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Effects of Sodium Removal on Acid Secretion by the Frog Gastric Mucosa.* (31398)

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In various studies of ionic requirements of the bathing solutions for H^+ and Cl^- transport in the frog gastric mucosa, K^+ and Ca^{++} have both been shown to be essential for acid secretion(1,2) while Na^+ free solutions have been claimed to support normal secretion in the mucosa(3) and 75% substitution of Na^+ by choline was shown to be without effect(4). The results presented here show that when the intracellular Na^+ concentration is reduced to very low levels, there is significant inhibition of acid and chloride secretion which is reversed by readmission of Na^+ to the nutrient but not to the secretory side.

Methods. Rana pipiens gastric mucosa was mounted between 2 lucite chambers as described previously(5). The potential difference (PD) was measured with a pair of calomel electrodes with renewable KCl junctions. Resistance was calculated from the change in PD obtained by sending 10 microamps of current in either direction and short circuit current was determined as the current necessary to reduce the PD to 0 within 30 seconds. Acid secretion was determined using the pH stat method(6) and Cl^{36} tracer fluxes were measured using the Nuclear Chicago liquid scintillation counter in which quench correction was performed using the channels ratio method. The bathing solutions were of the following composition: Nutrient: Na^+ 118 mM, K^+ 4 mM, Ca^{++} 1.7 mM, Mg^{++} 0.8 mM, Cl^- 109 mM, HCO_3^- 18 mM, glucose 10 mM; secretory: Na^+ 105 mM, K^+ 4 mM, Cl^- 109 mM. In the Na^+ free experiments choline was substituted for Na^+ and in the Na^+ and Cl^- free experiments SO_4^{--} was additionally substituted for Cl^-. In all the substitution experiments the membrane was washed 4 times over a period of 5 minutes with the appropriate solutions. The substitutions were carried out either on both sides simultaneously or one side followed by the other. In the experiments where the Na^+ was readmitted, aliquots of the choline nutrient or secretory solutions were withdrawn and replaced with aliquots of the Na^+

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TABLE I. Summary Table of Effects of Na^+ Removal on *in vitro* Frog Gastric Mucosa. (Cl^- fluxes 15 in either direction. Too few in individual groups for adequate statistics.)

Solution		No. exp	H^+, μEq hr^{-1} cm^{-2}	PD, mv	Resistance, Ω cm^2	Short circuit current, μa cm^{-2}	Cl^- flux, μEq hr^{-1} cm^{-2} N→S	Cl^- flux, μEq hr^{-1} cm^{-2} S→N
Na^+			3.10	26.7	223	115.8	—	—
Na^+-free	(a)	7	1.21 ± .34	8.4 ± 3.5	348 ± 41	26.9 ± 5.3	—	—
(± SEM)	(b)	8	1.12 ± .44	0	437 ± 93	0	—	—
	(c)	15	0	0	625 ± 48	0	—	—
All Na-free			0.55	3.2	499	9.8	2.59 ± .54	1.78 ± .31
Change			−2.55*	−23.5*	+276*	−106.0*		

* $P < .01$ by t test.

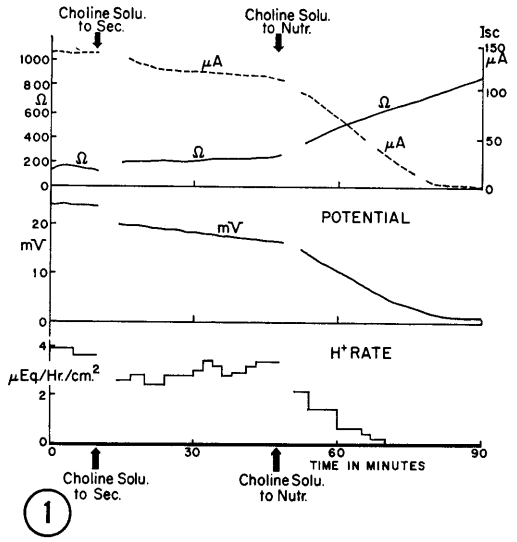
nutrient or secretory solution until virtually all of the choline had been replaced. Na^+ and K^+ analyses were performed on membranes treated in various ways. In some experiments the membranes were exposed after 3 washings to Na^+ free solutions in small beakers and gassed with 95% O_2-5% CO_2 for 30 minutes. In other experiments the membranes were set up in the standard secretory chamber and washed as usual with Na^+ free solutions. Secretory values were recorded and the membranes removed from the chamber for analysis. The Na^+ and K^+ analyses were performed by digestion according to Davenport's method(3), using internal standard flame photometry for Na^+ and K^+ analysis.

