Effects of Methylprednisolone on Hydrogen Ion Absorption in the Canine Stomach

RAPHAEL S. K. CHUNG, MICHAEL FIELD, and WILLIAM SILEN, *Department of Surgery, Veterans Administration Hospital and University of Iowa College of Medicine, Iowa City, Iowa 52242, and Departments of Medicine and Surgery, Harvard Medical School and Beth Israel Hospital, Boston, Massachusetts 02215*

ABSTRACT The effect of methylprednisolone (2 mg/kg per day given parenterally for 3 doses, 2 wk or 12 wk) on the permeability of mammalian gastric mucosa to hydrogen ion (H^+) was examined with denervated fundic pouches in dogs with antrectomies. Transmucosal electric potential difference (PD) and net fluxes of H^+ and Na^+ were determined for luminal [H^+] from 20 to 160 mM and [Na^+] from 1 to 140 mM ([H^+] and [Na^+] were varied reciprocally). The PD was 50–60 mV lumen negative and was constant over the entire range of Na^+ and H^+ concentration tested. Net H^+ flux varied linearly with [H^+]. Extrapolation indicated apparent H^+ loss at zero luminal concentration, suggesting a basal HCO_3^- secretion. Addition of acetylsalicylic acid (ASA) or taurocholate decreased the PD to 30–40 mV and increased threefold the slope of the relation between net H^+ flux and [H^+] (k_H). Calculation of PD-independent permeability constants for H^+ (P_H) with the Goldman constant field equation indicated that this increase in k_H could not be attributed solely to the associated decrease in PD. Prednisolone administered for 3 doses had no effect on either the basal mucosal permeability to H^+ or the altered permeability induced by ASA or taurocholate. Chronic administration induced a low rate of basal acid secretion (at 12 wk) but had no effect on either PD or k_H. However, the increase in k_H and P_H that developed upon addition of ASA or taurocholate in chronically treated dogs was more than one and a half times that of controls. These data suggest that prolonged treatment with glucocorticoids increases susceptibility of the gastric mucosa to damage by agents that increase permeability to H^+.

INTRODUCTION

Ingle reported over 30 years ago that administration of large doses of glucocorticoids to rats resulted in a high incidence of gastric ulceration (1, 2). For years it was held that glucocorticoids were potentially ulcerogenic, but recent reviews of randomized, double-blind trials provide no support for this contention (3, 4). As Conn and Blitzer (4) point out however, the lack of proof for an association between glucocorticoids and peptic ulcers does not summarily exclude a role for steroids in ulcerogenesis because in controlled trials an increased incidence of peptic ulcers was observed in patients with nephrotic syndrome (5) or cirrhosis (6) who received glucocorticoids, or in other patients receiving a total dose which exceeded 1,000 mg. It has been suggested that the reduced serum binding capacity resulting from the hypoalbuminemia in nephrosis and cirrhosis may lead to high blood levels of unbound steroids. Prospective clinical studies may never conclusively settle the issue because a very large number of patients suffering from conditions marginally benefited by steroid would be required for such a trial (4).

Laboratory studies of the effects of glucocorticoids on gastric secretion have produced conflicting results. Chronic glucocorticoid administration to animals with intact adrenals has no effect on gastric secretion in the rat (7), increases acid and pepsin secretion in the dog (8), and produces equivocal findings in man (7). Whether chronic administration of glucocorticoids affects the rate of H^+ reabsorption from the stomach is not known. Cooke et al. (9) measured gastric absorption of H^+ at a single luminal concentration and showed no significant alteration in the rate of

Part of this work was presented at the Annual Meeting of the American Gastroenterological Association in Boston, Mass. 1970. *Gastroenterology.* **58:** 1038. (Abstr.)

Dr. Chung is a Clinical Investigator in the Veterans Administration Research and Educational Career Development Program.

Received for publication 30 June 1977 and in revised form 20 March 1978.

H^+ absorption in denervated fundic pouches in adrenalectomized dogs treated for 1 mo with desoxycorticosterone alone, desoxycorticosterone plus hydrocortisone, or just supplemental intake of sodium chloride. Chvasta and Cooke (10) later showed that topical instillation of hydrocortisone or cortisone acetate also had no effect on the net H^+ or electrolyte fluxes or the potential difference across the canine gastric mucosa. In view of the postulated role of increased permeability of the gastric mucosa to H^+ as a mechanism for gastric mucosal injury, we further studied in dogs the effects of acute and chronic administration of high doses of methylprednisolone either alone or in combination with acetylsalicylic acid (ASA)[1] or taurocholate on the permeability of the gastric mucosa to H^+.

METHODS

Antrectomy and denervated fundic pouches were prepared in 20 beagle dogs weighing 13–16 kg as previously described (11). 6 wk after operation the animals were feeding well and had maintained a stable weight. Food but not water was withheld for 18 h before each study. The fundic pouch, equipped with a Thomas cannula, was perfused with electrolyte solution in a recirculating circuit at 37°C at a hydrostatic pressure of less than 5 cm H_2O as described previously (11). Four sets of solutions of graded H^+ concentrations (20, 40, 80, 120, and 160 mM) were employed. One set contained 10 mM ASA in each of the solutions, the second set contained 20 mM ASA, the third set contained 20 mM Na taurocholate, and the fourth contained HCl alone. Except for the 160 mM solution, all were brought to 300±2 mosmol per kg H_2O with NaCl. They also contained polyethylene glycol (PEG, Carbowax 4000, Union Carbide Corp., New York), 10 g per liter, and [^{14}C]PEG 100 mg per liter (sp act 0.4 μCi per mg, New England Nuclear, Boston, Mass.) as a volume marker. The osmolality of the solutions containing 160 mM of H^+ and either ASA or taurocholate was 330±2 mosmol per kg H_2O. To begin the experiment, 50 ml of solution was pipetted into the reservoir and circulated by a roller pump. 5-ml samples were taken half hourly. In about two-thirds of the experiments, including all the ASA and taurocholate experiments, the solutions remained in the pouch for 60 min, whereas the rest had 90 min of perfusion for each solution. At each change of solutions, the pouch was emptied and was rinsed with a solution identical in composition to the solution to be instilled next except that it contained no [^{14}C]PEG. 10 min were allowed for complete drainage. All drainage and rinsings from the pouch were collected and measured to 0.1 ml and assayed for ^{14}C for calculation of PEG recovery. Not more than three test solutions were instilled on the same day. The sequence of instillation was randomized with respect to ascending or descending order of H^+ concentrations. The resting character of the pouch (i.e., absence of H^+ secretion in the basal state) was established in all dogs at 6 wk after operation before commencement of the main study. These preliminary tests were carried out with instillation of 50 ml of a solution of 300 mM NaCl containing volume marker, with 1-ml samples taken at 30-min intervals for detection of any net H^+ secretion.

