Effect of pH on Muscle Calcium and Magnesium.* (26399)

DANIEL L. GILBERT† (With technical assistance of JoAnna McGann)
Department of Physiology, Albany Medical College of Union University, Albany, N. Y.

It has been pointed out that in maintenance of plasma pH, the role of the ion movement across cell membranes is not to be ignored(1). The relationship of permeating ion concentration to the membrane potential can be expressed by the following equation (2): $\frac{C_1}{C_2} = r^z q$. In this equation, subscripts 1 and 2 refer respectively, to intracellular and extracellular compartments; C is concentration of the ion; z = valence of the ion; $r = \text{antiln} \frac{F}{RT} E_m$ (F is the Faraday constant, R is the gas constant, and E_m is the membrane potential or difference in electrical potential of the extracellular compartment minus the intracellular compartment); and $q = \text{antiln} \frac{W_q}{R_T}$ (W_q is the active ion transport potential difference, *i.e.*, extracellular minus intracellular). Addition of acid causes the net valence of the intracellular non-permeating ions to increase by accepting the hydrogen ions, and this in turn causes the value of r to decrease(3). If extracellular ion concentration, C_2, and the ion work factor, q, are both constant, then intracellullar concentration, C_1, should be proportional to r^z. Thus, it is to be expected that acid addition to muscle cells should cause a decrease in intracellular concentration of the ion if the ion is a cation (*i.e.*, z is positive) and similarly alkali addition should cause an increase in intracellular concentration of the cation. If water shifts are ignored, then an increase in intracellular concentration indicates a cation shift into the intracellular water phase, and likewise a decrease in intracellular concentration indicates a cation shift out of the intracellular water phase. Thus, the cation would buffer the extracellular phase by exchanging with the hydrogen ion across the cell membrane. The purpose of this study was to determine if changes in extracellular pH would cause the expected shifts of muscle calcium and magnesium.

Methods. Frog muscles were dissected out and were maintained overnight in the control solution at 5°C. Generally, a muscle set consisted of the tibialis anticus longus, ileofibularis, semitendinosus, and the sartorius (occasionally the peroneus). Then one muscle set and its contralateral set were transferred respectively to the experimental and the control flasks, each of which contained 50 ml of solution. Tris(hydroxymethyl)-aminomethane was used as a buffer(4). All solutions contained 2 mM of $CaCl_2$, 2 mM of $MgCl_2$, 65 mM of NaCl, 2.7 mM of KCl, and 50 mM of tris(hydroxymethyl) aminomethane (Sigma 7-9 buffer, obtained from Sigma Chemical Co., St. Louis, Mo.). The pH was regulated by additions of various amounts of HCl. Five solutions were prepared which contained 60, 50, 40, 30, or 0 millimoles HCl per liter. The resulting pH of these solutions was 2.2, 6.8, 7.4 (control solution), 8.0, and 10.0, respectively. After 5 hours with gentle agitation at a temperature of about 23-26°C, the individual muscle sets were blotted on filter paper moistened with the control solution and weighed on a torsion balance. Each muscle set was placed in a platinum crucible, dried at about 100°C for approximately 1 hour, ashed overnight at 500°C, and dissolved in HCl. In one set of experiments, after precipitating with ammonium oxalate, calcium content was determined by an ethylenediamine tetraacetic acid (EDTA) titration using a calcium indicator (5). In another set of experiments, after precipitating with oxine, magnesium content and an approximate calcium (referred to as metal) content were determined by an EDTA titration using both a calcium and

* This work was supported by U. S. Public Health Service grant.

† Present address: Dept. of Physiology, Jefferson Medical College, Philadelphia, Pa.

TABLE I. Effect of pH on Calcium and Magnesium in Muscle.

Muscle analysis	Exp. pH*	Millimoles/kg wt — Control	Experimental	Exp./Control, %	"P" value, %
Calcium	2.2	2.42 ± .04	1.55 ± .02	63.9 ± 1.46	.0
	6.8	2.30 ± .03	2.18 ± .02	94.9 ± 1.54	.7
	8.0	2.28 ± .05	2.35 ± .05	103.1 ± .95	.8
	10.0	2.38 ± .05	5.87 ± .08	247.5 ± 2.96	.0
Magnesium	2.2	6.52 ± .05	1.67 ± .04	25.7 ± .67	.0
	6.8	6.08 ± .04	5.10 ± .04	83.9 ± .72	.0
	8.0	6.15 ± .06	6.64 ± .06	108.4 ± .93	.0
	10.0	6.34 ± .04	7.76 ± .07	123.3 ± .60	.0
Metal	2.2	2.25 ± .07	1.42 ± .06	63.7 ± 3.24	.0
	6.8	2.28 ± .09	2.35 ± .11	103.3 ± 3.77	39.8
	8.0	2.35 ± .09	2.29 ± .11	98.1 ± 4.53	68.3
	10.0	2.17 ± .08	5.96 ± .35	280.6 ± 1.98	.0

* No. of experiments for each pH was equal to 12.

magnesium indicator(5,6). "P" values were obtained by comparing the per cent experimental/control values with 100%, and using a previously described "t" table(7).

Results. Results are given in Table I. The low pH of 2.2 caused a decrease of 4.85 millimoles magnesium/kg muscle and a decrease of only 0.87 millimoles calcium/kg muscle. On the other hand, the high pH of 10.0 caused an increase of 3.49 millimoles calcium/kg muscle and an increase of only 1.42 millimoles magnesium/kg muscle. Although the differences were not as pronounced for the 6.8 and 8.0 pH experiments, the results show that in experiments with a pH below 7.4 there was a decreased calcium and magnesium in the muscle, and in experiments with a pH above 7.4, there was an increased calcium and magnesium in the muscle. The metal determinations are used as confirmatory evidence for the calcium values.