Results. Secretion: The parameters obtained with the standard NaCl solutions are shown in the first row of Table I. Previous solutions used in this and other laboratories have generally contained inorganic phosphate. It appears from these data that omission of phosphate has little effect on the secretory parameters of the frog gastric mucosa. When choline solutions were substituted for the Na^+ solutions on the secretory side only slight changes were observed. These changes could be accounted for by the presence of a Na^+ gradient in the direction nutrient to secretory (Fig. 1). The removal of Na^+ from the nutrient side produced a marked reduction of the short circuit current, potential difference and secretory rate, accompanied by a rise in resistance (Fig. 1).

It appeared that the results fell into 3 groups (Table I). In one group (7/30 exp.) although there was a marked reduction in PD, this did not fall to 0, and there was residual finite acid rate, short circuit and the usual rise in resistance. In the second group, (8/30 exp.) in spite of the absence of both PD and short circuit current, there was a residual finite acid rate (Fig. 2), sensitive to SCN^-. In the experiments described above with PD = 0 and a finite acid rate, the acid rate fell to 0 within 3 to 5 minutes when the tissue was made anoxic by bubbling 95% nitrogen and 5% CO_2. During this fall there was no observed change of the PD. When oxygen was readmitted acid secretion was reestablished. At no time could any change in the PD be noted. The third and largest (15/30 exp.) group showed neither PD nor acid secretion. There appeared to be a progressive rise in resistance from Group 1 to Group 3. The mean net chloride flux corresponded closely to the sum of the mean residual H^+ rate and residual short circuit current. It appears then that there was little transport of chloride other than that associated with HCl or Cl^- secretion, for instance in the form of choline chloride.

Na^+ readmission: When Na^+ was readmitted on the secretory side there was no significant reversal of the inhibitory effects. However, when Na^+ was readmitted to the nutrient side, the effects were reversed as shown in Fig. 3. Degree of reversal was related to degree of Na^+ re-substitution on the nutrient side. Small amounts of Na^+ (up to 6 mM) on the nutrient side did not reverse the effects on H^+ secretion. However, there was partial reestablishment of the PD, and a fall in resistance. At 12 mM Na^+, acid secretion was initiated. Further addition of Na^+ resulted in progressive increase in H^+ rate and PD with a fall in resistance. At 45 mM Na^+, the H^+ rate and the PD had

reverted to normal, but resistance was 400 Ω cm^2 compared to the 200 Ω cm^2 in Na^+ solutions initially. Readmission of Na^+ on the secretory side further lowered the resistance, and with a return to normal



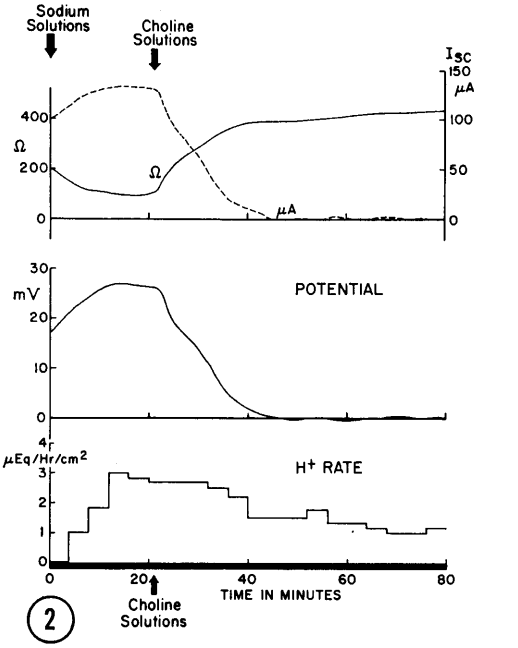


FIG. 1. Effect of choline substitution for Na^+ on secretory and nutrient side of *in vitro* frog gastric mucosa.

