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Effect of Severe Anoxia on the Permeability of Gastric Mucosa[1] (38517)

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There is evidence that ischemia by causing anoxia, may play a part in increasing hydrogen ion back diffusion and in the formation of gastric stress ulceration (1–4). However, the evidence is conflicting (5, 6) and the relative roles of anoxia or diminution of blood flow are uncertain, perhaps because the degree of ischemia and anoxia are difficult to control *in vivo*. However, if anoxia is pathogenetically important in increasing hydrogen ion back diffusion, then severe anoxia should have a marked effect. For that reason, the effect of severe anoxia on the hydrogen ion back diffusion and the permeability of the gastric mucosa to erythritol was investigated using an *in vitro* preparation of rabbit gastric mucosa.

Methods. Hydrogen ion back diffusion experiments. Five to six lb male New Zealand white rabbits were sacrfiiced by a blow to the back of the head, and a piece of fundic gastric mucosa was excised, stripped of the muscle layers, and mounted in an Ussing chamber of surface area 1.96 cm^2. Each side of the tissue was perfused separately with a Ringer's solution containing 136 m*M* NaCl, 5 m*M* KCl, 3.6 m*M* $CaCl_2 \cdot 2H_2O$ and 1.2 m*M* $MgCl_2 \cdot 6H_2O$. The perfusing solutions were oxygenated and circulated by a humidified stream of 100% oxygen, and kept at 37° by a water jacket. The pH of both the mucosal and serosal solutions were maintained independently at pH 7.4 by the addition of either 0.01 *N* hydrochloric acid or 0.01 *N* sodium hydroxide from two separate pH stats (Radiometer, Copenhagen).

The potential difference across the mucosa in millivolts was measured on a digital voltmeter (Keithley Instruments) using salt agar bridges and calomel electrodes. A second set of silver–silver chloride electrodes were used to pass a current to short-circuit the tissue and reduce the electrical gradient to zero. The tissue resistance was calculated in ohm cm^2 from the magnitude of deflection of the potential difference read within 15 sec of passing a standard current to 100 μm amps across the tissue. There was no evidence of polarization.

Back diffusion studies were performed after the electrical properties of the tissue had stabilized, which was about 1 hr. The $[H^+]$ of the mucosal solution was then altered to 0.1, 1.0, 2.0 and 3.2 mEq/L in separate experiments, and the back diffusion of hydrogen ions measured as the amount of 0.05 *N* hydrochloric acid added to maintain the pH constant. The volume change from adding HCl was always less than 3% and therefore was not taken into account. Higher concentrations of HCl were not used because preliminary studies indicated that they were uniformly associated with rapid and severe disintegration of the tissue. All back diffusion measurements were made over a period of 1 hr with the perfusing solutions bubbled with 100% oxygen, and a further 1.5 hr with the tissue rendered severely anoxic by gassing with 100% nitrogen in place of oxygen. All measurements were taken in the short-circuited state.

Erythritol flux experiments. The tissue was mounted in a manner similar to that used in the back diffusion experiments, but both perfusing solutions were 15 ml of phosphate buffered Ringer's solution at pH 7.0 containing 1 m*M* erythritol. At the start of the flux period 5 μCi of ^{14}C-erythritol (Amersham/Searle) were added to the mucosal solution, and at 15 min intervals 1000λ samples were taken from the serosal solution for scintillation counting, and replaced with 1000λ of Ringer's solution containing 1 m*M* erythritol to maintain the volume and erythritol concentration constant. 10λ samples were taken from the mucosal solution for counting at the beginning and at the end of the experiment. These data were used to

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work out the specific activity of the mucosal solution and the transmucosal flux of erythritol for each 15 min period. The flux of erythritol was expressed in pmoles/cm²/sec. The samples for counting were added to 15 ml of Bray's solution, cooled to 4° and dark-adapted for 1 hr before counting each sample for 10 min in a liquid scintillation counter (Nuclear Chicago).