The samples were analyzed for H^+ by titrating 1-ml aliquots with 0.1 NaOH to pH 7.00 with an automatic titrimeter (Radiometer Co., Copenhagen, Denmark). Sodium was determined by flame photometry (Instrumentation Laboratories, Inc., Lexington, Mass.). Duplicate aliquots of 1 ml were pipetted into albumin-coated planchets, dried under an infrared lamp, and counted in a gas-filled, low-background planchet counting chamber (model 1943, Nuclear-Chicago Corp., Des Plaines, Ill.). In ~two-thirds of the experiments, [^{14}C]PEG was determined by counting duplicates of aliquots of 500 μl in a liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif., LS-230) connected to on-line computer until the variability was 0.2%. Reproducibility of both methods was the same and was sufficient to detect a volume change of 0.1–0.2 ml. The PEG recoveries for over 100 experiments were 99.2±1.7 (mean ±SD) for base-line studies (including prednisolone treatment) and 98.6±2.4 for ASA and sodium taurocholate studies.

Net flux calculations were performed according to the following set of equations programmed into a digital computer. Volume (V_0) just before removing zero-time sample: $V_0 = V_i(P_i/P_0)$, where i refers to the instilled solution, and P refers to the concentration of PEG.

Volume (V_j) just before removing j^{th} sample: $V_j = (V_{j-1} - Vs)(P_{j-1}/P_j)$, where Vs refers to the sample volume (5 ml).

Net ion flux (I_j): $I_j = V_jC_j - (V_{j-1} - Vs)C_{j-1}$, where C_j refers to the concentration of the ion at the j^{th} period.

The signs of net flux measurement indicate movement into (+) or out of (−) the gastric lumen.

Gastric absorption of ASA (14 dogs) and sodium taurocholate (2 dogs) was determined by measuring the concentration of these substances in the samples of perfusate. Salicylate was determined by a colorimeteric method employing a ferric salt as reagent as modified by Trinder (12), and sodium taurocholate was determined by a dehydrogenase method according to Talalay (13).

Permeability characteristics of the gastric mucosa to hydrogen were evaluated by plotting net H^+ fluxes in μmol/30 min against the average luminal H^+ concentration for each 30-min flux period. The resulting relationships were characterized by simple linear regression analysis and the slopes were compared by analysis of covariance (14). Four sets of initial studies (namely, base-line, 10 mM ASA, 20 mM ASA, and taurocholate) were completed. Methyprednisolone sodium succinate (Solu-Medrol, Upjohn Co., Kalamazoo, Mich.) was then given by intramuscular route in three different schedules, each to one group of dogs. For single-dose experiments, an i.m. injection of methylprednisolone at 2 mg/kg was given to 4 dogs 16 h before each of the following studies conducted in sequence: ASA (20 mM), basal, taurocholate, taking 2 days for each study. These dogs thus received a total of 3 doses over a 6-day period. In the second treatment schedule, methylprednisolone was given intramuscularly at 2 mg/kg for 19 days to 4 other dogs. Beginning on the 14th day, ASA (20 mM), basal, and taurocholate studies were repeated in that order. In the third treatment schedule, another three dogs received a depot form of the steroid methylprednisolone acetate (Depo-Medrol, Upjohn Co.) at 7 mg/kg by weekly i.m. injections for 13 wk. This dose corresponds to 1 mg/kg per day of methylprednisolone sodium succinate. The ASA studies were repeated beginning 12 wk after treatment. The control ani-

[1] *Abbreviations used in this paper*: ASA, acetylsalicylic acid; ASAH, undissociated form of acetylsalicylic acid; k_H, concentration-independent coefficient for H^+ loss from the gastric lumen; PD, transmucosal electric potential difference; PEG, polyethylene glycol; P_H, PD-independent permeability constant for H^+.

mals, given intramuscular saline injections for 1 dose (two dogs), 2 wk (four dogs), and 12 wk (three dogs), were similarly tested on an identical schedule. Serum electrolytes measured before and after treatment period remained unchanged in both prednisolone-treated and control animals. No apparent ill effects were observed in the animals during the treatment period.

Transmucosal electric potential difference (PD) was measured as previously described (11). The PD probes (3-M KCl bridges) were placed in the gastric lumen and a cephalic vein. Junctional PD between Ringer's solution and various test solutions were determined with the same bridges and measuring instruments, and was found never to exceed 0.5 mV.

Gastric mucosal biopsies of the pouches performed through the Thomas cannula in the control and prednisolone-treated (both long and short terms) dogs showed no detectable differences or abnormality on light microscopy. Nor was there any readily detectable difference between biopsies obtained 6 wk after operation and 4 mo after operation in the control animals.

