Ion Requirements for Gastric Acid Secretion.* (23291)

HORACE W. DAVENPORT
Department of Physiology, University of Michigan, Ann Arbor.

Absolute ion requirements for gastric acid secretion have not been determined, although Gray and Adkison(1) and others(2) found that changes in concentrations of Ca, Mg or K in their salt solutions affected secretion by frog gastric mucosae or mouse stomachs *in vitro*. *In vivo* Cl can be almost completely replaced by Br with no adverse effect(3), and mouse stomachs secrete well if 97% of Cl is replaced by Br or I. The law of electrical neutrality of solutions requires that anions accompany the secreted cation, and the mucosa actively secretes Cl in the direction serosa to mucosa(4). There may exist an obligatory coupling between acid and Cl (or halide) secretion. Although Hogben(4) did not find active Na transport in frog mucosa, Rehm (unpublished results) claimed that the dog's stomach pumps Na from mucosal to serosal sides. In the kidney acid secretion appears to be linked with Na transport in the opposite direction(5), and a similar association between acid and Na may occur in the stomach.

To determine ion requirements, O_2 uptake and acid secretion by frog gastric mucosae were measured in a variety of salt solutions in which the ions customarily present in "physiological salt solution" were varied in concentration or eliminated. Frog mucosa was studied because it appears to be less fastidious than mammalian tissue, and it should reveal the minimum requirements of the secretory mechanism.

Methods. Observations were made from January to April on *R. pipiens* obtained from a dealer and kept in a refrigerator. The stomach was removed from a pithed frog, and the muscularis was stripped off and discarded. The mucosa was washed with salt solution and tied and trimmed at junction of stomach and esophagus. The sac was filled with solution and tied slightly proximal to the pyloric sphincter. The filled mucosa was placed in a large Warburg flask, gassed with O_2 at about 730 mm Hg, and incubated with shaking at 25.2°C. After approximately 15 minutes equilibration measurement of O_2 uptake was begun and continued for an hour when contents of the sac were removed and titrated. O_2 uptake was calculated as qO_2, micromoles of O_2 consumed per mg dry weight per hour, and acid secretion was calculated as qH^+, micromoles of acid secreted per mg dry weight per hour. These are given in text and tables without repetition of units and with the standard errors of the means. Salt solution used to fill the preparations was an unbuffered one containing 240 mOM of salt calculated without regard to activity coefficients and an additional 11 mM glucose. When changes in composition were made isotonicity was maintained by proper adjustment of the major component. The 10 ml on the serosal side was identical except that it was buffered with 10 mM of tris(hydroxymethyl) aminomethane (Tris). Buffering was necessary, for secretion of alkali on the serosal side otherwise raised the pH to 9 or 10 and reduced secretion. Tris was titrated to pH 7.35 with HCl or other acids, and its osmolar concentration was calculated from its freezing point depression. Concentrations given in the tables are those of solutions as they were prepared. When those of Na and K were measured (by Dr. Richard Malvin) in the serosal solutions at end of incubation the true concentrations are given in parentheses.

Results. Calcium and magnesium. Results at top of Table I show that only the Ca and Mg brought to the solution with the tissue are necessary. Addition of 1 mM ethylenediaminetetraacetate (EDTA) abolishes secretion. The effect is reversed by washing the mucosae in 1 mM Ca. Furthermore, inhibition does not occur if EDTA is chelated with a slight excess of Ca. Traces of Ca removable by EDTA are essential for secretion. No conclusion is possible about the role of

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TABLE I. Respiration and Acid Secretion by Frog Gastric Mucosae.

Major salt	Variables, mM	n	qO_2	qH^+
NaCl	1 Ca, .9 Mg, 4.6 K	8	.11 ± .01	.18 ± .02
	0 .9 4.6	6	.12 ± "	.18 ± .03
	1 0 4.6	6	.12 ± "	.20 ± .02
	0 0 3	6	.10 ± "	.17 ± "
	0 0 3 1 EDTA	6	.08 ± "	0
	1.2 .9 3 1 EDTA	6	.13 ± "	.29 ± .03
NaCl	1 Ca, .9 Mg, 0 K (.3)	10	.08 ± .01	.04 ± .02
	1 .9 1 (1.2)	5	.13 ± .02	.22 ± .04
	1 .9 2	6	.11 ± .01	.19 ± .02
	1 .9 3	6	.14 ± .03	.25 ± .03
Choline	0 Na, washed (.6)	6	.06 ± .01	.07 ± .02
	0 (1.3)	12	.07 ± "	.06 ± "
	2 (3)	10	.07 ± "	.06 ± .01
	10	12	.09 ± "	.15 ± .02
	32	11	.10 ± "	.14 ± .01
	111 Li	6	.10 ± "	.14 ± .03
Na	.2 Cl, 116 Br, 2 NO_3	12	.10 ± .01	.18 ± .01
	.2 116 I, 2	8	.08 ± "	.07 ± .02
	0 118	5	.09 ± "	.11 ± .01
	10 118	5	.08 ± "	.05 ± .02
	32 86	6	.11 ± "	.11 ± "
Na_2SO_4	0 Cl	5	.09 ± .01	.05 ± .01

other metals which chelate with EDTA, for the concentration, if any, of EDTA within the cells is not known.

