# Critical *In Vitro* Factors in Evaluation of Gastric Antacids II

## Inhibition of Neutralization Rate of Dried Aluminum Hydroxide Gel

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**It was determined that the *in vitro* neutralization rates of dried aluminum hydroxide gel are dependent on the amount of antacid used. Neutralization rates of aluminum hydroxide were inhibited by a variety of materials, including proteins, enzymes, polypeptides, amino acids, and certain organic acids. Several possible inhibition mechanisms are explored.**

THE MARKED INHIBITORY effect of peptone and pepsin on the *in vitro* activity of noneffervescent antacids has recently been reported (1). The significance of these findings is that similar inhibition might occur in the stomach. Since antacids are most frequently administered after meals, it is conceivable that a nonspecific, protein-induced inhibition of certain alkalizing agents could result in considerable loss of antacid activity. In view of the importance of gastric antacids in modern therapeutics, it was decided to continue the investigation of this phenomenon.

Previous work has indicated that aluminum hydroxide, of all antacids tested, demonstrates the highest susceptibility to the inhibitory effect of polypeptides. Therefore, the possible inhibitory effect of various proteins, polypeptides, and amino acids on the *in vitro* activity of dried aluminum hydroxide gel U.S.P. is considered in this report. Certain aspects of the inhibition mechanism are also explored.

### EXPERIMENTAL

Rate of neutralization and buffering capacity studies were carried out in accordance with the specifications reported by Desai *et al.* (1). All experiments, except where noted, were conducted using 1.0 Gm. of dried aluminum hydroxide gel U.S.P.

**Relationship of Rate of Neutralization to Buffering Capacity.**—It has been proposed that the polypeptide inhibitory effect on the buffering capacity of noneffervescent antacids indicates a kinetic or reversible phenomenon rather than an irreversible state (1). This conclusion was based on the observation that the polypeptides had no effect on the total volume of acid neutralized but reduced the maximum pH attained during the course of the experiments. Therefore, it was reasoned that the over-all effect of polypeptides on the antacid buffering capacity could be ascribed to an inhibitory influence on the rate of neutralization. This reasoning is supported by the results depicted in Figs. 1 and 2. A definite relationship exists between these two evaluatory procedures. A more prolonged neutralization time results in a lower maximum pH value in an analogous buffering capacity determination.

Past reports have considered the buffering capacity of a given antacid as involving an equilibrium state. Therefore, if antacid compound A is capable of neutralizing $X$ ml. of 0.1 $N$ HCl, and antacid compound B consumes an equal quantity, these compounds have been considered comparable. Since the rate of secretion of HCl *in vivo* is a significant variable, it must be appreciated that the integral, $d\,\mathrm{pH}/d$ ml. acid, is a more valid criterion of antacid activity. Thus, if one were to consider the plots in Fig. 2 as representative of hypothetical compounds A, B, and C, it may be noted that although all antacids have the same buffering capacity (*i.e.*, consume the same quantity of 0.1 $N$ HCl), they are far from equivalent. Compound A would most likely be the more effective antacid. If the total area under the curve is a more valid indication of antacid activity, then the situation is no longer equilibrium dependent. Rather it becomes kinetic in nature, and the rate of reaction of the antacid with HCl is the determining factor.

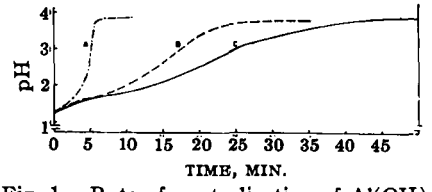


Fig. 1.—Rate of neutralization of $Al(OH)_3$ in 50 ml. 0.1 $N$ HCl. Key: A, 1.0 Gm. $Al(OH)_3$; B, 1.0 Gm. $Al(OH)_3$ + 250 mg. pepsin; C, 1.0 Gm. $Al(OH)_3$ + 250 mg. peptone.

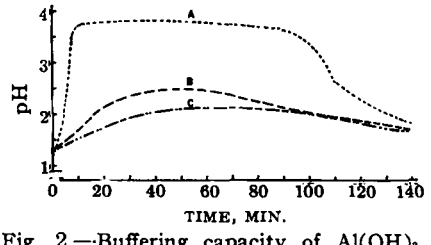


Fig. 2.—Buffering capacity of $Al(OH)_3$ in an initial quantity of 50 ml. 0.1 $N$ HCl + 0.1 $N$ HCl added at rate of 2.0 ml./min. Key: A, 1.0 Gm. $Al(OH)_3$; B, 1.0 Gm. $Al(OH)_3$ + 250 mg. pepsin; C, 1.0 Gm. $Al(OH)_3$ + 250 mg. peptone.

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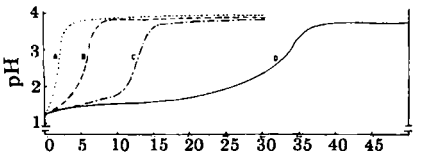


Fig. 3.—Rate of neutralization of various doses of $Al(OH)_3$ in 50 ml. 0.1 *N* HCl. Key: A, 2.0 Gm.; B, 1.0 Gm.; C, 0.5 Gm.; D, 0.25 Gm.

