needed since the third decimal is sufficient to demonstrate the kinks. If the curve is plotted on graph paper in such a way that a fraction of a millimeter represents the fourth decimal place, the experimental errors are not seen at all. Any deviations from the smooth path must be due to intrinsic causes.

Of all substances known to the author, water seemed an exception in that it alone seemed free of these discontinuities. However, experiments performed recently

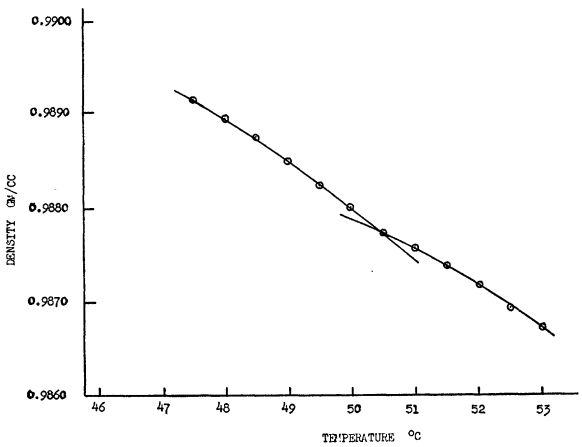

FIG. 1.

by the co-author show that this is not true, and that kinks are easily noticed in the density-temperature curve for ordinary distilled water. A 25-cc pycnometer was

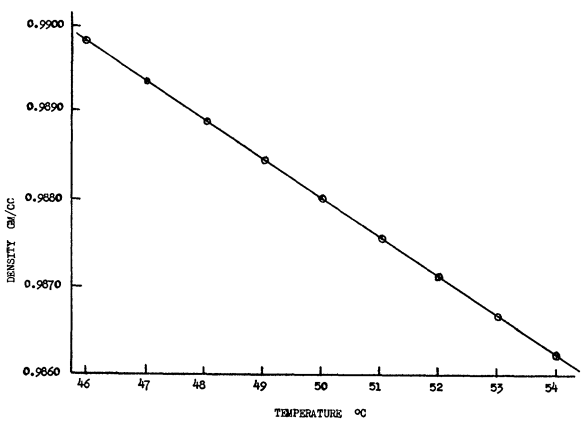

FIG. 2.

used in these measurements, and densities determined for the range of temperatures from 47° to 53° C in a thermostat regulated to .002° C. There is a smooth stretch of the curve between 47° and 50° and another between 50° and 53°. These intersect just a little above 50° C, giving a well defined kink (see Fig. 1). Below 47 another smooth stretch of the curve begins, which is not shown on the drawing. Fig. 2 was drawn from the data given in the Smithsonian Tables. As may be seen, the graph is perfectly smooth with only a slight curvature.

There can be no doubt that the experimental results were smoothed by applying the generally accepted rules for drawing a representative curve amidst erratic points due to experimental errors. In this case, however, it is not legitimate to do so because, as explained, the effect is well above the limits of experimental errors, and, on a drawing of the scale shown, the errors do not appear.

This is not the only case in which the figures given in tables are adulterated. There are some instances, especially in the study of liquids, in which the entire experimental work ought to be done afresh.

References

1. ANTONOFF, G. *Phil. Mag.*, 1925, **1**, 256.
2. ANTONOFF, G. *J. phys. Chem.*, 1944, **48**, 80.
3. YOUNG, SIDNEY. *Proc. Dublin roy. Soc.*, 1910, **12**, 374.

A Study of Gastric HCl Formation[1]

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The mechanism whereby parietal cells secrete HCl against a concentration gradient has previously been investigated in preparations of isolated gastric mucosa (*6–10*). In the present study a modification of earlier apparatus has been introduced to permit simple continuous measurement of pH difference across the wall of the isolated stomach of the rat. The experimental procedure entailed the opening of the abdominal wall under barbiturate anesthesia and the injection, into the exposed stomach, of chilled phosphate buffer at pH 7.4. The entire stomach was then removed, opened along the lesser curvature, and rinsed with several changes of the solution with which the apparatus was to be filled. The rugated portion of the stomach was so clamped between the smoothly ground faces of two half-cells (Fig. 1) that it served as a membrane separating the apparatus into two compartments. Ten ml of solution (see below) was then pipetted into each half-cell and a glass electrode immersed on each side. O_2 saturated with water vapor was bubbled through both sides to effect oxygenation as well as mixing. The potential difference generated between the two glass electrodes was read on a Beckman pH meter (Model H). More stable readings were obtained when the cell was placed in a grounded metal box and the housing of the meter was grounded. It was repeatedly observed that the pH difference, as computed from the total potential difference, and as calculated from individual determinations of pH on both sides of the membrane, agreed within 0.2 pH unit. With the development of a pH difference, it was invariably found that the solution in contact with the mucosal surface became acid and simultaneously the solution in the serosal compartment became alkaline.