RESULTS

Effect of prednisolone on basal acid secretion in the denervated pouch. None of the dogs showed any secretion in the basal fasting state when tested 6 wk to 3 mo after operation. This remained unchanged when retested after treatment with prednisolone for 1 dose or for 2 wk. However, dogs treated with prednisolone for 12 wk showed a basal secretion of 34±11 μmol/30 min ($n = 6$) at the end of the treatment period, significantly different from before treatment ($P < 0.01$).

Effect of luminal H^+ concentration on net H^+ flux under base-line conditions and in the presence of ASA and taurocholate. As illustrated in Fig. 1, a linear relationship was obtained when net H^+ fluxes were plotted against $[H^+]$. The slope of this line provides a concentration-independent coefficient for H^+ loss from the gastric lumen (k_H). Values of k_H were not corrected for the surface area of each fundic pouch because each dog served as his own control and because pouches in all dogs were of approximately the same size, holding 35–37 ml of solution in the nondistended state. Mean values for k_H, correlation coefficient, and y-intercept for 14 dogs under base-line conditions are listed in Table I, and all individual data are included in Fig. 1. The negative y-intercept, which indicates apparent net loss of H^+ in the absence of luminal H^+, suggests that some HCO_3^- (or OH^-) is secreted under base-line conditions. This observation has also been made by others (15–17).

Addition of either ASA (20 mM) or taurocholate (20 mM) to the perfusate produced a threefold increase in k_H whereas the relation between net H^+ flux and $[H^+]$ remained linear (see Fig. 1 and Table I). Addition of 10 mM ASA produced a smaller increase in k_H with no effect on the linearity of the JH/$[H^+]$ plot (Table I). The mean y-intercepts were negative in all experiments, suggesting HCO_3^- secretion or absorption of ASAH or taurocholic acid (see Discussion). However, the magnitude of the intercepts was not significantly different from that measured under base-line conditions. The smaller increase in k_H seen with 10 mM ASA was associated with smaller

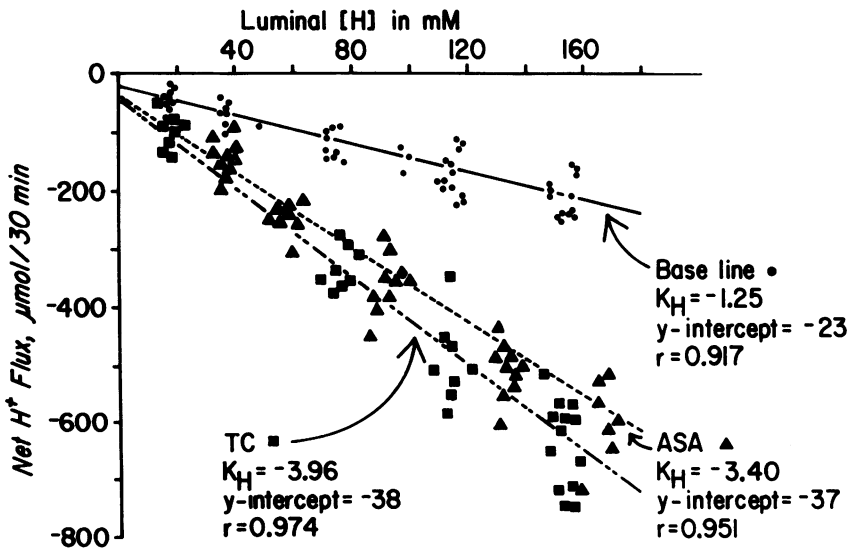

FIGURE 1 Effect of luminal $[H^+]$ on net H^+ flux under base-line conditions and when challenged with ASA and taurocholate (TC). Each point represents the mean of two flux periods. The values are slightly different from those in Table I (which gives the means of the regression statistics of individual dogs), whereas the calculations in the figures were made by fitting into one line the data from all dogs. The ASA plot refers to experiments with 20 mM ASA. Experiments with 10 mM ASA were not plotted for the sake of clarity.

TABLE I

ASA and Taurocholate-Induced Changes in k_H

Condition	n	r^2	k_H	y-Intercept	P
			ml/30 min	*μmol/30 min*	
Base-line	20	0.88–0.96	−1.18±0.23	−21±11	—
ASA, 20 mM	14	0.92–0.99	−3.40±0.37	−39±38	<0.001
ASA, 10 mM	6	0.89–0.94	−2.67±0.31	−58±18	<0.001*
Taurocholate	14	0.96–0.98	−3.92±0.64	−40±46	<0.001

Values are mean±SE; r^2 given in ranges indicate the proportion of the variance of y that can be attributed to its linear regression on x.

P refers to F test comparison between k_H's of ASA or taurocholate to base line in each individual dog.

* ASA (10 mM) < ASA (20 mM), $P < 0.01$.

amounts of ASA absorbed (78±17 μmol/30 min, $n = 12$).

Changes in k_H produced by ASA and taurocholate in prednisolone-treated dogs. Base-line k_H's determined when prednisolone had been given for 1 dose, 2 or 12 wk, were unchanged from that before treatment (see k_H column in Table II). This was also confirmed by comparison with control groups on identical schedules of saline injections, a regimen which similarly did not alter base-line k_H. A single dose of

TABLE II

PD-Independent Permeability Constants (P_H)*

Condition	n	k_H	PD	P_H	P
		ml/30 min	*mV*	*ml/30 min*	
Base-line	20	−1.20±0.24	56±3	4.07±0.25	—
ASA, 10 mM	6	−2.67±0.31	34±2	5.39±0.31	<0.01‡
ASA, 20 mM	16	−3.44±0.37	34±3	6.94±0.24	<0.001§
Taurocholate	16	−3.90±0.51	33±3	7.69±0.46	0.001§
Prednisolone, 3 doses	4	−1.16±0.31	54±3	3.75±0.40	—
Prednisolone, 3 doses ASA, 20 mM	4	−3.68±0.41	32±4	7.10±0.37	NS‖
Prednisolone, 3 doses Taurocholate	4	−3.84±0.32	35±5	7.93±0.29	NS‖
Prednisolone, 2 wk	4	−1.32±0.29	57±3	4.59±0.32	—
Prednisolone, 2 wk ASA, 20 mM	4	−5.21±0.58	34±4	10.50±0.52	<0.01¶
Prednisolone, 2 wk Taurocholate	4	−5.99±0.47	35±4	12.36±0.54	<0.01¶
Prednisolone, 12 wk	3	−1.26±0.21	56±4	4.28±0.23	
Prednisolone, 12 wk ASA, 20 mM	3	−4.89±0.40	32±4	9.43±0.55	NS**

Values are mean±SE, number of animals in second column.

