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The influence of in-vivo carbonation on GI physiological processes and drug permeability[1]

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Abstract

Oral effervescent formulations were originally developed as a means to mask unpleasant tastes typically associated with drug compounds. Although these delivery systems provide a palatable saline taste, effervescence may also produce physiological changes within the body. When effervescent solutions (50 ml) were administered to a cannulated mixed breed canine, there was an increase in pressure gradient across the gastroduodenal junction resulting in an altered pattern from fasted to a 'pseudo-fed' state. These solutions also created an increase in total discharge volume and mean solid content when compared with controls. The results are indicative of an increase in mucosal and gastric secretions along with stripping of the mucus layer due to mechanical turbulence. In-vitro diffusion studies showed an increase in benzoic acid permeability across rabbit ileum tissue in the presence of carbon dioxide (CO_2). This effect also occurred with nitrogen indicating the enhancement mechanism is not unique to the chemical entity CO_2, but rather to epithelial disruption which was verified by electrophysiology measurements. In-situ rat single-pass perfusion studies also showed an increase in benzoic acid absorption with relatively rapid (20 min) tissue recovery after CO_2 exposure. © 1997 Elsevier Science B.V.

Keywords: Effervescent; Carbon dioxide; Benzoic acid; Oral drug delivery; Absorption enhancement; Tissue recovery

1. Introduction

Effervescent preparations have been used in delivering therapeutic agents for more than 200 years. Generally, effervescent formulations include a carbonate containing compound, such as sodium bicarbonate and an acidifying agent, such as citric and tartaric acid. The resultant chemical reaction produces rapid evolution of carbon dioxide (CO_2) gas. The widespread acceptance for effervescent delivery systems may be traced to their characteristic palatable saline taste which can mask the unpleasant taste typically associated with many drug compounds.

In the eighteenth century, saline cathartics were the first known effervescent formulation to be used. Since that time, preparations utilizing effervescent technology have included; lozenges, stomach distress medications (e.g. Alka-Seltzer), analgesics, dental cleansers, adsorbents, soft drinks, and vitamin supplements (e.g. calcium). Early use of therapeutic formulations consisted of separate packets of acid and base powders which would be mixed together within a glass of water. Today, the identical principle applies except the components are usually combined within a capsule or tablet. Whether CO_2 production initially occurs outside the body (i.e. in a glass of water) with subsequent ingestion or within the body (i.e. in the stomach), this paper will focus on the effects effervescence produces within the gastrointestinal tract. These will include physiological

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Table 1
Test solutions and amounts of effervescent mixture components

Test solution	Sodium bicarbonate (g)	Citric acid (g)	Water (ml)	Theoretical molar CO_2 conc.
Water	—	—	50	—
EFF I (control)	0.656	0.500	50	—
EFF I	0.656	0.500	50	6.70×10^{-3}
EFF II (control)	1.312	1.000	50	—
EFF II	1.312	1.000	50	1.33×10^{-2}
EFF III (control)	1.968	1.500	50	—
EFF III	1.968	1.500	50	2.00×10^{-2}

effects in addition to an examination of the enhancement of drug permeability across the gastrointestinal epithelium.

2. Materials and methods

2.1. Materials

Citric acid, sodium bicarbonate, and benzoic acid were obtained from Sigma Chemical Company (St. Louis, MO). ^{14}C-benzoic acid and ^{3}H-polyethylene glycol (PEG) were purchased from NEN Research Products (Wilmington, DE). Halothane was supplied by Halocarbon Laboratories (North Augusta, SC). Ketamine was obtained from Fort Dodge Laboratories (Fort Dodge, IN). Xylazine was obtained from Phoenix Pharmaceuticals (St. Josephs, MO). All other reagents were analytical grade and were used as received.