In every experiment the solutions introduced on both sides of the membrane were initially identical, and when

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agents were added in the course of an experiment, similar additions were made to both compartments. The standard solution employed was a modified, lightly buffered Kreb's solution (*14*) in which the concentration

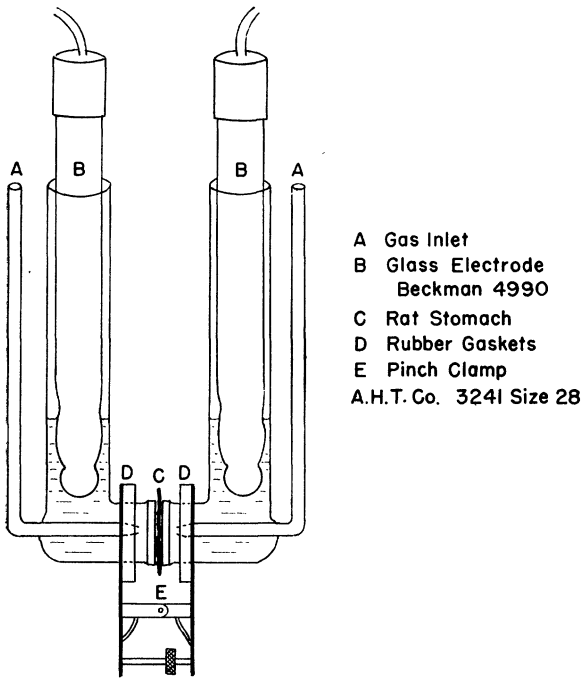

FIG. 1. Cell for the continuous measurement of pH difference across gastric wall.

of phosphate was reduced to approximately physiological levels (942 ml of 0.9% NaCl; 38 ml of 1.15% KCl; 10 ml of 1.18% $MgSO_4$; 13 ml of isotonic phosphate buffer at pH 7.4; 2 gm glucose). Under these conditions, the observed potential difference increased over a period of 3 to 6 hrs from an initial value of approximately zero to a value of about 250 mv, corresponding to a pH difference of about 4 units (Curve A, Fig. 2). Failure of development of a potential difference usually proved to be due to perforations in the membrane.

Successive elimination of the various ionic constituents from the medium revealed that acid production proceeded nearly as well in a medium of 0.9% NaCl solution as in the more complex media. In fact, in a medium containing only 5% glucose and no added salts, acid production proceeded actively for about one hour. It was further found that the presence or absence of glucose in the standard medium had no effect upon the capacity to generate acid, a finding which is attributed to the presence of intracellular nutrient adequate for the duration of the experiment.

In accord with observations of others (*7, 9*), continuous oxygenation of the fluid in the mucosal compartment was found to be essential to prolonged acid production. Interruption of the stream of O_2, or its replacement by N_2 (Curve B, Fig. 2), resulted in a decrease in the rate of acid secretion, although characteristically this decrease was not marked until 15 or 20 min of anoxia had elapsed. Reoxygenation after a period of 40 to 60 min of anoxia resulted in a limited degree of resumption of acid formation. Here again a short time lag was noted.

In agreement with reports in the literature (*15*), a high degree of inhibition of gastric acid formation was obtained with cyanide (0.0003 M) (Curve C, Fig. 2), fluoride (0.01 M), arsenite (0.001 M), and iodoacetate (0.003 M). In addition, tetramethyl *p*-phenylenediamine, reported to inhibit pyridine nucleotide systems (*12*), was found to poison in 0.002 M concentration. Histamine (0.005 M) gave an inconstant stimulation of acid production, while Benadryl (0.004 M), malonate (0.01 M), and phlorhidzin (saturated solution) were without demonstrable effect.

