Proceedings of the Iowa Academy of Science

Volume 77 | Annual Issue Article 20

1970

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Failure of Thiocyanate to Influence The Flux of Sodium Through The Isolated Frog Gastric Mucosa

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Abstract. Isolated stomachs from mammals showed substantial active absorption of sodium (Am. J. Dig. Dis. *14*:221-238, 1969) in contrast to isolated frog gastric mucosa. Could the difference have been due to a "micro-environment" in the latter preparation based on fine structure features that maintained high H-ion concentration at the site of active transport? To answer this, H-ion secretion was inhibited in frog gastric mucosa with 15 mM thiocyanate. Unidirectional fluxes of sodium were not materially affected. These results cast doubt on a possible depression of active sodium absorption by a high H-ion concentration in the "micro-environment" of the isolated amphibian mucosa.

A description of the major ionic movements through the gastric mucosa which are associated with its electrophysiology and the production of HCl is beginning to emerge. Apart from the elasmobranchs (Hogben, 1967), the mucosae of vertebrates not only secrete acid but generate a characteristic transepithelial potential difference of about 35 mv, oriented such that the luminal face is negative with respect to the interstitial surface. Depending on the species and the preparation being studied, there appears to be two different origins for the generation of this electrical potential.

In the case of the isolated frog gastric mucosa, for which most information is available, the situation is reasonably clear (Hogben, 1965). The transepithelial potential difference arises from a current of Cl^- ions that are being secreted towards the lumen or mucosal surface at a rate in excess of the secretion in the same direction of H^+ ions. A net movement of Na^+, from mucosa to serosa, does not contribute significantly to the electrical current (Kitahara et al, 1969). There is a highly efficient transport of K^+ from mucosal to serosal surface but it is quantitatively insignificant (unpublished observations).

When the surface of the secreting gastric mucosa of the dog *in situ* is bathed by iso-osmotic HCl, a more or less similar picture obtains (Rehm, 1953). Since neither Na^+ or K^+ are initially present at the mucosal surface, there can not be a net ionic movement of either species from mucosa to serosa. Rather each moves or is secreted in the direction of interstitial fluid to lumen. Consequently, the electrical current must arise once again from a discrepancy between the rates at which Cl^- and H^+ are being secreted into the lumen, the former being favored.

To our surprise the ionic movements encountered in the isolated mammalian stomach differ in an important respect. To a degree,

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depending on the species and the mode of study, the active transport of Na^+ from mucosa to serosa contributes importantly or is almost exclusively responsible for the generation of the electrical potential difference (Kitahara et al, 1969). These *in vitro* preparations only secrete acid at a modest rate and under the conditions of study, the pH of the solution bathing the mucosal surface did not fall below 4-3. By contrast the actively secreting dog stomach *in situ*, whose mucosal surface is bathed by iso-osmotic HCl, is being bathed by a solution with a pH of less than 1. Unlike the *in vitro* canine gastric mucosa, the resting dog stomach *in situ* does actively absorb Na^+ from mucosa to serosa (Bornstein et al, 1959).

There appears to be a way to explain the difference between the absorption of Na^+ by the *in vitro* canine gastric mucosa and the secreting dog stomach *in situ*. Acidification of the solution bathing the mucosal or luminal surface of the canine stomach *in situ* suppresses the flux of Na^+ from mucosa to serosa, and by implication suppresses the active absorption of Na^+ (Code et al, 1963). Our own tentative results suggest that acidification of the mucosal solution bathing the *in vitro* canine gastric mucosa also inhibits the active transport of Na^+ (Kitahara et al, 1969).

I would have liked to have gone a step further and attempted to reconcile the observations of the *in vitro* frog gastric mucosa with those of the *in vitro* dog gastric mucosa. As we have carried out our studies on the *in vitro* amphibian gastric mucosa, the pH of the mucosal solution bathing the mucosal surface does not fall below 4-3. By the standards necessary to inhibit Na^+ transport by the canine mucosa this would appear to be insufficient, yet Na^+ transport is insignificant. The question becomes, does the concentration of H^+ bathing the site of transport determine whether there is significant transport of Na^+ contributing to the generation of the electrical current?

Elsewhere I have developed the argument that for the isolated frog gastric mucosa, the critical pH is not that of the bulk solution bathing the mucosa but that of a "micro-environment" at the site of ionic transport that is relatively discrete from the bulk solution (Hogben, 1962). To test the hypothesis that the concentration of H^+ at the site of transport might determine whether there is a significant active absorption of Na^+ at its site of transport, I have chosen to inhibit the secretion of H^+ with thiocyanate and thus, presumably, to raise the pH of the "micro-environment" of the isolated frog gastric mucosa.

For this purpose I have employed techniques previously described (Mohammed and Hogben, 1964). The gastric mucosa of the bullfrog, *R. catesbiana* was stripped from the thick muscle coat and mounted as a flat membrane between two halves of a

plastic flux chamber. Each surface of the mucosa was bathed by identical bicarbonate-buffered electrolyte solutions having the common ions in their usual concentrations and 5 gm of glucose per liter. The solutions were gassed with 95% O_2, 5% CO_2. To inhibit hydrogen ion secretion, 15 mM SCN was incorporated in the solutions. There was provision for monitoring the transmucosal electrical potential difference and also for voltage clamping that potential at any desired value. In these experiments the transmucosal electrical potential difference was abolished or the mucosa was maintained in the short-circuited state such that there was no difference of electro-chemical potential. Under these circumstances, the unidirectional fluxes of an ion should be equal in the two directions unless the mucosa itself were using metabolic energy to "pump" an ion in one direction

To determine the unidirectional flux of Na^+, ^{22} Na was added to one side of the mucosa and, after a period allowing attainment of isotopic steady state, the opposite side of the mucosa was sampled for the fraction of isotope that had moved through the mucosa leading to a calculation of the unidirectional flux.

Table 1.

Na Flux In the Presence of 15 mM NaSCN

	$M_{m \to s}$			$M_{s \to m}$		
	x	SE	n	x	SE	n
Flux μEq $cm^{-2}hr^{-1}$	0.36	±0.02	8	0.21	±0.01	4
I_{sc} μEq $cm^{-2}hr^{-1}$	1.95	±0.22	8	2.24	±0.43	4
Terminal PD mv	40.87	±3.22	6	53, 49		2
Terminal Conductance m . mhos cm^{-2}	1.41	±0.11	6	2.0, 1.2		2
Flux successive 45′ periods μEq $cm^{-2}hr^{-1}$						
1	0.38	±0.03	8	0.20	±0.02	4
2	0.36	±0.02	8	0.21	±0.01	4
3	0.34	±0.02	8	0.20	±0.01	4
4	0.35	±0.02	8	0.21	±0.004	4

M = flux, m = mucosa, s = serosa

The results are given in Table 1. There is a statistically significant difference in the unidirectional fluxes in the presence of 15

mM NaSCN in the direction of an active absorption of Na^+ from the mucosal to the serosal surface. However, when compared with our previous study (Kitahara et al, 1969) in the absence of thiocyanate and a resultant inhibition of H^+ secretion, the fluxes are not very different and the net active absorption is insignificant. For the original hypothesis to be sustained, thiocyanate inhibition of H^+ secretion should have led to a 10 fold increase in the value of the unidirectional and net flux of Na^+.

It is concluded that the "micro-environment" hypothesis is not an adequate explanation for the difference between the isolated amphibian and mammalian stomachs.

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