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Effect of Ouabain on Acid Secretion and Electrolyte Content of Frog Gastric Mucosa.* (27595)

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We have described some aspects of the electrolyte composition of frog gastric mucosa as the first step in a study of the relation between cell composition and secretory ability (1). In this paper we show that the concentration of ouabain (G-strophanthin) which inhibits acid secretion also causes K in the tissue to fall and Na to rise. If the mucosa is incubated in a solution in which part of the Na is replaced by K, tissue concentrations are returned toward normal, and acid secretion is restored.

Methods. Our methods for measuring acid secretion by gastric mucosal sacs of *Rana pipiens* have been described(2). The only change in technic is that the tissues are now always incubated under a Po_2 of approximately 2,000 mm Hg which reduces aerobic glycolysis to a negligible rate. Likewise, our methods for incubating mucosas for determination of volumes of distribution of radioiodinated serum albumin (RISA), inulin and mannitol and our analytical methods for tissue electrolytes have been described, together with evidence showing that the 4-hour volume of distribution of RISA is probably a correct estimate of the extracellular space(1).

Results. The inhibitory effect of ouabain on acid secretion by frog gastric mucosa is shown in Fig. 1.

The effect of 10^{-4} M ouabain on electrolytes and spaces of RISA and inulin in frog gastric mucosa is shown in Fig. 2. $[Na]_o$ was 117 mM, $[K]_o$ 9 mM and $[Cl]_o$ 104 mM. The control values are the cumulative ones collected in our laboratory over a 2-year period, and include observations made in parallel with those in which ouabain was used. Consequently, the differences can be attributed to ouabain and not to seasonal variations or changes in methods. The climbing curves for RISA and inulin show diffusion of the substances into the tissue over the 4-hour period. The curves for H_2O, Na, K and Cl show the amounts in the tissues at time of analysis. Ouabain produces no change in total H_2O, but reduces both RISA and inulin spaces. Four-hour mannitol spaces, not shown in the figure, were for control mucosas 545 $\pm$ SEM 17 ($n = 30$) and for ouabain-treated mucosas 410 $\pm$ SEM 10 ($n = 20$). The parallel reduction in these 3 spaces without increase in total H_2O shows that the cells swelled at the expense of extracellular fluid space. During incubation tissue Na rose and K fell, doubtless reflecting similar changes in intracellular concentrations. On the assumption that the 4-hour RISA space is true extracellular volume the intracellular concentrations can be calculated. However, it seems likely that there are at least 2 cell types in

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TABLE I. Rates of Acid Secretion and Water, Sodium and Potassium Contents of Frog Gastric Mucosas Incubated 1 Hr in Salt Solutions with or without 10^{-4} M Ouabain.

	Ouabain	$[K]_o$ 9 $[Na]_o$ 120	29 100	44 85
qH^+	—	285 ± 8 (20)†	275 ± 16 (25)	209 ± 11 (15)
pM/mg dry wt/hr	+	113 ± 13 (19)	258 ± 14 (25)	184 ± 12 (14)
H_2O	—	827 ± 5 (15)	839 ± 3 (14)	841 ± 2 (15)
g/kg wet wt	+	836 ± 3 (14)	849 ± 3 (15)	838 ± 2 (14)
RISA space*	—	235 ± 6 (94)	191 ± 13 (10)	151 ± 15 (10)
g/kg wet wt	+	133 ± 11 (20)	143 ± 11 (10)	164 ± 11 (10)
Na	—	71 ± 3 (13)	51 ± 1 (15)	43 ± 1 (14)
meq/kg wet wt	+	91 ± 3 (15)	63 ± 1 (15)	52 ± 1 (15)
K	—	54 ± 2 (14)	64 ± 2 (15)	67 ± 1 (15)
meq/kg wet wt	+	34 ± 1 (15)	50 ± 1 (15)	57 ± 1 (15)

* 4-hr value. † Mean ± SEM (n).

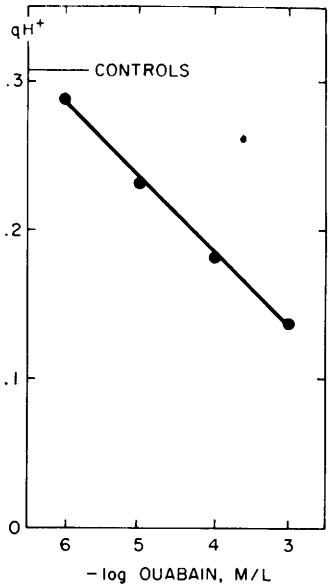

FIG. 1. Ordinates: rates of acid secretion by frog gastric mucosas in μM/mg dry wt/hr at 25°C. Abscissae: -log concentration of ouabain in M/l. Each point is the mean of 5 or more determinations.

the frog gastric mucosa: i) those similar to striated muscle in composition contained high K and low Na, and ii) those containing high Na and Cl(1). We have suggested that the first kind are the surface epithelial cells and the second the oxyntic cells. In the absence of a sound method of partitioning the electrolytes among the cell types any calculation of "intracellular" concentrations must give a nearly meaningless average.