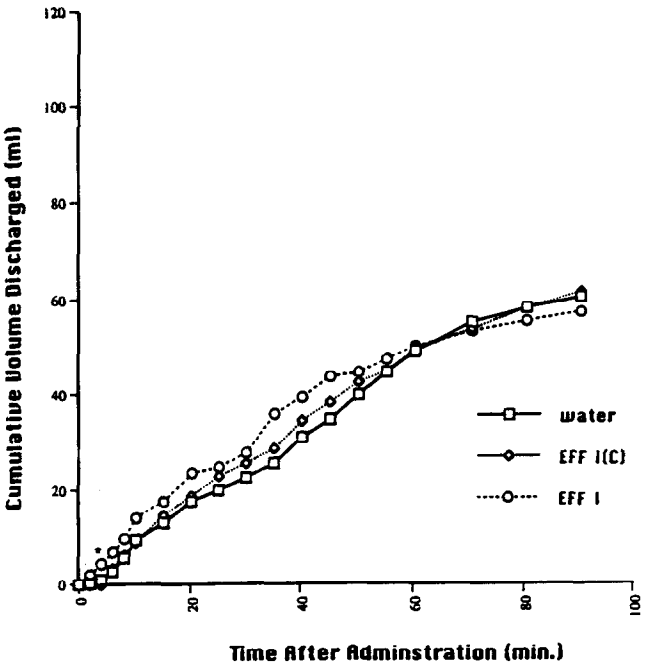

Fig. 1. Gastric emptying of 50 ml of water, EFF I, and its control solution administered during phase I in the fasted dog. Cumulative volume recovered from the duodenal cannula has been plotted as a function of time. (*) Indicates a statistical difference at $P = 0.01$.

2.2. Methods

2.2.1. Preparation of cannulated dog

As reported in previous studies from this laboratory [1,2], the surgical procedure of Reinke et al. was followed [3]. The dogs were anesthetized with 1% halothane USP followed by laparotomy under aseptic conditions. A modified Thomas cannula made of Acetal (O.D. 17 mm, I.D. 16 mm; University of Wisconsin Physical Plant Machine Shop, Madison, WI) was implanted in the duodenum through a longitudinal cut ≈ 15 cm from the gastrointestinal junction and free of mesenteric blood supply. The cannula was exteriorized through an opening in the abdomen and fixed to the abdominal wall at a site ≈ 4 cm below the last rib and 2 cm from the midline cut. A surgical recovery period of 3 weeks was allowed before studies commenced. The dogs were trained to stand quietly and to accept oral administration of liquids and solids by natural swallowing while supported by slings.

2.2.2. Preparation, administration, and collection of test solutions

Effervescent I, II, and III (EFF I, EFF II, and EFF III) solutions were prepared just prior to administration to the dogs. Table 1 shows a list of test solutions and amounts of each component. The control solutions were stirred for 1 h prior to administration in order to eliminate excess carbon dioxide.

Before each experiment, the dog was fasted for 16–20 h with a free supply of water. Three to four glass spheres, 2 mm in diameter, were administered by placement in the back of the mouth followed by natural swallowing. The cannula was opened and duodenal discharge was allowed. Discharge of the glass spheres as well as the presence of bile and mucus was used to ascertain phasic activity. After one complete phasic activity cycle was allowed to pass, an additional 15 min period of no discharge was allowed to make sure that the GI motility of the dog was in phase I. This time was considered time 0, at which either control or test solu-

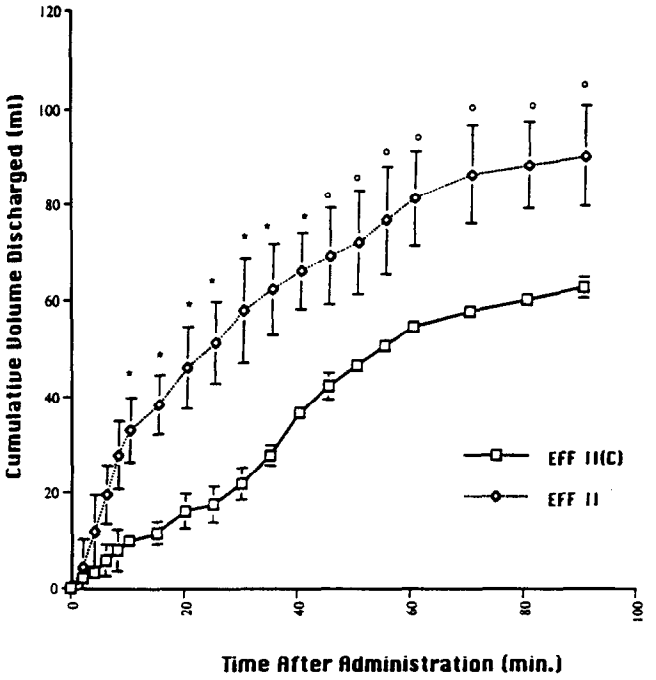

Fig. 2. Gastric emptying of 50 ml of EFF II, and its control solution, administered during phase I in the fasted dog. Cumulative volume recovered from the duodenal cannula has been plotted as a function of time. (*) Indicates a statistical difference at $P = 0.01$, (°) indicates a statistical difference at $P = 0.05$.