To account for the above experimental results as well as many observations by others, a hypothesis is offered for the mechanism of gastric HCl formation. Consideration has been given to the following additional and pertinent facts: carbonic anhydrase (*2, 3*) and niacin-containing coenzyme (*1*) are abundantly present in the

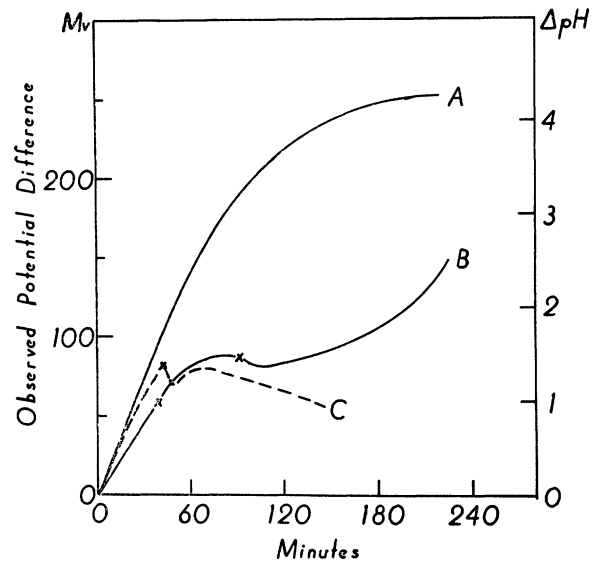

FIG. 2. The development of pH difference across the rat gastric wall. Curve A, standard preparation; Curve B, O_2 replaced by N_2 during interval x – x; Curve C, KCN (0.0003 M) added at x.

parietal cells; niacin deficiency results in hypochlorhydria (*17*); during periods of HCl secretion the venous drainage of the stomach is enriched with regard to $NaHCO_3$ (*5, 11*); an electrical potential difference between inert electrodes, wherein the mucosa is negative to the serosa in an external circuit, exists across the gastric wall (*16*). Thus chemically and electrically, as well as histologically, the cells under consideration are clearly oriented, and it is suggested that this orientation is a reflection of intracellular stratification of the several enzyme systems, in contrast to a condition in which the enzyme systems are well intermixed. The postulated arrangement of the enzyme systems concerned is roughly diagrammed in Fig. 3.

The simplest picture that will account for the secretion

of HCl against a concentration gradient is that, at the expense of energy derived from the oxidation of some carbohydrate intermediate, RH_2, a local high concentration of hydrogen ions develops at some point close to the gastric lumen. Thence, by diffusion, HCl enters the gastric juice. An obvious and abundant source of hydrogen ions is the oxidation of RH_2 by a pyridine nucleotide coenzyme (cf. Fig. 3). The assignment of the site of reversible hydrogenation of pyridine nucleotide to the *ortho* double bond of pyridine is in fact based upon the

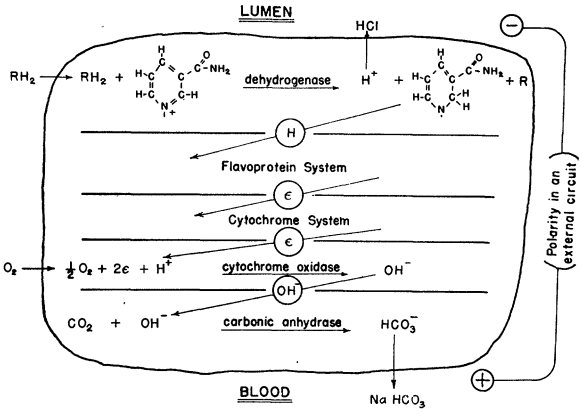

FIG. 3. Postulated stratification of enzymes in parietal cell and mechanism of HCl formation.

appearance of an extra mol of acid during its reduction (*12, 18*), and we offer the postulate that this reaction is the actual source of the hydrogen ions in the present process. Adjacent to the pyridine nucleotide enzyme system, flavoprotein, cytochromes, and cytochrome oxidase may be pictured as lying in successive strata, with carbonic anhydrase intervening between the cytochrome oxidase and the vascular border of the cell. The hydrogen atoms which accumulate on the pyridine nucleotide as it is reduced by RH_2 can then be passed on to the adjacent flavoprotein, which can serve as a reductant of the neighboring cytochromes. Utilizing electrons supplied to it by cytochrome, cytochrome oxidase can now catalyze the formation of hydroxyl ions from gaseous O_2. Whereas in a less oriented system these hydroxyl ions are doubtless neutralized by hydrogen ions arising from the reduction of pyridine nucleotide, in the present hypothesis this neutralization is precluded by the anatomical remoteness of the two processes; instead, the accumulating hydroxyl ions are "detoxified" by reaction with CO_2 in the presence of carbonic anhydrase. The HCO_3^- thus formed diffuses into the blood stream as $NaHCO_3$. Note that in this picture Na^+ and Cl^- do not participate but merely passively accompany the other ions in their diffusions (*4*).