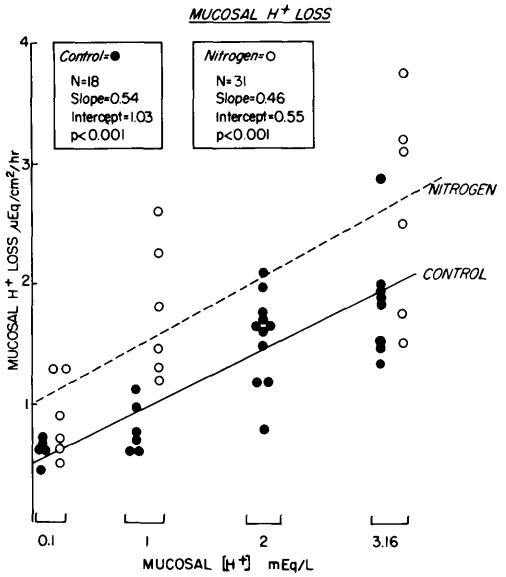

FIG. 1. The relationship of loss of hydrogen ion from the mucosal solution to hydrogen ion concentration of the mucosal solution in control and anoxic tissue. The solid and dashed lines are drawn through the average values of H^+ loss at the various mucosal H^+ concentrations.

The flux measurements were conducted over a 3-hr period. For the first hour the perfusing solutions were bubbled with 100% oxygen, and for the second and third hours the tissue was rendered severely anoxic by gassing with 100% nitrogen. Control tissues were gassed with 100% oxygen for 3 hr. Experiments were carried out with the mucosal solution at either pH 7.0 or pH 3.0, and the serosal solution at pH 7.0 in all experiments.

Results. Back diffusion of hydrogen ions. In normal oxygenated tissue hydrogen ion back diffusion increased as the pH of the mucosal solution fell. When hydrogen ion back diffusion is plotted against the hydrogen ion concentration of the mucosal solution, the resulting regression is 0.46 ($P < 0.001$) (Fig. 1). There is a similar pattern in anoxic tissue with a regression of 0.54 ($P < 0.001$) (Fig. 1).

After 1 hr of severe anoxia hydrogen ion back diffusion was increased when compared with the controls for the same pH, but the increase only became statistically significant at all pH values after 1.5 hr of anoxia (Table I).

Flux of erythritol. The flux rate of erythritol from the mucosal to the serosal solution remained constant for 45 minutes after the start of severe anoxia, following which the rate rose progressively although these values never became statistically significantly different from those of tissues exposed continuously to 100% oxygen (Fig. 2). However,

TABLE I. THE EFFECT OF ANOXIA ON THE LOSS OF HYDROGEN ION FROM THE MUCOSAL SOLUTION AT pH 4.0, 3.0, AND 2.5.[a]

	Mucosal H^+ loss μEq/cm²/hr		
	$[H^+]$ of mucosal solution (mEq/L)		
	0.1	1.0	3.2
100% Oxygen $N = 31$	0.60 ±0.04	0.91 ±0.13	1.89 ±0.15
100% Nitrogen 1 hr $N = 18$	0.89 ±0.30 ($P < 0.05$)	1.77 ±0.23 ($P < 0.005$)	2.63 ±0.37 N.S.
100% Nitrogen 1.5 hr $N = 16$	0.88 ±0.10 ($P < 0.05$)	1.90 ±0.22 ($P < 0.005$)	3.32 ±0.41 ($P < 0.05$)

[a] ($P \lesssim 0.05$) significantly different from oxygenated tissue at same $[H^+]$. N.S. = Not significantly different from oxygenated tissue at same $[H^+]$.