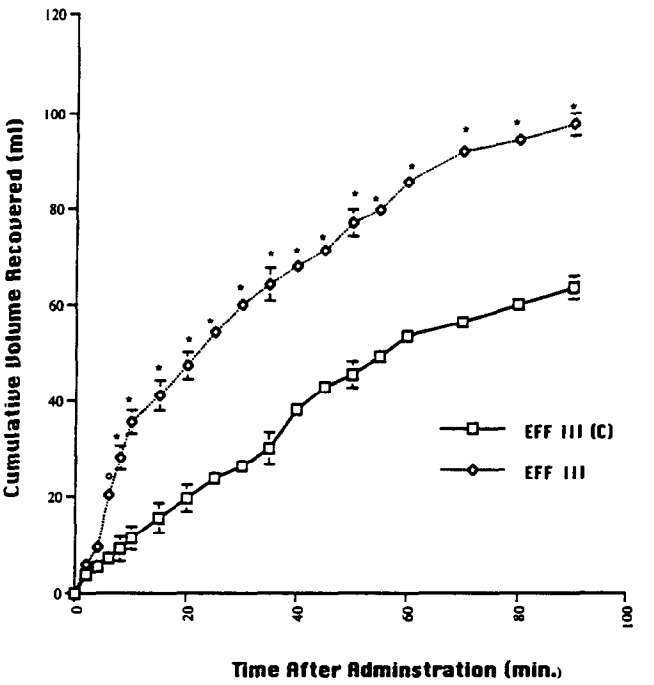

Fig. 3. Gastric emptying of 50 ml of EFF III, and its control solution, administered during phase I in the fasted dog. Cumulative volume recovered from the duodenal cannula has been plotted as a function of time. (*) Indicates a statistical difference at $P = 0.01$, (°) indicates a statistical difference at $P = 0.05$.

tion was administered to the back of the mouth by a flexible tube attached to a syringe. The dog's mouth was held up and administration occurred at a rate ≈ 250 ml/min to allow comfortable swallowing.

The volume discharged from the stomach and the first 15 cm of duodenum was collected. The total volume discharged after one complete cycle for each animal was collected while nothing was administered. The samples were taken at pre designated time intervals. The effluent volume was measured. Each experiment was repeated three to eight times on each dog.

Samples, 20 ml, were taken from the total discharge and placed into tarred petri dishes. The dishes were placed within a dura-dry corrosion resistant freeze-dryer (FTS System, Stone Ridge, NY).

2.2.3. *In-vitro permeability study*

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Madison, WI, USA) weighing 325–349 g were maintained on an unrestricted diet with standard caging facilities and a 12 h light/dark cycle. The rats were sacrificed by exposure to carbon dioxide. An 8 cm ileum segment was excised and placed in ice cold saline. The epithelium was exposed via a longitudinal incision along the mesenterium, washed with ice cold saline, and immediately mounted into a modified using diffusion apparatus. Drug-free Sorenson's buffer, 7 ml, (pH 6.8, 300 m Osm) was added to the receiver chamber. Buffer, 7 ml, with 0.82 mM benzoic acid + 1.5 μCi ^{14}C-benzoic acid was placed in the donor chamber. Solutions were prewarmed and maintained at 37°C by a circulating water bath. In control experiments, stirrers were placed ≈ 1.0 cm from the tissue surface in both chambers to ensure mixing. With CO_2 or nitrogen bubbling experiments, stirrers in the donor chamber were replaced with a 1.5 mm diameter tubing allowing direct bubbling (rate ≈ 100 ml/min) onto the epithelial surface. The gas contained $< 1.0\%$ oxygen necessary for tissue survival. Samples, 1 ml, were removed from the receiver chamber at specific time intervals and replaced with an equal amount of drug-free Sorenson's buffer. Permeability coefficients (P_m) were determined by using Eq. (1):

Table 2
Mean weight residues after freeze drying of 20 ml samples taken from discharge (± S.D.)