* $P_H = k_H \times 0.02673 \times (1\text{-}3^{37.4(PD)})/PD$.

‡ Unpaired Student's t test between ASA (10 mM) and ASA (20 mM).

§ Student's t test of paired variates between ASA and base line, taurocholate and base line.

‖ Unpaired Student's t test between prednisolone (3 doses) + ASA (20 mM) and ASA; prednisolone (3 doses) + taurocholate, and taurocholate.

¶ Unpaired Student's t test between prednisolone (2 wk) + ASA, and ASA; prednisolone (2 wk) + taurocholate and taurocholate.

** Unpaired Student's t test between prednisolone (12 wk) + ASA and prednisolone (2 wk) + ASA.

methylprednisolone did not affect the change in k_H induced by ASA or taurocholate (Table II). However, both ASA and taurocholate produced significantly greater increases in k_H after 2 wk of prednisolone treatment than before treatment (each dog compared to itself; $P < 0.001$). Also, dogs given 2 wk of prednisolone showed a greater k_H when challenged with ASA or taurocholate than dogs given saline (Table III, Figs. 2 and 3) ($P < 0.01$).

Table IV compares the changes in k_H produced by ASA in three dogs which received depot prednisolone (Depo-Medrol) at 7 mg/kg per wk for 12–13 wk, with the findings in three dogs which received saline injections. Significantly greater k_H values were again observed in the prednisolone-treated group ($P < 0.01$). A positive y-intercept was found in the group of dogs subjected to 12 wk of prednisolone injections (Fig. 4). This was significantly different from zero and from the saline-injected controls ($P < 0.01$). ASA-induced k_H's after 12 wk of prednisolone were significantly greater than before treatment (each dog compared to itself; $P < 0.001$).

TABLE III

Effect of 2 Wk of Prednisolone Treatment on ASA and Taurocholate-Induced Changes in k_H

Condition	Dog no.	k_H	y-Intercept	r^2	P
		ml/30 min	*μmol/30 min*		
Prednisolone	1	−1.42±0.06	−39±6	0.99	NS
	2	−1.64±0.15	−1±16	0.96	
	3	−0.94±0.16	−40±15	0.86	
	4	−1.27±0.02	−27±2	0.99	
Saline	5	−1.62±0.03	−10±2	0.99	NS
	6	−1.34±0.12	−36±12	0.97	
	7	−1.21±0.04	−6±4	0.99	
	8	−1.18±0.04	−22±5	0.98	
Prednisolone ASA, 20 mM	1	−5.00±0.43	−32±50	0.97	<0.01
	2	−5.89±0.19	7±22	0.99	
	3	−4.53±0.27	11±31	0.98	
	4	−5.40±0.44	−4±40	0.97	
Saline ASA, 20 mM	5	−2.93±0.67	−65±3	0.83	<0.01
	6	−4.74±1.00	−91±5	0.85	
	7	−3.66±0.21	−18±4	0.99	
	8	−3.06±0.29	−33±3	0.97	
Prednisolone Taurocholate	1	−5.49±0.44	−39±45	0.97	<0.01
	2	−5.83±0.44	−56±45	0.98	
	3	−6.00±0.56	−69±48	0.97	
	4	−6.62±0.46	−16±42	0.99	
Saline Taurocholate	5	−3.15±0.40	−82±32	0.94	<0.01
	6	−3.74±0.28	−66±26	0.98	
	7	−2.89±0.11	−110±12	0.99	
	8	−3.10±0.37	−54±27	0.95	

Values are expressed as mean±SE (n = 20–24).
P refers to F test comparison of k_H's of appropriate groups.

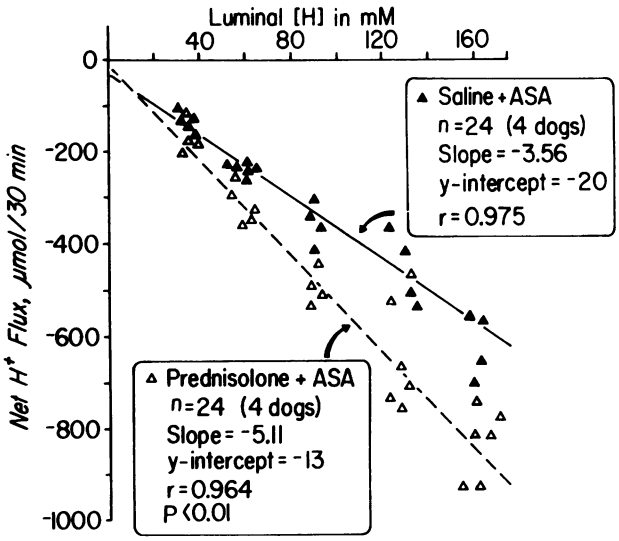

FIGURE 2 Effect of prednisolone (2 wk) on ASA-induced changes in k_H. For regression statistics of each individual animal, see Table III. Each point represents the mean of two flux periods.