FIG. 2. A typical experiment where choline replacement of Na^+ resulted in a finite acid rate with a zero PD (group b, Table I)—see text.

TABLE II. Na^+ and K^+ Levels of Control and Choline Washed Tissues (± SEM).

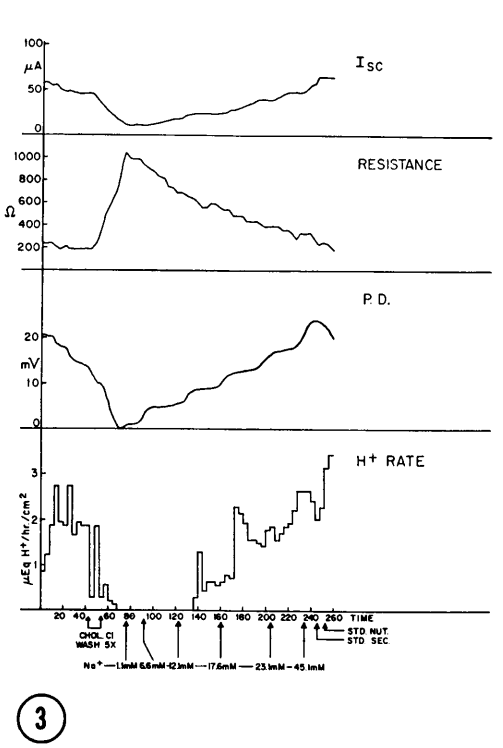
Membrane taken from:	No. of membranes	mEq/kg wet wt Na^+	mEq/kg wet wt K^+
Sodium solutions	6	48.7 ± 3.6	47.3 ± 3.5
Choline "	10	.33 ± .31	48.5 ± 1.8

Na^+ conditions, the resistance fell to the original value. Addition of K^+ up to 40 mM did not reverse the choline effects.

Na^+ levels: The data obtained by the Na^+ and K^+ analyses of the membrane showed that the technique of washing reduced the sodium concentration to less than 1% of control. There was no significant effect, however, on potassium concentration (Table II).

Na^+ and Cl^- substitution: The choline sulfate experiments were of 2 types. In the first type (6 exp.) the membrane was initially exposed to Na^+ = containing solutions and then choline chloride was substituted for sodium chloride on both sides. In this set of experiments when SO_4^{--} was additionally substituted for Cl^- there was maintained secretion with an inversion of PD. This secretion rate was maintained through a period of 13.6 ± 3.0 minutes then fell off progressively and reached 0 after 25.8 ± 3.4 minutes (mean of 6 exp. ± SEM) (Fig. 4). During the decline of H rate to 0 there was an approximately ($r = .729$, $n = 21$) linear relationship between the PD and the secretory rate. These data confirm Rehm's observations of a direct relationship between the PD and the hydrogen ion rate in sulfate(8) using dinitrophenol.

In the second type, both Cl^- and Na^+ were replaced by sulfate and choline simultaneously following exposure to $NaCl^-$ containing solutions. In this group, the choline sulfate data initially were virtually indistinguishable from results obtained with sodium sulfate-containing solutions. There was a rise in resistance, an inversion of the PD and a reduction of the H^+ secretory rate (which eventually fell to 0), as in the experiments above. The resistance obtained in the choline sulfate experiments was extremely high, in some experiments reaching as high as 1,800