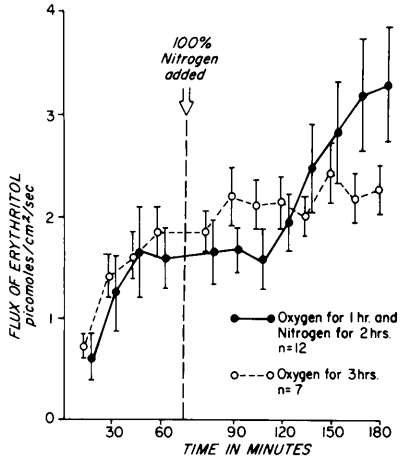

FIG. 2. The effect of anoxia on the flux of erythritol from mucosa to serosa at pH 7.0. Note that open circles represent tissues exposed to 100% oxygen for 3 hr. Nitrogen was used only in tissues represented by the closed circles.

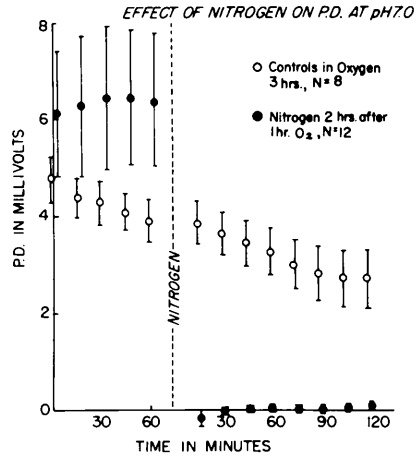

FIG. 3. The effect of anoxia on potential difference.

when comparing the same tissues before and after the introduction of anoxia (closed circles Fig. 2), there was a statistically significant increase from baseline values in the flux rates of erythritol after 150 min of anoxia $P < 0.05$.

The pattern of the transmucosal flux of erythritol at pH 3.0 was the same as that for pH 7.0, there being no statistical difference.

Electrical properties. 100% nitrogen caused an immediate fall in potential difference to zero in contrast to the slow gradual decline in potential difference observed in control tissue throughout the experiment (Fig. 3). A similar fall in potential difference was recorded in anoxic tissue at pH 3.0.

The tissue resistance rose immediately in response to anoxia and after 30 min fell progressively reaching preanoxic levels after 90–120 min. This is a marked contrast to the steady resistance maintained over 3 hr in control tissue (Fig. 4). The response of the resistance to anoxia is not statistically different at pH 3.0 and pH 7.0. At both pH 3.0 and pH 7.0, there was an immediate rise in resistance for 30 min with a subsequent fall reaching preanoxic levels after 90–120 min of severe anoxia (Fig. 4).

Discussion. Using *in vitro* rabbit gastric mucosa we have previously shown the transmucosal flux of erythritol to be a reliable indicator of mucosal permeability (7).

These experiments show that anoxia increased mucosal permeability only when prolonged to an extent that is unlikely to be met in an *in vivo* situation. Our findings indicate also that the effect of anoxia was not dependent upon the pH of the mucosal solution between pH 2.5 and 7.0. Good evidence that our tissues were indeed anoxic was the immediate and marked fall in potential difference which was a result of inhibition of active cellular transport mechanisms. The cause of the rise in resistance occurring immediately after the introduction of anoxia is uncertain, but it could be the result of obliteration of intercellular channels caused by cellular swelling recently demonstrated in anoxia piglet gastric mucosa by Machen and Forte (8). The subsequent fall in resistance reaching preanoxic levels by 2 hr may be due to mucosal cell damage. The prolonged maintenance of resistance in the presence of severe anoxia has also been observed in the frog gastric mucosa by Heinz and Durbin (9), who noted that the measured total conductance only rose after 18 hr of anoxia.