Comparison of the results of the two chronic prednisolone treatment schedules showed that ASA produced similar k_H's in both groups but the y-intercept was greater (positive) in the group treated for a longer duration ($P < 0.01$). The new intercept, both in the direction and magnitude, is compatible with our findings in the base-line secretory studies in which long-term prednisolone treatment resulted in a small increase in base-line acid secretion.

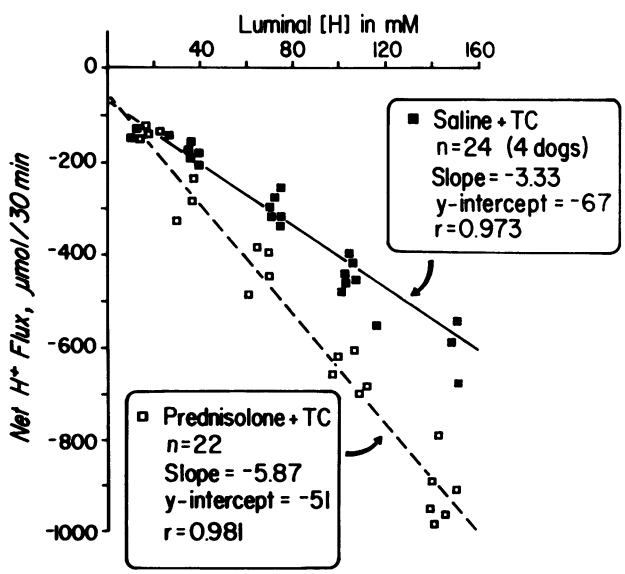

FIGURE 3 Effect of prednisolone (2 wk) on taurocholate-induced changes in k_H. For regression statistics of each individual animal, see Table III. Each point represents the mean of two flux periods.

TABLE IV

Effects of Depot Prednisolone (12 wk) on ASA-Induced Changes in k_H

Condition	Dog no.	k_H	y-Intercept	r^2	P
		ml/30 min	*μmol/30 min*		
Prednisolone	9	−5.22±0.41	52±24	0.98	<0.01
× 12 wk	10	−4.44±0.46	38±18	0.97	
ASA, 20 mM	11	−5.01±0.56	59±29	0.96	
Saline	12	−2.74±0.18	−74±25	0.98	<0.01
× 12 wk	13	−3.28±0.46	−60±19	0.92	
ASA, 20 mM	14	−3.22±0.30	−58±24	0.94	

Values expressed as mean±SE (n = 15–18).
P refers to F test comparison of k_H's of the two groups.

Effect of prednisolone and luminal acidity on gastric absorption of ASA and taurocholate. The mean rates of absorption of ASA and taurocholate from solutions of different acidity are shown in Tables V and VI. The ASA values do not differ significantly from each other. The administration of prednisolone for either 2 or 12 wk (pooled in the Table) resulted in no significant changes in absorption of ASA.

Repeated determinations of luminal absorption of taurocholate in two dogs (Table VI) showed that absorption varied with luminal acidity. Significantly greater absorption occurred in 160 mM HCl than 80 mM than 20 mM ($P < 0.01$). Prednisolone did not alter the rate of absorption of taurocholate at any concentrations of H^+ tested.

Transmucosal electric potential difference. PD was unaffected by replacing H^+ with Na^+ over the range of H^+ concentrations employed (20–160 mM). This was true for the base-line condition and also when ASA and taurocholate were added. The base-line PD varied from 50–60 mV. ASA and taurocholate decreased the PD by about 20 mV. The time course of PD change upon addition of ASA or taurocholate confirmed previous observations (18). The PD decreased within the first 15 min after ASA or taurocholate were added to about 30 mV and then remained constant. The PD under base-line conditions and after ASA and taurocholate was the same in prednisolone-treated as in control animals.

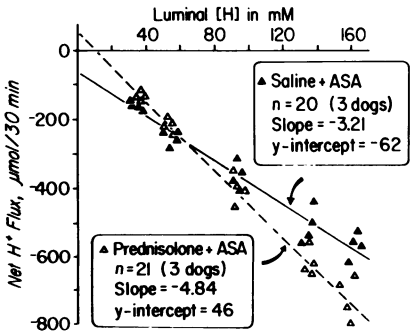

FIGURE 4 Effect of prednisolone (12 wk) on ASA-induced changes in k_H. For regression statistics of each individual animal, see Table IV. Each point represents the mean of two flux periods.

TABLE V

Lack of Effect of Prednisolone on Gastric Absorption of ASA

Condition	No.	Acid solutions* 20	40	80	120	160
Base-line	24	109±9	122±12	112±10	125±14	119±11
Prednisolone	12	121±12	136±10	112±10	121±11	129±16
Saline	12	98±11	124±12	126±11	119±16	132±15

Values are ASA absorbed given as mean±SE μmol/30 min. These are the mean rates of absorption obtained by dividing the sum of two consecutive 30-min absorptions by two. The values in each column are not significantly different from values in other columns.
* Solutions are measured in millimoles.

Determination of PD-independent permeability constant for H^+ (P_H). The linearity between net H^+ flux and luminal $[H^+]$ observed at constant PD under a variety of conditions suggests that the predominant mode of egress of H^+ from the stomach is simple ionic diffusion. Although unchanged by varying luminal $[Na^+]$ and $[H^+]$, the PD was affected by the presence of ASA and taurocholate, and the direction of this effect was such as to increase cation diffusion from the lumen. To determine whether the effects of these agents on k_H were "real" (i.e., effects which cannot be explained by the changes in PD alone) or only apparent (i.e., changes in k_H attributable entirely to changes in PD), the P_H's were calculated. The Goldman constant field equation, as derived by Hodgkin and Katz was used (19). Thus,

$$I_H = P_H \cdot \frac{F^2V}{RT} \cdot \frac{H_b - H_l \cdot e^{-VT/RT}}{1 - e^{-VF/RT}}$$

TABLE VI

Lack of Effect of Prednisolone on Gastric Absorption of Taurocholate

Condition	No.	Acid solutions* 20	40	80	120	160
Base-line	8	16±2	29±5	46±4	54±8	56±8
Prednisolone	4	20±4	29±4	39±6	52±7	54±8
Saline	4	22±4	31±5	42±6	54±8	54±6

Values are μmol/30 min of sodium taurocholate absorbed given as mean±SE.
Student's t test: 160 > 80 > 20, $P < 0.025$ for all three conditions.
* Solutions are measured in millimoles.

where I_H represents the diffusional flux of H^+ in μmol/30 min, H_b and H_l are the H^+ concentrations in blood and lumen, respectively, V is the transmucosal PD with the lumen as the reference potential, and F, R, and T have their usual connotations.