Discussion. These experiments point out that both calcium and magnesium ions in muscle can contribute to the pH buffering of the extracellular phase by moving out of the muscle when the extracellular phase is acid and by moving into the muscle when the extracellular phase is alkaline. In solutions of moderate pH changes, calcium and magnesium shifts are not too pronounced, and probably other ions are quantitatively more important in tissue buffering. There appears to be more than one state of calcium(2,8) and magnesium(6) in muscle tissue and the main ion exchanges may not involve the intracellular water phase. In the case of calcium, Swan recently observed that acid solutions caused an efflux of Ca^{45} from muscle, which he interpreted to be due to an exchange taking place on the surface of the muscle(9). In reference to magnesium, previous studies have shown that only about one-fourth of the intracellular magnesium is exchangeable with Mg^{28}, a fraction which includes the free magnesium ion in the intracellular water(6). Since a lowering of pH to 2.2 has been found to release some 75% of the intracellular magnesium, we have clear support for the inference that extracellular acidity is capable of freeing magnesium from its non-exchangeable phase. On the other hand, it appears that the high pH of 10 was acting significantly to increase the binding of calcium. The failure of these 2 divalent ions to exhibit quantitatively equivalent shifts with changes in pH possibly relates in part to the different binding properties of the *in vivo* binding sites for these ions. Microincineration studies have been interpreted to indicate that calcium and magnesium are intimately associated with the contractile machinery of the myofibril(10). Perhaps this location represents a part of the non-exchangeable phase of these ions.

It should also be recognized that there appear to be active transport mechanisms for both calcium(2,11) and magnesium(6) and it is possible that changes in pH might influence this transport mechanism. However, measurements of membrane potential, ion flux, and ionic concentration of the various phases would be required to dissociate quantitatively this possibility from the conse-

quences of ionic binding in the various muscle phases.

Summary. These *in vitro* experiments show that both magnesium and calcium in muscle can contribute to the buffering of pH. In low pH solutions there is a decrease in both calcium and magnesium, and in high pH solutions there is an increase in both calcium and magnesium in muscle.

1. Gilbert, D. L., *Yale J. Biol. Med.*, 1960, v32, 378.

2. Gilbert, D. L., Fenn, W. O., *J. Gen. Physiol.*, 1957, v40, 393.

3. Gilbert, D. L., *Bull. Math. Biophys.*, 1960, v22, 323.

4. Gomori, G., PROC. SOC. EXP. BIOL. AND MED., 1946, v62, 33.

5. Gilbert, D. L., McGann, J., *ibid.*, 1958, v97, 791.

6. Gilbert, D. L., *J. Gen. Physiol.*, 1960, v43, 1103.

7. ———, *Mathematical Tables and Other Aids to Computation,* 1951, v5, 85.

8. Shanes, A. M., Bianchi, C. P., *J. Gen. Physiol.*, 1959, v42, 1123.

9. Swan, R. C., *Communications of XXI Internat. Congr. Physiol. Sc.,* Buenos Aires, 1959, 267.

10. Draper, M. H., Hodge, A. J., *Austral. J. Exp. Biol. Sci.*, 1950, v28, 549.

11. Hodgkin, A. L., Keynes, R. D., *J. Physiol.*, 1957, v138, 253.

Received December 21, 1960. P.S.E.B.M., 1961, v106.

Measurement of the Vascular Factor Attending Drug-Altered Sensitivity to Peptic Digestion.* (26400)

ARNOLD S. LEONARD, WARD O. GRIFFEN, JR., DEMETRE M. NICOLOFF, RAYMOND C. DOBERNECK AND OWEN H. WANGENSTEEN

Department of Surgery, University of Minnesota Medical School, Minneapolis

The susceptibility of esophageal, gastric, and duodenal mucosa to acid peptic digestion depends upon pH and pepsin concentration of the gastric juice, the mucous barrier, tissue vascularity, and inherent cellular resistance. Clinical and experimental evidence has shown abetment of the acid peptic ulcer diathesis by various drugs including reserpine(1), serotonin, cortisone(2), pitressin (3) and nitroglycerin(4). It has been shown recently that reserpine increases susceptibility of the esophageal mucosa to acid peptic digestion(5).

The first segment of the experiment concerned assessment of the role of serotonin, ACTH(6) and hydrocortisone(7,8) which are released by reserpine, in the augmented sensitivity of the esophagus to injury by acid peptic juice. Pitressin, nitroglycerin, and chlorpromazine also were evaluated in this respect.

In the second segment of the experiment, the effect of reserpine, chlorpromazine, pitressin, and nitroglycerin on local esophageal blood flow was measured in an attempt to evaluate the importance of the vascular factor in potentiation of acid peptic digestion. A modification of Saperstein's technic was utilized(9).

Method. Adult cats under pentobarbital anesthesia (30 mg/kg) were used. Respiration was maintained by means of an automatic respirator through a cervical tracheostomy. In the first portion of the study, the animals were divided into 7 groups. The technic of perfusion, with different segments of the same cat's esophagus as the control and test preparation was used(5). In Group 5A, however, separate cats were used for control and test preparation. In the latter group, the perfusions using the same patient's gastric juice were started immediately after injecting pitressin intravenously into the test animal. At the end of the procedure, the mucosal surface of the esophagus was inspected. On removal of the esophagus, degree of injury to both segments was graded

* This work was supported by U. S. Public Health Service Peptic Ulcer Grant and Donald J. Cowling Fund for Surgical Research.