Potassium. When K was omitted rates of secretion ranged from 0 in four instances up to maximum .12. The serosal fluid at the end of incubation contained .3 mM K. Optimal concentration of K is near 3 mM.

Sodium. NaCl was replaced by choline chloride, and all solutions contained 1 mM Ca, 9 mM Mg and 4.6 mM K. The lowest Na (.6 mM) was attained by washing the mucosae twice. At this concentration secretion ranged from .04 to .14, the highest rate occurring in a solution found to contain .5 mM Na. Raising the concentration to 10 or 32 mM does not restore secretion to maximum. When Li replaced Na as the major cation secretion occurred at two-thirds maximum.

Sodium and adrenal cortical extract. Villarreal, Ganong and Gray(6) found that increased adrenal cortical activity after a latent period of three to four hours was followed by increased acid secretion *in vivo*. We(7) failed to find stimulation of acid secretion *in vitro* by either desoxycorticosterone or cortisone, and disoxycorticosterone at high concentrations inhibited secretion. Some effect might be found at low Na concentrations where the hormones could promote efficient utilization of the ion. To detect action of any cortical hormones, Upjohn's aqueous extract (generously supplied by Upjohn Co.) was added to both filling and bathing solutions at 1 ml of extract to 100 ml of fluid. The extract used had activity equivalent to .1 mg hydrocortisone per ml, and this gave .011 or more mg of hydrocortisone equivalent per mucosa. Because the extract itself contained NaCl, absence of Na could not be attained. Na concentrations of 5, 12, 32 and 111 mM were used, and the solutions also contained 1 mM Ca, .9 mM Mg and 3 mM K. Results in Table II show that at high Na concentrations the extract reduced secretion, and at low concentrations it had no effect.

Bromide, Iodide, Nitrate and Sulfate. Solutions were made with NaBr, NaI or $NaNO_3$ replacing NaCl. The source of Ca in all instances was $Ca(NO_3)_2$. Tris for the serosal solution was titrated with acetic acid to pH 7.35. KBr, KI or KNO_3 was present at 4.6 mM, and results are to be compared with those in the first line of Table I. Histamine phosphate was used in solutions containing 118 mM NO_3 to eliminate halide. Results show that secretion occurs at control level

TABLE II. Effect of Aqueous Adrenal Cortical Extract on Respiration and Acid Secretion of Frog Gastric Mucosae.

	Controls			Adrenal cortical extract		
Na, mM	n	qO_2	qH^+	n	qO_2	qH^+
5	10	.07 ± .01	.11 ± .02	9	.08 ± .01	.10 ± .02
12	9	.10 ± "	.14 ± "	11	.11 ± "	.16 ± .03
32	10	.12 ± "	.18 ± "	11	.10 ± "	.12 ± .02
111	6	.14 ± .03	.25 ± .03	11	.11 ± "	.19 ± "

with Br replacing Cl, but it is reduced with I. Secretion at a reduced rate occurs with NO_3 as the only anion, and replacing NO_3 with Cl to the extent of 32 mM does not increase it. To determine whether secretion can occur with divalent SO_4 as the only anion, the solution on the mucosa side contained 78.3 mM Na_2SO_4, 1.5 mM K_2SO_4 11 mM glucose and .1 mM histamine phosphate. The serosal solution was the same except that 10 mM Tris titrated to pH 7.35 with H_2SO_4 replaced an equivalent osmotic amount of Na_2SO_4. Ca was omitted on account of its insolubility. In these solutions O_2 uptake was at control levels, and acid secretion definitely occurred although its rate was one-fifth maximal.

Discussion. The results show that secretion can occur when Na or Cl (or halide) are very low in solutions bathing the mucosa. Reduction of secretion below maximal rate under this condition could be explained by lack of essential Na or Cl, and the fact that secretion actually does occur could be attributed to highly efficient utilization of traces of Na or Cl. However, raising the concentrations of Na or Cl does not cause a really substantial increase in secretion until the concentration is more than 10 mM. If Na or Cl is essential one would expect that making more Na or Cl available by raising its concentration only a little would greatly increase secretion. Dependence of acid secretion upon adventitious Na and Cl appears to be relative rather than absolute, and the essential process in gastric acid secretion is the secretion of acid.

Summary. Acid secretion and O_2 uptake measured in frog gastric mucosae *in vitro* occur but at reduced rates when Na in bathing solutions is .6 mM or less. Both are raised towards maximal when Na is 10 mM. Secretion occurs at 75% maximal when Li replaces Na. Adrenal cortical extract reduces secretion with high Na and has no effect with low Na. Neither added Ca nor Mg is necessary for secretion, but secretion is reversibly inhibited by 1 mM ethylenediaminetetraacetate. Secretion occurs when K is .3 mM but is maximum at 3 mM. Secretion occurs at 20% maximal when Cl is .2 mM or less. Secretion is unaffected by replacing Cl by Br, but it is reduced when Cl is replaced by I.

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