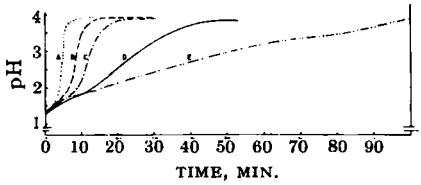


Fig. 4.—Effect of peptone on neutralization rate of 1.0 Gm. $Al(OH)_3$ in 50 ml. 0.1 *N* HCl. Key: A, control; B, 50 mg. peptone; C, 100 mg.; D, 250 mg.; E, 500 mg.

## RESULTS AND DISCUSSION

**Effect of Antacid Dose on Neutralization Rate.**—The neutralization of aluminum hydroxide by HCl is an extremely complex process which has not been elucidated completely. A schematic representation of this reaction may be

*a*, aluminum hydroxide (solid, excess) → aluminum hydroxide (solution)

*b*, aluminum hydroxide (solution) + $H^+$ → aluminum salts + $H_2O$

The demonstration has been made in our laboratories that both the $K_{sp}$ and rate of step *a* is pH dependent. In addition, step *b* is instantaneous. Therefore, although the kinetics of such a reaction are extremely involved, it would appear that the over-all process is rate-limited by step *a*. However, exploratory experiments have indicated that the neutralization rate is independent of rate of agitation above a given minimum, which is considerably lower than the stirring rate employed throughout this investigation. It would appear, therefore, that this model is oversimplified. Considering the hydrophobic nature of aluminum hydroxide, an alternate scheme would assign the rate-limiting step to the wetting of the solid surface by the test fluid. Stated in another way, the rate-limiting step would involve the diffusion of protons to the surface of the poorly soluble antacid. This proposition seems to be the more valid.

In either case, the rate of neutralization would be dependent on the total surface area of the antacid and in turn on the total amount of antacid employed. This concept is contrary to conclusions in a recent report (2) which evaluated aluminum salts of water-insoluble aliphatic acids. These authors stated, (with respect to *in vitro* testing) "the exact amount (of antacid) added is not important provided an excess is present; however, it must be in an immediately dispersable form." Although the conclusions reached may be valid for the antacids tested in the cited study, it was believed that this was not the case for the more commonly used antacids. To verify this belief, the rate of neutralization of various excess amounts of aluminum hydroxide was studied. The results depicted in Fig. 3 clearly reveal the strong dependence of neutralization rate on the amount of antacid employed. Experiments using magnesium trisilicate and calcium carbonate yield equivalent results.

These data indicate that, under similar *in vivo* conditions, the onset of action of noneffervescent antacids will be dependent on the dose administered. According to these findings, it is quite plausible that a double dose of an antacid would have considerably more than twice the antacid activity of a single dose. Additional experiments established that the rate of neutralization of effervescent antacids are independent of the amount used.

**Effect of Amount of Inhibitor on Rate of Neutralization.**—As indicated in Fig. 4, the extent of inhibition increases proportionately with the amount of peptone[1] employed. This relationship held for all inhibitors tested. It is clear that even relatively small amounts of polypeptide would essentially negate the antacid activity of aluminum hydroxide by depressing the neutalization rate.

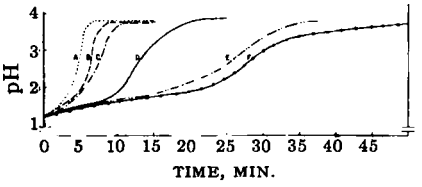


Fig. 5.—Effect of various protein inhibitors on the reaction rate of 1.0 Gm. $Al(OH)_3$ in 50 ml. 0.1 *N* HCl. Key: A, control; B, 100 mg. amylase; C, 250 mg. gelatin; D, 100 mg. cellulase; E, 100 mg. protease; F, 100 mg. Tryptone, a polypeptide prepared by Difco Laboratories, Detroit, Mich.

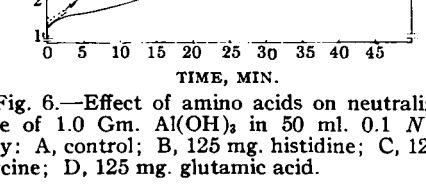


Fig. 6.—Effect of amino acids on neutralization rate of 1.0 Gm. $Al(OH)_3$ in 50 ml. 0.1 *N* HCl. Key: A, control; B, 125 mg. histidine; C, 125 mg. glycine; D, 125 mg. glutamic acid.

**Protein Inhibition.**—Newey (3) was the first investigator to point out that a combination of pepsin and peptone incorporated in the test fluid acted to inhibit the *in vitro* buffering capacity of aluminum hydroxide. The present study reveals that a combination of these two materials is not a necessary condition for the inhibitory process but that each can independently exert an inhibitory effect (Fig. 1). The polypeptide, peptone, appears to be an inhibitor more effective than pepsin when the two are compared at equal concentrations.