Test solution	Weight of the residue (g)
No solution	0.375 (0.019)
EFF I (control)	0.439 (0.036)
EFF I	0.453 (0.045)
EFF II (control)	0.496 (0.065)
EFF II	0.673 (0.041)*
EFF III (control)	0.542 (0.053)
EFF III	0.874 (0.071)*

* Significant difference between test and control solutions at $P = 0.01$.

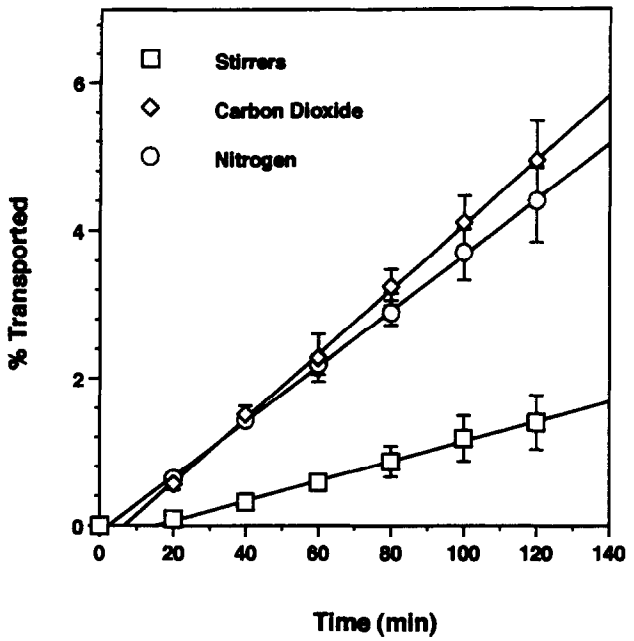

Fig. 4. % Benzoic acid transported across rabbit ileum tissue vs. time. Error bars represent standard error of mean for four to seven replications.

$$P_m = V/AC_0(dC/dt) \quad (1)$$

Where V = initial volume (7 ml), A = tissue area (0.196 cm^2), C_0 = initial concentration (100%), and dC/dt = steady state slope of percentage transported versus time curve.

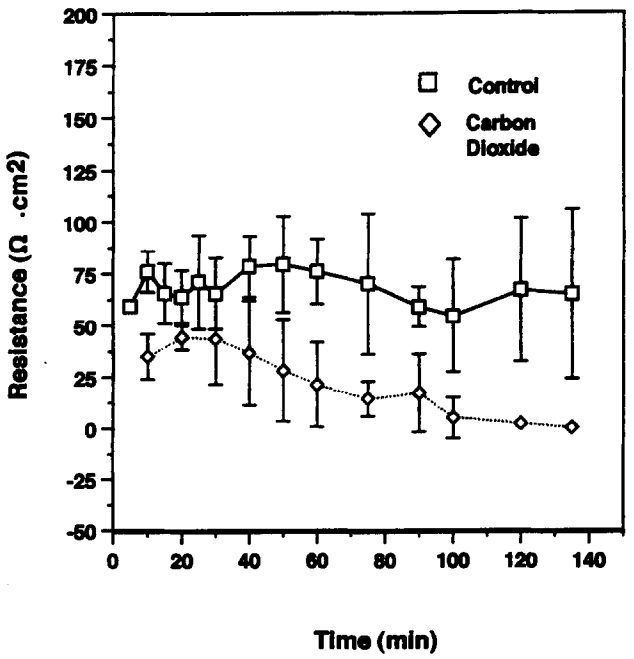

Fig. 5. Resistance vs. time profile of rat duodenal tissue. Error bars represent the standard deviation of four replications.

