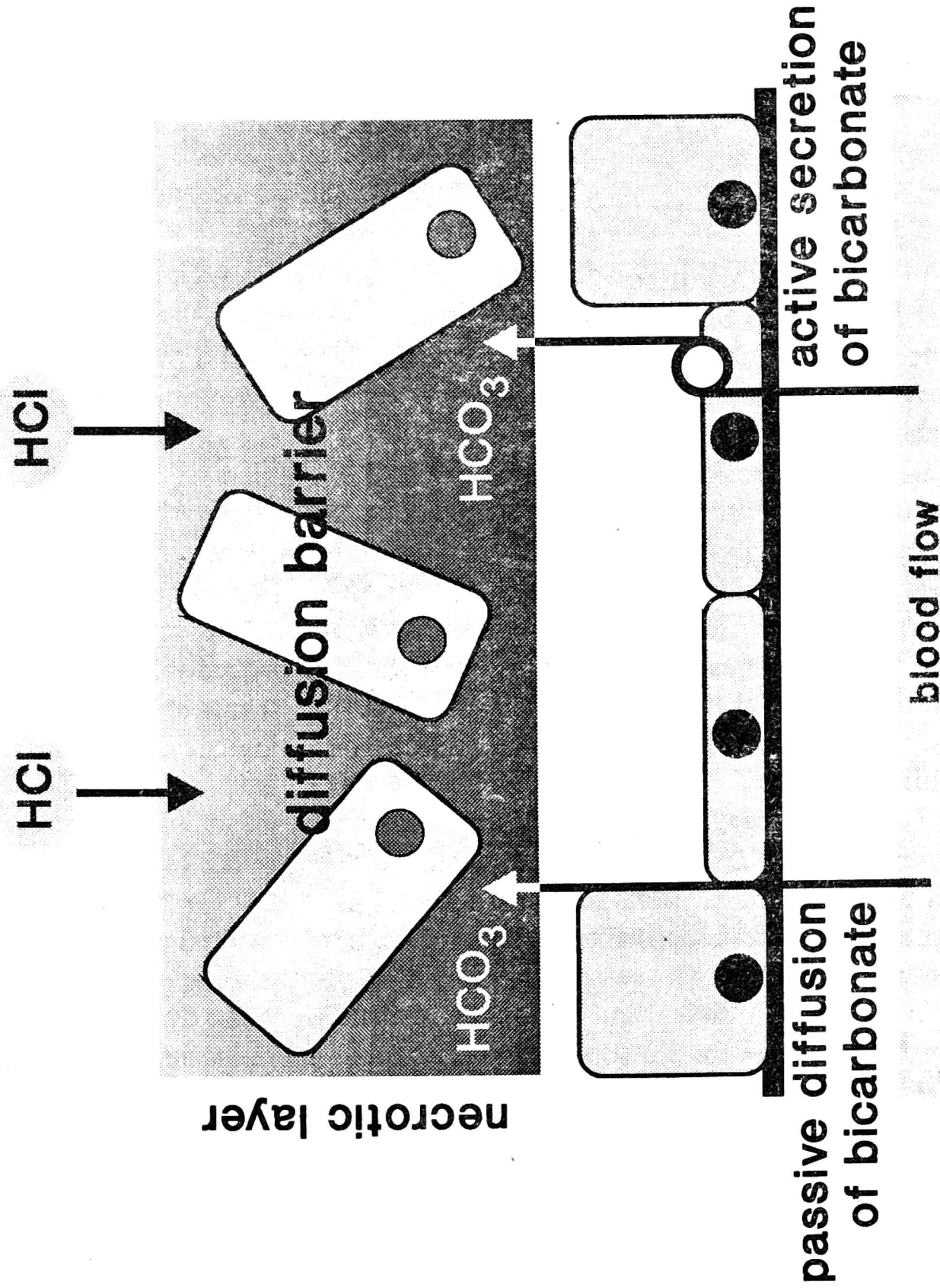
Under physiological conditions both the barriers of the duodenal and the colonic mucosa are exposed to bile salts. From our results the duodenal mucosa has a higher resistance against the luminal bile salt sodium — deoxycholate (Na — DOC) than the colon. There was no difference of damage when duodenal mucosal sheets were bathed luminally with Na — DOC solution after acid exposure, whereas this procedure caused inhibition of restitution of the colonic epithelial barrier. This seems to be of physiological importance since the colon is only under pathological conditions exposed to bile.

Recent studies demonstrated the importance of calcium for restitution of the gastric (12) and small intestinal (10) epithelial barriers. Our results support these observations for the duodenal as well as for the colonic mucosal epithelium. In both epithelia absence of calcium resulted in inhibition of restitution. According to the observations of other authors (10, 12) this supports the fact that calcium is required for the restitution of the duodenal and colonic barrier. Calcium is important for migration and cell adhesion in both mucosae.

An interesting result was obtained in the duodenum. Rapid restitution was nearly completely inhibited by inhibition of alkaline secretion or at least impaired by removal of the necrotic layer upon the damaged epithelium (5). Since the inhibition of rapid restitution was only observed at an acidic pH of 3, the presence of a neutral or alkaline microenvironment seems to be necessary (*Fig. 4*).

Rapid restitution in the duodenum has probably important clinical implications in the pathogenesis of peptic ulcer disease. In the colon failure of restitution might cause bacterial translocation (13) or chronic inflammatory disease.

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The Alkaline Micro-Environment

HCl

HCl

diffusion barrier

HCO₃

HCO₃

necrotic layer

passive diffusion
of bicarbonate

blood flow

active secretion
of bicarbonate

W.Feil

Fig. 4. Proposed model for the importance of an alkaline microenvironment for rapid restitution in the presence of luminal acid.

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