Because $H_b = 0$ and $k_H = I_H/H_l$,

$$P_H = k_H \cdot \frac{(1 - e^{VF/RT})}{VF/RT}$$

Values for P_H are shown in Table II. ASA and taurocholate significantly increased P_H. Prednisolone had no apparent effect on P_H in base-line experiments. Prednisolone-treated dogs challenged with ASA or taurocholate resulted in greater P_H's than before treatment. In the case of ASA, 2 or 12 wk of prednisolone produced equal increments in P_H. These data indicate that the increase in gastric mucosal permeability to H^+ induced by ASA and taurocholate, with and without prednisolone treatment, cannot be attributed solely to effects of the simultaneous decrease in PD on cation diffusion.

Variation of net Na^+ fluxes with changes in luminal $[H^+]$ and $[Na^+]$. Net Na^+ fluxes under varying conditions are shown in Fig. 5. Net secretion of Na^+ occurred under all circumstances, increasing as luminal $[H^+]$ was increased. Base-line Na^+ fluxes were the same in both control and prednisolone-treated dogs. ASA and taurocholate substantially increased Na^+ secretion, the largest secretory rates occurring in prednisolone-treated dogs.

DISCUSSION

The present study has examined the ionic permeability characteristics of the in vivo mammalian gastric mucosa, and how these characteristics are altered by corticosteroids, ASA, and sodium taurocholate.

The transmucosal PD remained constant (50–60 mV, lumen negative) over a wide range of luminal $[Na^+]$ (1–140 mM) and $[H^+]$ (20–160 mM), an observation made as early as 1937 by Quigley (20). Kitahara et al. (21) suggested on the basis of in vitro studies with gastric mucosa from several animals including the dog, that when the luminal pH is 2 or lower, active Na^+ absorption ceases and the PD is generated solely by active chloride secretion. This also appears to be true for canine stomach in vivo as noted by Code et al. (22). The present findings are consistent with the previous observations and suggest further that the contribution of active chloride secretion to the PD is probably unaffected by wide variations in the luminal $[Na^+]$ and $[H^+]$. Transmucosal differences in concentration of Na^+ and H^+ also do not appear to generate significant diffusion potentials across the gastric mucosa in vivo.

Net H^+ flux varied linearly with luminal $[H^+]$ under all conditions tested (base-line, pretreatment

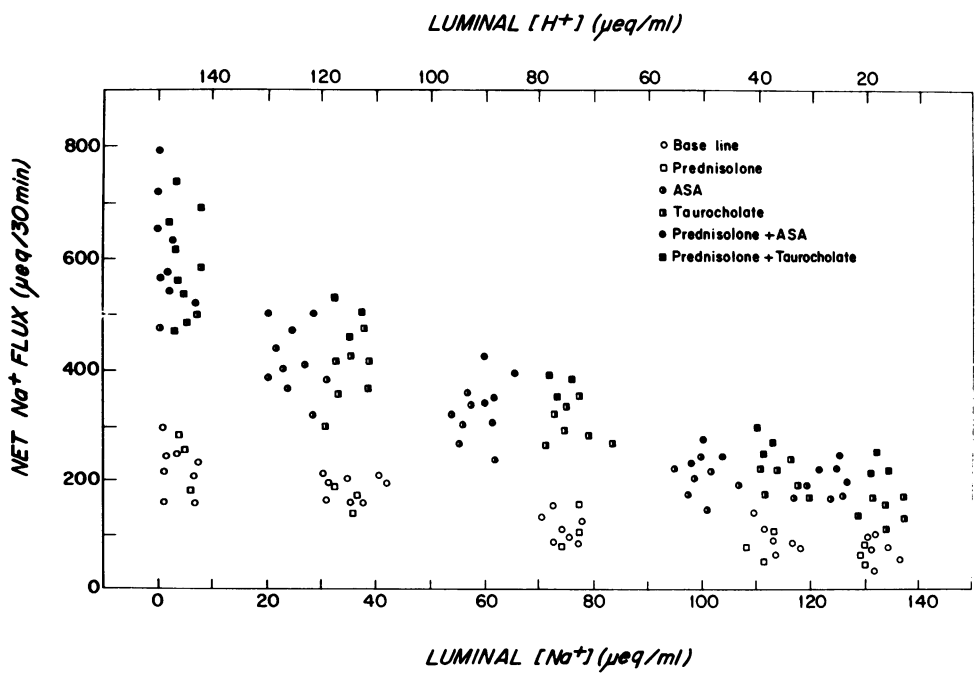

FIGURE 5 Variation of net Na^+ fluxes with luminal H^+ and Na^+ concentrations. Values from two dogs were plotted for each condition. Each point represents the average of two observations. All ASA experiments were done with 20 mM ASA.