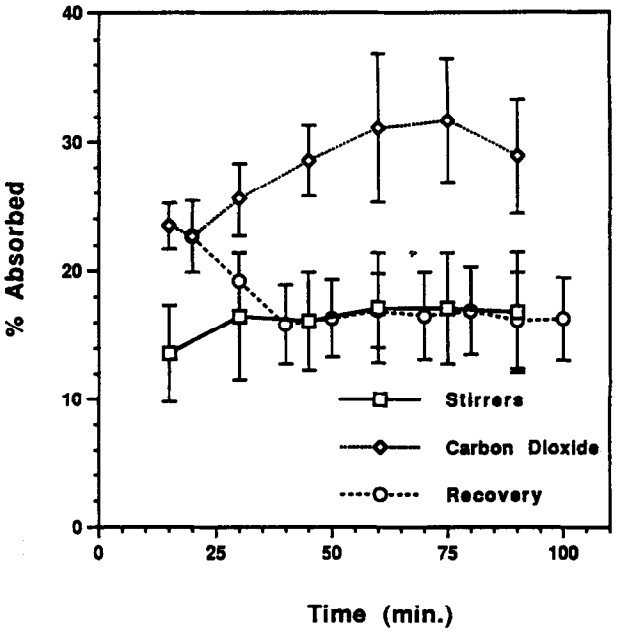

Fig. 6. Single pass perfusion tissue recovery of 10 cm rat ileum segment after exposure to carbon dioxide bubbling.

2.2.4. Electrophysiology

Rat duodenum tissue, as previously described, was inserted between two diffusion cell chambers. Sorenson's buffer, 7 ml (pH 6.5, 300 m Osm) was placed in the receiver and donor chambers, the latter containing 0.82 mM benzoic acid. A dual Ag/AgCl electrode system was used to measure membrane resistance. Current ranging from 0 to 15 μA was applied by a dc power supply (Hewlett Packard. Palo Alto, CA) across the membrane in stepwise fashion. Resistance measurements were obtained from the resultant transmembrane potential values, taking into consideration resistance values of the bathing medium.

2.2.5. In-situ tissue recovery studies

Male Sprague-Dawley rats weighing 400–450 g were fasted for 20–24 h with an unrestricted supply of water prior to each study. The rats were anesthetized with a 0.4 ml intramuscular injection of ketamine (100 mg/ml) and xylazine (10 mg/ml) mixture with additional 0.1 ml injections every 30–45 min as necessary to maintain anesthesia. The rat's body temperature was maintained at 37°C by a heating pad and all solutions were prewarmed to 37°C prior to perfusion. A 10 cm ileum segment was cannulated ending 15 cm proximal to the ileo-cecal valve and was performed so as not to inhibit blood flow. The segment was reinserted into the abdominal cavity and the abdominal wall sutured to prevent heat loss. The solution medium was prepared by dissolving benzoic acid (0.82 mM) in isotonic Sorenson's buffer (pH 6.8) with the addition of ^{14}C-benzoic acid and ^{3}H-(PEG) as a non-absorbable marker of

water flux. Drug-free buffer was perfused through the segment by a peristaltic pump at a constant rate of 0.5 ml/min for 30 min at which time the perfusate containing drug entered the cannulated segment.

In test experiments, CO_2 was bubbled at a rate of 5 ml/min into the proximal portion of the segment after the cleansing period. For tissue recovery, CO_2 was bubbled for the first 20 min. then discontinued. Samples, 1 ml, were assayed for each time interval. The fraction (F_a) benzoic acid (BA) absorbed was calculated by using Eq. (2):

$$F_a = 1 - [(^3H - PEG_{in}/^3H - PEG_{out}) \times (^{14}C - BA_{out}/^{14}C - BA_{in})] \quad (2)$$

where in = inlet concentration and out = outlet concentration.