The present hypothesis accounts for the obligatory aerobic nature of the process of HCl formation, the dependence of the process upon dietary niacin, and the difference in electrical potential across the secreting gastric wall. It assumes stoichiometrical equivalence of the HCl secreted into the lumen and $NaHCO_3$ discharged into the venous blood, an equivalence recently demonstrated experimentally by Davies (*5*), and it permits the Q_{HCl} to exceed the Q_{O_2} by a factor of as much as 100%, which is also in accord with observations of this author. These relationships become apparent if one considers the over-all balanced expression for the sum of the several reactions postulated in Fig. 3:

$$2\ RH_2 + O_2 + 2CO_2 \longrightarrow 2R + 2H^+ + 2HCO_3^-$$

The hypothesis finds further support in the observed high concentrations of pyridine nucleotide and carbonic anhydrase in the parietal cells. No novel reactions have been postulated, but rather some degree of lamination of well-known enzymes, resulting in a separation of hydrogen ions known to arise from the reduction of pyridine nucleotide and hydroxyl ions which arise from the reaction of reduced cytochrome oxidase with oxygen.

Histochemical attempts to demonstrate the postulated stratification of enzymes have thus far not succeeded, nor have attempts to assemble synthetic acid-secreting membranes along the lines of the foregoing hypothesis. The identity of the hydrogen donor, RH_2, is at present undetermined, but it may be pointed out that any or all substrates susceptible of oxidation by pyridine nucleotide could satisfy the requirements of the present hypothesis.

Thus, a hypothesis for gastric HCl formation is offered, predicated upon two basic assumptions: that the H ions of the gastric juice arise from the reduction of pyridine nucleotide; and that the enzymes of the oxidation-reduction systems in the parietal cell are arranged in successive strata. The hypothesis which follows from these assumptions appears to be in accord with all pertinent facts relating to gastric HCl formation.

References

1. BRADFORD, N. M., DAVIES, R. E., ELLIS, E., and HUGHES. D. E. *Commun. biochem. Soc.* (266th Meeting), April 1948.
2. DAVENPORT, H. W. *J. Physiol.*, 1939, **97**, 32.
3. DAVENPORT, H. W. *Amer. J. Physiol.*, 1940, **128**, 725.
4. DAVENPORT, H. W., and FISHER, R. B. *Amer. J. Physiol.*, 1940, **131**, 165.
5. DAVIES, R. E. *Biochem. J.*, 1948, **42**, 609.
6. DAVIES, R. E., CRANE, E. E., and LONGMUIR, N. M. *Biochem. J.*, 1946, **40**, proc. xxxvi.
7. DELRUE, G. *Arch. internat. Physiol.*, 1930, **33**, 196.
8. DELRUE, G. *Arch. internat. Physiol.*, 1933, **36**, 129.
9. GRAY, J. S., ADKISON, J. L., and ZELLE, K. *Amer. J. Physiol.*, 1940, **130**, 327.
10. GRAY, J. S., and ADKISON, J. L. *Amer. J. Physiol.*, 1941, **134**, 27.
11. HANKE, M. E., JOHANNESEN, R. E., and HANKE, M. M. *Proc. Soc. exp. Biol. Med.*, 1931, **28**, 698.
12. KARRER, P., SCHWARZENBACH, G., BERZ, F., and SALMSSEN, U. *Helv. Chim. Acta*, 1936, **19**, 811.
13. KENSLER, C. J., DEXTER, S. O., and RHOADS, C. P. *Cancer Res.*, 1942, **2**, 1.
14. KREBS, H. A., and EGGLESTON, L. V. *Biochem. J.*, 1940, **34**, 442.
15. LUTWAK-MANN, C. *Biochem. J.*, 1947, **41-1**, 19.
16. REHM, W. S. *Amer. J. Physiol.*, 1943, **139**, 1.
17. RUFFIN, J. M., and DICK, M. *Ann. int. Med.*, 1939, **12**, 1940.
18. WARBURG, O., and CHRISTIAN, W. *Biochem. Z.*, 1936, **287**, 291.