Mucosas were incubated 1 hour in a series of salt solutions whose K concentrations were elevated in 5 mM steps and whose Na concentrations were correspondingly reduced. Results are given in Fig. 3 where it can be seen that at $[K]_o$ of 29 mM or higher acid secretion in the presence of 10^{-4} M ouabain is almost as fast as in its absence. Electrolyte data at 3 K concentrations are given in Table I.

Discussion. The effect of ouabain on the gastric mucosa is similar to its action on the kidney where it inhibits acid secretion and causes intracellular Na to rise and K to fall (3,4). Ouabain and related cardiotonic glycosides have the general property of blocking intracellular accumulation of K and extrusion of Na(5,6), and there is a question whether

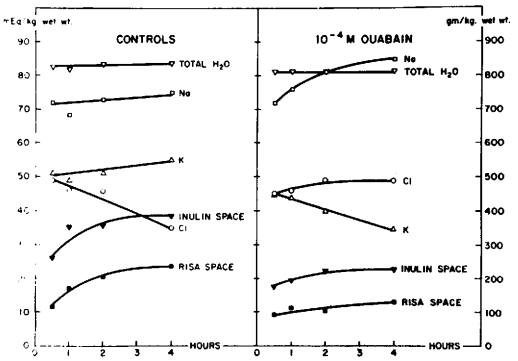

FIG. 2. Ordinates: left, Na, K, and Cl in frog gastric mucosa in meq/kg wet wt; right, H_2O and volumes of distribution of RISA and inulin in g/kg wet wt. Abscissae: time of incubation at 25°C. Incubation solution contained Na 117 mM, K 9 mM, Cl 102 mM, histamine 0.1 mM and glucose 11 mM. Control points are means of from 9 to 94 observations made over a period of 2 yr. Ouabain points are means of from 19 to 25 observations made concurrently with many of the control observations.

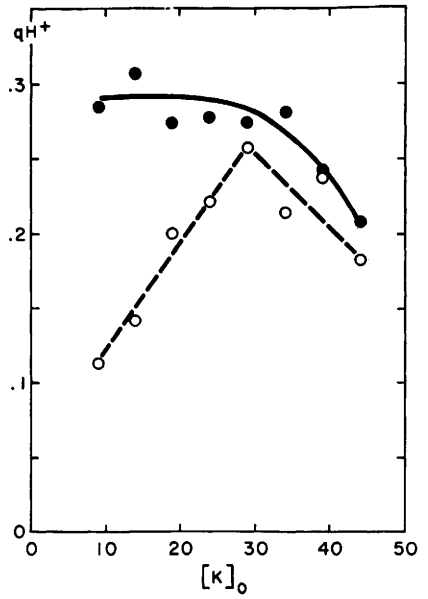

FIG. 3. Ordinates: rates of acid secretion by frog gastric mucosas in μM/mg dry wt/hr at 25°C. Abscissae: K concentrations in mM. Sum of K and Na in all solutions was 129 mM. Each point is the mean of 10 to 25 observations. Solid line is for results without ouabain; interrupted line is for results with 10^{-4} M ouabain.

other effects of the drugs are primary ones or are merely secondary to their alteration of intracellular electrolyte patterns. Burg and Orloff(4) have given a thorough analysis of this problem in the case of kidney tissue, and have demonstrated insofar as is experimentally possible that inhibition by strophanthidin of PAH accumulation by kidney slices is secondary to K depletion. Although they present much other pertinent evidence, their major point is that depression of PAH accumulation caused by strophanthidin can be reversed by increasing the K content of the medium and thereby of the cells. We present similar evidence to explain the inhibition of gastric acid secretion by ouabain, and although other explanations remain possible if not plausible we conclude that at least part of the drug's effect is expressed through alteration of intracellular environment. If we were able to give a valid calculation of intracellular composition of the oxyntic cells under our experimental conditions we could specify the intracellular electrolyte composition required for acid secretion. Until this becomes possible we present our data with the expectation that they will become useful when the basis of such a calculation is at hand.

Summary. Ouabain in concentrations from 10^{-3} to 10^{-6} M inhibits acid secretion by frog gastric mucosas *in vitro* and at 10^{-4} M causes tissue Na to rise and K to fall. Increasing K concentration in the medium from 9 to 29 mM largely abolishes the inhibitory effect of 10^{-4} M ouabain. We conclude that at least some of the inhibitory effect is the result of alteration of intracellular electrolyte pattern.

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Effect of Erythropoietin on the Uptake and Utilization of Iron by Bone Marrow Cells *in vitro*.* (27596)

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During the last decade numerous studies have demonstrated that plasma from severely anemic or severely hypoxic mammals contains an erythropoietic factor, the so-called erythropoietin(1,2,3). This factor is undoubtedly of importance in adjustment of red

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