with prednisolone, ASA, taurocholate, and combinations thereof). When these two variables are plotted (i.e., JH^+ vs. $[H^+]$), projection of the resulting line to the intercept with the y-axis indicates apparent H^+ loss at a luminal H^+ concentration of zero. This is consistent with the theory of a basal HCO_3^- or OH^- secretion, accounting for one-half of the net H^+ disappearance from the lumen at 20 mM $[H^+]$ but only 10% at 160 mM H^+. Our results confirm those of Altamirano (15) who exposed the canine gastric mucosa to different concentrations of HCl in the absence of NaCl, thus disregarding changes in osmolality. Therefore, neither luminal $[Na^+]$ nor tonicity appear to affect the fundamental relationship between H^+ flux and $[H^+]$, suggesting that H^+ disappears from nonsecreting gastric pouches as a result of both simple ionic diffusion and neutralization by secreted HCO_3^- or OH^-, the former process predominating at H^+ concentrations greater than 20 mM. Recently Flemstrom also reported active alkalinization of fundic mucosa in the bullfrog (17) amounting to 10% of maximal H^+ secretion in that species. The results of the experiments with ASA and taurocholate are those that would be predicted for agents that alter passive permeability: an increase in the slope of the $JH^+/[H^+]$ relationship and a decrease in PD presumably because of decreased resistance. Calculation of the P_H indicates that the ASA and taurocholate-induced increase in slope cannot be attributed solely to the associated decrease in PD. Indeed it is likely that the decrease in PD reflects the overall increase in passive ion permeability caused by these agents. These effects of ASA and taurocholate on PD and H^+ back diffusion are consistent with the observations of several other investigators (18, 23–25).

Net Na^+ movement into the gastric lumen was substantially increased by ASA and taurocholate. The highest rates of Na^+ secretion were observed in prednisolone-treated animals in the presence of ASA and taurocholate. Results for Na^+ are therefore entirely consistent with those obtained for H^+. The several factors influencing the net Na^+ flux (active secretion, filtration flow, diffusion, and cellular loss) are sufficiently complex to preclude a clear-cut determination of diffusional and nondiffusional components of the net Na^+ flux.

The absorption of both ASA and taurocholate are known to be pH dependent so that the linearity of the $JH^+/[H^+]$ relationship may theoretically be disturbed in two ways. First, increased absorption of undissociated organic acid at lower pH contributed more to net H^+ absorption than at higher pH. Second, increased absorption of both ASA and taurocholate may further increase mucosal permeability. In the case of ASA, which has a pK_a of 3.5, essentially all of the ASA was present in the lumen in the undissociated form over the full range of H^+ concentrations employed (20–160 mM), so that the rate of absorption may not be affected. Direct measurement of ASA absorption (Table V) showed little change over this range of H^+ concentrations. As k_H was determined from the slope of the relationship between luminal $[H^+]$ and net H^+ flux, ($JH = k_H[H^+] + k_{ASAH}[ASAH]$, where k_{ASAH} is the diffusion constant for undissociated form of acetylsalicylic acid, ASAH), k_H was not influenced by the flux of H^+ in the form of ASAH. Furthermore, as there was no demonstrable difference in ASA absorption over the whole range of $[H^+]$ studied, k_H did not further change secondary to changing rates of absorption of ASA. As taurocholic acid is 50% ionized at 25 mM H^+, taurocholate absorption would be expected to vary nonlinearly with H^+ concentration in the 20–160 mM range, as indeed it did (see Table VI). Taurocholate absorption was small, however, compared to overall H^+ absorption. The measured rate of taurocholate absorption varied from 13% of the net H^+ flux at 20 mM $[H^+]$ to 8.6% of the net H^+ flux at 160 mM $[H^+]$. Thus, the loss of linearity because of nonionic diffusion of taurocholic acid was less than 5%, which may not be seen on the plots because of experimental variations. However, the increase in k_H from increased absorption of taurocholate may well have made the graphs curvilinear, although this was not borne out from our data. It is not known how much of an increase in k_H would be caused by the demonstrated increase of taurocholate absorption at lower pH.

Neither 1 dose nor 2 wk of treatment with prednisolone induced basal acid secretion from the fundic pouch, but 12 wk of treatment did. The mechanism of this increase is unknown, but increase in basal acid secretion has also been reported in in vivo, canine, gastric secretory studies in the literature (8, 9). A single in vitro study of the mammalian gastric mucosa also demonstrated a marginally increased acid secretion in addition to increased ionic conductance when the mucosa was exposed to desoxycorticosterone (25).

Although administration of prednisolone did not by itself increase the ionic permeability, it did potentiate the action of ASA and taurocholate. The potentiation was as great after 2 wk of treatment as after 3 mo of treatment. As increased permeability induced by ASA (Table I) and bile salts (26) appeared to be related to the amount absorbed, the potentiation effect of glucocorticoid could have been explained by increased absorption of ASA or taurocholate. However, our data indicate that prednisolone had no effect on absorption of either agent thereby excluding this possibility as the explanation for the observed potentiation. Thus glucocorticoids may truly

increase the susceptibility of the gastric mucosa to damage by other agents. A possible explanation for the present physiologic observations is suggested by studies of the effects of ACTH and glucocorticoids on the rate of cell renewal in the gastric mucosa. Rasanen reported that administration of glucocorticoids to the rat resulted in a decrease in the frequency of mitotic figures in the gastric mucosa (27, 28). Loeb and Sternschein found that the administration of cortisone markedly suppressed thymidine incorporation into DNA in the gastric mucosa of young rats within 24 h (29). Max and Menguy (30) showed that after 2–4 wk of treatment with ACTH, adult dogs displayed a reduction in the mitotic index in the gastric mucosa and also a reduction in the rate of exfoliation of the mucosal cells. A steroid-induced reduction in the rate of turnover of gastric mucosal cells increases the average age of these cells. It is conceivable that the older cells are more readily damaged (or caused to exfoliate) by ASA and taurocholate than are younger cells.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Geoffrey M. Johnson, Veterans Administration Hospital, Iowa City, Iowa and the statistical advice and computation assistance of Barbara Broffitt, Department of Biostatistics, University of Iowa.