3. Results and discussion

3.1. Effect of effervescence on stomach emptying

Gastric emptying of liquids is proportional to the amount of liquid remaining in the stomach (i.e. first order kinetics) [2]. When liquids are administered while the stomach is in the fasted state, the Migrating Motor Complex (MMC) is affected only by large volumes. With volumes of 100 ml or less, the gastrointestinal pressure gradient is insignificant so that the MMC will not be disrupted and emptying will occur mostly during phase II and/or phase III. When large volumes of 150 ml or more are administered, an increase in pressure, caused by distention, within the proximal stomach is sensed. Therefore, the MMC is disrupted and immediately converts the stomach into the fed state. Liquid emptying patterns from the stomach are very important in that the absorption profile of an oral drug may be altered. Effervescent formulations provide a method for conversion of the stomach from a fasted to fed state. The rate of stomach discharge for 50 ml volumes of non-carbonated water and control solutions were compared with that of a carbonated solution of equal volume (Fig. 1, Fig. 2, and Fig. 3). The rate of discharge for the non-carbonated and control solutions were consistent with previously reported findings by Gupta and Robinson [2]. The non-carbonated solutions did not induce a pressure gradient across the gastroduodenal junction, therefore, the stomach remained in its relaxed state (phase I). Fig. 1 illustrates that the solution with the lowest amount of carbonation (EFF I) had discharge rates similar to the non-carbonated water and control solutions. The other carbonated solutions, EFF II and EFF III, produced greater quantities of CO_2 and followed a completely different fluid discharge pattern as compared with controls (Fig. 2 and Fig. 3). Both solutions discharged immediately after administration and most of the fluid was emptied within the first 20 min. This pattern followed previously reported findings for large volume solutions by Hinder et al. [4] and Gupta et al. [2]. The $t_{1/2}$ for EFF I, EFF II, EFF III, which is the time at which half of the administered volume is discharged, was 25.8, 7.4, and 7.2 min, respectively. When compared with the $t_{1/2}$ (35 min) for the non-carbonated water solution and for the EFF I, EFF II, EFF III controls, 30.50, 33.00, and 27.10 min, respectively, EFF II and EFF III seemed to create enough CO_2 in order to increase the pressure within the stomach. This created a change in the motility pattern from the fasted to 'fed-like' state (i.e. pseudo-fed).

3.2. Impact of effervescence on gastrointestinal mucus

The total volume discharged after administration of 50 ml of each solution was calculated. For all solutions, the total volume discharged after 90 min was 20% higher and is attributed to normal gastric and pancreatic secretions. EFF I solution (Fig. 1) did not vary in total volume discharge when compared with 50 ml of water or its control solution. However, the higher carbonated solutions, EFF II and EFF III, showed a significant increase in total discharge volume, 89.74 and 97.40 ml, respectively, when compared with a total of 60.0 ml for their control solutions [5]. The average solids content (Table 2) was also obtained after freeze-drying of 20 ml samples which were taken from total homogenate cannula discharge effluent 90 min after administration. The mean solids content obtained from EFF II and EFF III were significantly greater than their corresponding control solutions. Again, EFF I showed insignificant difference from its control [5]. These results may be explained in terms of mechanical turbulence caused by CO_2 production. The turbulence created by the bubbling action causes a thinning and/or stripping of the stagnant unstirred water layer (mucus). Additionally, alteration of the mucus layer will produce stimulation of mucus and gastric secretions which may account for some of the increase in total discharge volume and weight residues seen with EFF II and EFF III.

3.3. Permeability and membrane alteration

In the literature, numerous studies have indicated that effervescent dosage forms have the potential for enhancing the absorption of various therapeutic agents [6–8]. Various mechanisms have been postulated to explain the effervescent effect including; increase in drug dissolution rate, mucus thinning, buffering effect, alteration of the pH gradient across the epithelium,

fluid flow, and epithelial disruption. As shown in Fig. 4, a significant increase ($P < 0.001$) in the benzoic acid permeability coefficient from 1.9×10^{-5} to 6.5×10^{-5} cm/s occurred with CO_2. This effect has also been shown with caffeine, tetracycline, diazepam, and mannitol (data not shown). In addition, tissue damage was not indicated by various techniques including MTT assay and electrophysiology. Tissue resistance values (Fig. 5) declined in the presence of CO_2 which strongly indicates an opening of the transport pathway (i.e. epithelial disruption). In addition, Fig. 4 shows the effect of an inert gas, nitrogen, on benzoic acid permeability. The permeability coefficient increased to 5.7×10^{-5} cm/s. The difference was significant ($P < 0.001$) when compared with stirrers but insignificant relative to CO_2. Therefore, it seems that the mechanism of CO_2 is not unique in that other gases provide the same magnitude of enhancement most likely due to epithelial disruption, increase in fluid flow, and/or mucus layer thinning.

Fig. 6 shows tissue recovery from an in-situ single-pass perfusion experiment. Benzoic acid absorption increased when CO_2 was bubbled into the segment. However, when the bubbling was stopped, a relatively rapid linear decline in benzoic acid absorption to control levels was observed. Therefore, the disruptive effects of CO_2 bubbling on the epithelium seem to be transient in nature and the epithelial barrier properties are reestablished in a relatively short period of time.

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