On the other hand, the increase in back diffusion of H^+ observed after 1 hr of anoxia might be explained by the smaller size of the hydrogen ion in comparison with erythritol, so that a small increase in mucosal permeability might be accompanied by an increase in hydrogen back diffusion before an increase in transmucosal flux of erythritol is detectable. In addition, it must be remem-

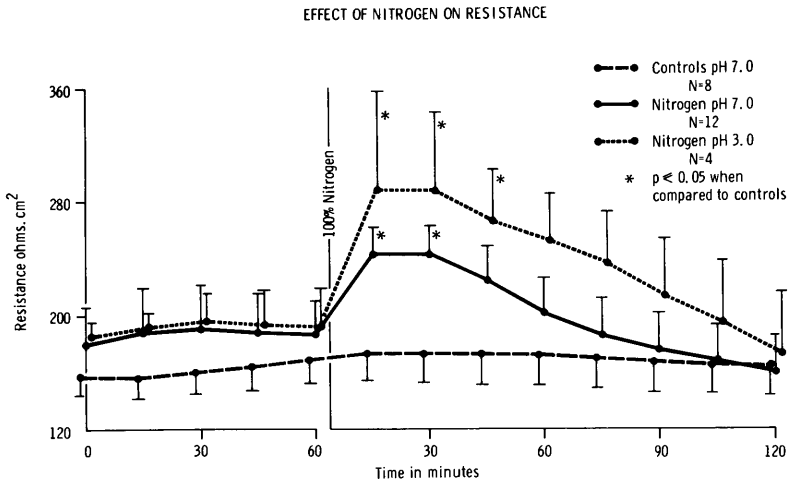

FIG. 4. Effect of anoxia on tissue resistance at pH 7.0 and 3.0 compared with controls at pH 7.0.

bered that in our experiments back diffusion of hydrogen ions was estimated by measurement of hydrogen ion loss from the mucosal solution whereas the movement of erythritol was measured by its appearance in the serosal solution. The two measurements are therefore not directly comparable.

Our method of estimation of back diffusion, as in all previous studies, suffers from an inability to differentiate between changes in secretion of hydrogen ions and changes in back diffusion. The apparent increase in back diffusion in our experiments could reflect to some extent the depression of acid secretion caused by anoxia. However, as acid secretion is almost immediately depressed by anoxia, a false increase in back diffusion would have been detectable sooner than 1 hr.

In rabbits subjected to hemorrhagic shock, Harjola and Sivula demonstrated mucosal vasoconstriction and ulceration, and Skillman *et al.* (4) showed increased hydrogen ion back diffusion and ulceration. Both of these groups suggested that anoxia was at least partially responsible for the observed effects. However, Mullane *et al.* (5) showed no effect of anoxia on the gastric mucosal barrier unless combined with circulatory changes. Hamza and Den Besten (10) could only demonstrate ulceration in dogs subjected to hemorrhagic shock when bile was allowed to reflux through the pylorus or when bile salts and acid were added to the stomach. When bile reflux was prevented by pyloric ligation, ulceration was not observed.

It is unlikely that the *in vivo* stomach is ever subjected to the severe and prolonged anoxia used in our current experiments *in vitro* in which an increase in mucosal permeability and hydrogen ion back diffusion was found. It is therefore probable that if anoxia plays a role in increasing back diffusion and/or causing formation of stress ulcers, it must act in concert with other factors.

Summary. The effect of severe anoxia produced by gassing with 100% nitrogen on gastric mucosal permeability and hydrogen ion back diffusion was investigated using an *in vitro* preparation of rabbit fundic gastric mucosa mounted in an Ussing chamber. Permeability was estimated by determination of the flux of the water soluble, nonlipid soluble molecule erythritol from the mucosal to serosal solution. The flux rate across normal tissue was 2.80 ± 0.41 pmoles/cm²/sec, and rose to 3.32 ± 0.57 pmoles/cm²/sec after 2 hr of severe anoxia. Hydrogen ion back diffusion was measured by determining with a pH stat the amount of hydrogen ion required to maintain the $[H^+]$ of the mucosal solution at 0.1, 1.0, 2.0 and 3.2 mEq/L in both normal and anoxic tissues. One hour of anoxia increased the back diffusion of H^+, but the changes only became statistically significant at all pH values after 1.5 hr. Anoxia did however cause an immediate fall in potential difference to zero, and a rise in resistance which after 30 min fell progressively to preanoxic levels. Anoxia produces a small

increase in gastric mucosal permeability, an effect which may be enhanced by other factors.

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