This work was supported by U. S. Public Health Service grants AM11079, AM13485, and AM05114 from the National Institutes of Health, Department of Health, Education and Welfare, a grant from the John A. Hartford Foundation, and by Veterans Administration Research grant MRIS 1404.01.

REFERENCES

1. Ingle, D. J., R. Sheppard, J. S. Evans, and M. K. Kuizenga. 1945. A comparison of adrenal steroid diabetes and pancreatic diabetes in the rat. *Endocrinology.* **37:** 341–356.
2. Ingle, D. J., M. C. Prestrud, and J. E. Nezamin. 1951. Effects of administering large doses of cortisone acetate to normal rats. *Am. J. Physiol.* **166:** 171–175.
3. Cushman, P., Jr. 1970. Glucocorticoids and the gastrointestinal tract: current status. *Gut.* **11:** 534–539.
4. Conn, H., and B. L. Blitzer. 1976. Nonassociation of adrenocorticosteroid therapy and peptic ulcer. *N. Engl. J. Med.* **294:** 473–479.
5. Black, D. A. K., G. Rose, and D. B. Brewer. 1970. Controlled trial of prednisone in adult patients with the nephrotic syndrome. *Br. Med. J.* **3:** 421–426.
6. Copenhagen Study Group for Liver Diseases. 1969. Effect of prednisolone on the survival of patients with cirrhosis of the liver. *Lancet.* **I:** 119–121.
7. Cooke, A. R. 1967. Role of adrenocortical steroids in the regulation of gastric secretion. *Gastroenterology.* **52:** 272–281.
8. Sun, D. C. H. 1969. Effect of corticotropin on gastric acid, pepsin, and mucus secretion in dogs with fistulas. *Am. J. Dig. Dis.* **14:** 107–112.
9. Cooke, A. R., R. M. Preshaw, and M. I. Grossman. 1966. Effect of adrenalectomy and glucocorticoids on the secretion and absorption of hydrogen ion. *Gastroenterology.* **50:** 761–767.
10. Chvasta, T. E., and A. R. Cooke. 1972. The effect of several ulcerogenic drugs on the canine gastric mucosal barrier. *J. Lab. Clin. Med.* **79:** 302–315.
11. Chung, R. S. K., M. Field, and W. Silen. 1973. Permeability of gastric mucosa to hydrogen and lithium. *Gastroenterology.* **64:** 593–598.
12. Trinder, P. 1954. Rapid determination of salicylate in biological fluids. *Biochem. J.* **57:** 301–303.
13. Talalay, P. 1960. Enzymic analysis of steroid hormones. *Methods Anal. Biochem.* **8:** 119–143.
14. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. 6th edition. Iowa State University Press, Ames, Iowa. 432.
15. Altamirano, M. 1970. Back diffusion of H^+ during gastric secretion. *Am. J. Physiol.* **218:** 1–6.
16. Bugajski, J., C. F. Code, and J. F. Schlegel. 1972. Sodium-hydrogen ion exchange across canine resting gastric mucosa. *Am. J. Physiol.* **222:** 858–863.
17. Flemstrom, G. 1977. Active alkalinization by amphibian gastric fundic mucosa in vitro. *Am. J. Physiol.* **233:** E1–E12.
18. Geal, M. G., S. E. Phillips, and W. H. Summerskill. 1970. The profile of gastric potential difference in man: effects of aspirin, alcohol, bile, and endogenous acid. *Gastroenterology.* **58:** 537–543.
19. Hodgkin, A. L., and B. Katz. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (Lond.).* **108:** 37–77.
20. Quigley, J. P., J. Barcroft, G. S. Adair, and E. M. Goodman. 1937. The difference in potential across gastric membranes and certain factors modifying the potential. *Am. J. Physiol.* **119:** 763–767.
21. Kitahara, S., K. R. Fox, and C. A. M. Hogben. 1969. Acid secretion, Na^+ absorption, and the origin of the potential difference across isolated mammalian stomachs. *Am. J. Dig. Dis.* **14:** 221–238.
22. Code, C. R., J. H. Higgins, J. C. Moll, A. L. Orvis, and J. F. Scholer. 1963. The influence of acid on the gastric absorption of water, sodium, and potassium. *J. Physiol. (Lond.).* **166:** 110–119.
23. Davenport, H. W. 1965. Damage to the gastric mucosa: effects of salicylates and stimulation. *Gastroenterology.* **49:** 189–196.
24. Ivey, K. J. 1971. The gastric mucosal barrier. *Gastroenterology.* **61:** 247–257.
25. Hogben, C. A. M., and D. R. Karal. 1973. *In* Transport Mechanisms in Epithelia. H. H. Ussing and N. A. Thorn, editors. Academic Press, Inc., New York. 240.
26. Black, R. B., D. Hole, and J. Rhodes. 1971. Bile damage to the gastric mucosal barrier: the influence of pH and bile acid concentration. *Gastroenterology.* **61:** 178–184.
27. Rasanen, T. 1962. Mitotic activity in rat epidermis and gastric mucosa after gluco- and mineralocorticoid administration. *Growth.* **26:** 1–14.
28. Rasanen, T. 1963. Fluctuations in the mitotic frequency of the glandular stomach and intestine of rat under the influence of ACTH, glucocorticoids, stress and heparin. *Acta Physiol. Scand.* **58:** 201–210.
29. Loeb, J. N., and M. J. Sternschein. 1973. Suppression of the thymidine incorporation into the gastric mucosa of cortisone-treated rats: possible relation to glucocorticoid-induced gastric ulceration. *Endocrinology.* **92:** 1322–1327.
30. Max, M., and R. Menguy. 1970. Influence of adrenocorticotropin, cortisone, aspirin, and phenybutazone on the rate of exfoliation and the rate of renewal of gastric mucosal cells. *Gastroenterology.* **58:** 329–336.