To determine the specificity of this inhibition, a number of protein materials, including undegraded proteins, enzymes, and polypeptides, was tested. The inhibition was not limited to pepsin and peptone but was a rather general phenomenon. Each of the

[1] Bacto-Peptone, Difco Laboratories, Detroit, Mich.

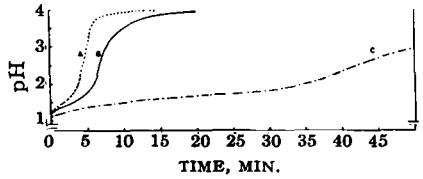


Fig. 7.—Effect of organic acids on neutralization rate of 1.0 Gm. $Al(OH)_3$ in 50 ml. 0.1 *N* HCl. Key: A, control; B, 1 meq. acetic acid; C, 1 meq. citric acid.

TABLE I.—GLUTAMIC ACID IN SOLUTION (PER CENT NITROGEN) DURING THE COURSE OF NEUTRALIZATION

| Sample Time, Min. | pH | N, % |
|---|---|---|
| Control | 1.25 | 0.022 |
| 15 | 2.17 | 0.022 |
| 30 | 3.10 | 0.022 |
| 45 | 3.40 | 0.022 |
| 60 | 3.66 | 0.022 |

protein materials tested was observed to exhibit some degree of inhibitory activity. The results of some of these tests are plotted in Fig. 5. It would appear likely that the existence of any protein or degraded protein in the stomach could result in the inhibition of the *in vivo* antacid activity of aluminum hydroxide.

**Amino Acid Inhibition.**—Experimental results indicated that partially degraded proteins were inhibitors generally more effective than intact proteins at equal concentrations. This aspect of the study was enlarged to examine the effect of amino acids on the neutralization rate. The results of this phase, summarized in Fig. 6, demonstrate that all three amino acids investigated have inhibitory activity at the concentration level employed.

The dicarboxylic compound, glutamic acid, was the most potent inhibitor of the three. A more imposing aspect is obtained when the inhibitory activities of the amino acids are compared on a molar basis. When 2 m*M* of each compound was tested, glycine and histidine each inhibited neutralization of aluminum hydroxide for 3 to 4 minutes. Glutamic acid under the same test conditions inhibited equilibrium to the extent of about 100 minutes.

**Organic Acid Inhibition.**—In view of the high inhibitory activity manifested by glutamic acid, a dicarboxylic amino acid, it was decided to determine the possible role of the carboxyl moiety in the inhibition process. Exploratory studies conducted with either acetic or citric acids demonstrated significant inhibition of the neutralization rate of aluminum hydroxide. Figure 7 indicates that, when compared on an equivalent basis the tricarboxylic citric acid was the more effective inhibitor.

It is important to note that neither organic acid had a perceptible effect on the initial pH of the test solution or on the equilibrium pH. Thus, a simple pH effect cannot be invoked in explaining the results.

**Existence of Insoluble Interaction Product.**—Among the possible explanations for the observed inhibition effects are (*a*) the formation of an insoluble aluminum hydroxide-inhibitor complex or (*b*) the adsorption of the inhibitor on the surface of the dispersed aluminum hydroxide particles. To check these hypotheses, the decision was made to determine accurately the amount of glutamic acid remaining in solution during the course of neutralization. Glutamic acid, 125.0 mg., was added to each of a series of flasks containing 50 ml. of 0.10 *N* HCl. Dried aluminum hydroxide gel, 1.000 Gm., was added to each; neutralization was allowed to proceed. After intervals of 15, 30, 45, and 60 minutes, the contents of successive flasks were rapidly filtered *in vacuo*, and the filtrate was analyzed for nitrogen content by a micro-Kjeldahl technique.[1] The results, summarized in Table I, show that the glutamic acid content in the solution had remained constant during the course of the reaction.

These experiments would seem to rule out the occurrence of an insoluble complex or the existence of inhibitor adsorption as possible mechanisms for the inhibitory process. However, there remains the possibility of a soluble complex formation between the aluminum hydroxide and a free carboxyl group, a moiety which has been present in each of the inhibitors tested. This interesting consideration is presently under investigation.

## SUMMARY

The inhibitory effect of pepsin and peptone on the buffering capacity of noneffervescent antacids may be attributed to a depression of the rate of neutralization of the antacid.

The dose of noneffervescent antacids was critical in determining the neutralization rate. The larger the dose employed, the faster the neutralization process.

The pepsin-peptone inhibition of aluminum hydroxide was observed to be a rather general phenomenon. Inhibition was noted with various proteins, enzymes, polypeptides, and amino acids.

Preliminary studies showed that the carboxylic acids, acetic and citric, were inhibitors of aluminum hydroxide neutralization.

The existence of an insoluble product of antacid and inhibitor, either *via* complex formation or inhibitor adsorption, was eliminated as a potential mechanism in the inhibition phenomenon.

## REFERENCES

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(2) Eriksen, S. P., Irwin, G. M., Swintosky, J. V., *ibid.*, **52**, 552(1963).
(3) Newey, A. E., *Pharm. J.*, **188**, 115(1962).

[1] The authors thank Dr. T. Cayle, Wallerstein Co., Staten Island, N. Y., for conducting these analyses.