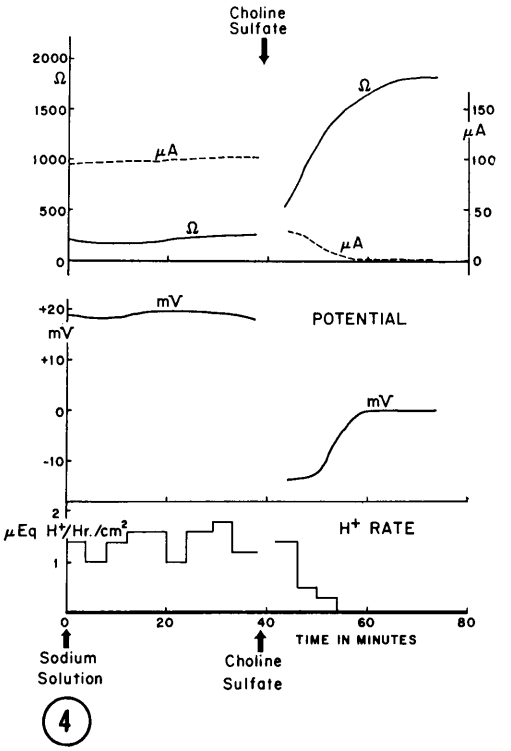


FIG. 3. Effect of Na^+ readmission on nutrient side of frog gastric mucosa bathed in choline solutions.

FIG. 4. Effect of choline sulfate substitution for NaCl in bathing solutions of *in vitro* frog gastric mucosa.

Ω cm^2. The acid secreted under choline sulfate conditions was inhibited by 10 mM SCN^-.

Discussion. It has become clear that there is an interdependence between K^+ concentration in the medium and H^+ secretion in the frog gastric mucosa(2). Davenport(3) has published an analysis of the various spaces of the gastric mucosa with reference to Na^+, K^+ and inulin, for example, and has demonstrated, using a tied-off sac of mucosa, that there is relatively little effect of choline substitution for Na^+ on acid secretion. These results are in contrast to the effects of K^+ removal on acid secretion where acid secretion is inhibited in the absence of K^+(2). With the sac technique none of the electrical parameters are measured and it is not clear why the data obtained with this method should differ from those obtained in the flux chamber. There are, however, various other points of difference, such as medium and washing procedure. There are differences of medium in washing, as we used a HCO_3^- buffered solution and in Davenport's experiments tris buffered solutions were used. In our experiments the tissue was washed exhaustively and from the Na^+ analysis the Na^+ content of the tissue was 0.33 mM/kg wet weight as estimated by Davenport's method, whereas he obtained a value of about 2.5 mM/kg wet weight residual Na^+ which is approximately 5% of the original value. It seems then that one possible explanation of the differences in results from the two laboratories involves an effect of the residual Na^+, and that to show an effect similar to the one we have described a lower Na^+ content has to be established. This is also suggested by the observation of Harris and Edelman and confirmed by us, that choline substitution up to 75% was virtually without effect on the mucosal parameters(4). That the effect is due to Na^+ removal rather than to a direct

action of choline, is also borne by the above observations. With respect to the reversal by KCl addition rather than NaCl addition (3) those experiments were performed in low Cl^- (*i.e.*, tris-mannitol) media and the optimal potassium concentration found, namely 36 mM, corresponds to the value found by other workers to be the optimal chloride concentration(7); thus this effect was presumably due to Cl^- addition, not K^+. The inhibition by choline solutions is readily reversible by the readmission of Na^+ but addition of KCl up to 40 mM had no effect in high Cl^- solutions. It may be concluded therefore that the inhibition is due to the removal of Na^+. Since in the absence of Na^+ a significant inhibition of both Cl^- and H^+ pumps is observed, it must be assumed then that there is some link between Na^+ levels and the H^+ and Cl^- transport mechanisms of the mucosa. The nature of this link is not known, but the primary effect may be on the Cl^- mechanism.

The question of the electrogenicity of the hydrogen ion pump is also raised by these data. The group of experiments in which finite acid rate with PD = 0 was obtained suggests the possibility that acid is secreted by a unitary mechanism under these conditions. Since with falling hydrogen ion rate an approximately straight line relationship was obtained between PD and acid, the hydrogen ion pump appears purely electrogenic in choline sulfate solutions, as it is in sodium sulfate solutions(8). Thus under choline chloride conditions, the pump is either unitary or there is a critical balance between the hydrogen and chloride electrogenic mechanism. This latter possibility is extremely difficult to exclude rigorously but anoxia and readmission of oxygen in the standard chloride solutions results in a differential effect on the hydrogen ion rate and PD (the hydrogen ion rate is reduced more rapidly than the PD) even after successive cycles of deoxygenation. Such treatment should lead to transient changes in PD in choline solutions, but, since no transient changes were observed, the simplest explanation of the data would be that the H^+ pump may have a unitary mechanism under these conditions (*i.e.*, low Na^+). In NaCl solutions, it is clear that the hydrogen ion mechanism acts as if it is at least partially electrogenic (from the fall in resistance on stimulation of hydrogen ion secretion and the PD changes with various inhibitors such as thiocyanate and amytal). Hogben has suggested that in the dogfish stomach the hydrogen ion mechanism is essentially nonelectrogenic(9), but in the frog stomach the hydrogen ion transport system may appear to be unitary, or partially electrogenic or purely electrogenic, depending on bathing solution composition.

The existence of 3 groups of data following Na^+ removal is a puzzling one. As far as possible the conditions of exposure of the membrane to choline solutions were kept consistent. The most reasonable explanation for these findings would be a variation in the amount of Na^+ retained in the different groups. Na^+ analyses however did not provide evidence for this hypothesis, but it should be pointed out that there are uncertainties in analytical procedures for Na^+ levels low in relation to K^+, and difficulty also in determining the extracellular water in frog gastric mucosa. These sources of error might mask critical variation in Na^+ levels which in any case are less than 1 mEq/kg wet weight of tissue.

The basic observation in this paper, namely, the inhibitory effect of sodium removal on Cl^- and H^+ transport, adds to the known cation requirements for gastric secretion. Thus Na^+, K^+, and Ca^{++} in the nutrient solutions all appear to be essential for acid and Cl^- secretion by the *in vitro* frog stomach. These ions, particularly Na^+ and Ca^{++}, may be acting essentially as activators of the H^+ and Cl^- transport system.

Summary. Removal of sodium from the frog gastric mucosa results in an inhibition of H^+ secretion and PD, short circuit current, Cl^- flux with a rise in the resistance. Choline sulfate experiments show an inverted PD with low hydrogen ion rates or 0 PD with 0 hydrogen ion rates. In some experiments in choline chloride acid secretion occurred in the presence of a 0 PD and this secretion appears likely to be by a unitary mechanism.

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Hyperresponsivity to Angiotensin Induced in Rats by Behavioral Stimulation.* (31399)

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Hyperreactivity to pressor stimuli is a characteristic of the hypertensive organism. Among the various pressor stimuli, those of psychological origin play a prominent role(1). It was of interest to us to determine whether psychological stimuli, which generally are studied in regard to their direct pressor effects, might also induce the hyperreactive state.

We have used previously the pressor response to angiotensin§ infusion in patients with benign essential hypertension as well as in hypertensive rats to demonstrate hyperreactivity(2,3). In the present experiment angiotensin was employed to study reactivity in normotensive animals after exposure to a noxious behavioral stimulus.

Materials and methods. Thirty-four male albino rats of the Holtzman strain weighing 175 to 400 g were fed standard rat chow, allowed tap water *ad libitum* and studied in groups of 4 to 5 experimental and control animals of the same weight. Systolic pressor response to intravenous injection of 0.1 μg of angiotensin was determined in each animal during light ether anaesthesia by an indirect technique which permits repeated determinations of the magnitude and duration of response, as described previously(3). Three to four responses were determined for each rat at each session and these were averaged. Immediately after the initial (pre-shock) determination, the animals were placed in an 18″ × 18″ × 18″ wooden box with an electric grid flooring. The experimental rats were exposed for 24 hours to a 25 milliampere electric shock paired in presentation with a bright light attached to the cage. This light-shock combination was delivered every 24 seconds for 3 seconds duration. Control animals were placed in the same box for 24 hours, but received no stimulation. Neither group received food or water while in the box. Immediately after the 24-hour period of shock, a second set of angiotensin responses was determined in both experimental and control groups. The rats then were returned to their colony cages and given food and water. A third set of angiotensin responses was determined 24 hours later.

Data on catecholamine excretion and organ content were obtained from an additional

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‡ Pre-doctoral trainee in Clinical Pharmacology; USPHS Training Grant HE-5467.

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