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Critical *In Vitro* Factors in Evaluation of Gastric Antacids I

Polypeptide Inhibition

By SAURABH DESAI, MILO GIBALDI, and JOSEPH L. KANIG

In vitro **investigation of a wide range of antacid materials contained in various commercially available dosage forms has indicated demonstrable differences in both the degree and rate of neutralization and in buffering capacities. Results obtained by using pH-titration curves reveal that the type of dosage form exerts an influence on the antacid properties of the same chemical compound. Inhibition of antacid activity of some compounds was observed when polypeptides were added to the artificial gastric fluid. It is thus conceivable that antacid activity could be completely curtailed if similar conditions are encountered in the human system.**

ANTACIDS are the object of considerable interest in therapeutics and in pharmacological and pharmaceutical research. Because of their widespread use in the treatment of peptic ulcers, hyperacidity, and gastric distress, numerous *in vitro* investigations have been instituted to evaluate antacid activity (1–11).

The increasing popularity of antacid therapy has led to the marketing of a large number of products which are based on the principle of neutralization of excess gastric acid. These commercially available products may be broadly divided into systemic and nonsystemic preparations. According to Fuchs (12) systemic antacids are defined as those which react with hydrochloric acid in the stomach to form by-products which are readily absorbed and, in turn, induce a systemic alkalosis by shifting the buffer balance of the blood. Nonsystemic antacids are said to form by-products which are insoluble in body fluids and therefore will not induce an alkalosis.

In vivo excretion data obtained from another study currently being conducted in our laboratories offer evidence that the supposed nonsystemic antacids may induce a mild but nevertheless measurable systemic alkalosis. Furthermore, almost all of the products in this category will react with HCl to form water soluble by-products such as magnesium chloride, calcium chloride, and aluminum chloride which are equally soluble in body fluids.

Since the possibility exists that the so-called nonsystemic antacids might induce systemic effects and to avoid conceptual misunderstandings because of erroneous terminology, antacids have been classified in this investigation on an entirely different basis. A study of the currently marketed antacids indicated that all products could be divided into two major product types—effervescent and noneffervescent.

Although a number of comparative studies in recent years have been reported (1–3, 7–10, 13) regarding antacid efficiency, a lack of information still exists on comparisons between (*a*) effervescent and noneffervescent antacids, (*b*) antacid products using doses recommended by manufacturers rather than equal weights of similar products or other arbitrary levels, (*c*) the different dosage forms of the same products at the recommended dosage levels for each.

Since it has the greatest effect on results, one of the most critical *in vitro* factors is the composition of the test fluid. Almost invariably a dif-

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TABLE I.—ANTACID PRODUCTS AND INGREDIENTS EVALUATED

Code Designation	Dosage Form	Recommended Dose[a] Min.	Max.	Composition
A	Effervescent tablet	1	2	Sodium bicarbonate, citric acid, monocalcium phosphate
B	Effervescent powder	1	...	Sodium bicarbonate, sodium potassium tartrate, tartaric acid
C	Effervescent granules	1	2	Sodium bicarbonate, potassium bicarbonate, citric acid, tartaric acid, calcium lactate, sodium phosphate, magnesium sulfate
D	Tablet	2	4	Aluminum hydroxide, magnesium hydroxide
D-L	Liquid	2	4	1 teaspoonful of D-L = 1 tablet of D
E	Tablet	2	4	Magnesium hydroxide, aluminum hydroxide
E-L	Liquid	2	4	1 teaspoonful of E-L = 1 tablet of E
F	Tablet	1/2	1	Aluminum hydroxide
F-L	Liquid	1	2	1 teaspoonful of F-L = 1/2 tablet of F
G	Tablet	1	2	Aluminum hydroxide, magnesium hydroxide
G-L	Liquid	1	2	1 teaspoonful of G-L = 1 tablet of G
H	Tablet	2	4	Magnesium trisilicate, aluminum hydroxide
H-L	Liquid	1	4	1 teaspoonful of H-L = 1 tablet of H
I	Tablet	2	4	Magnesium hydroxide
I-L	Liquid	1	3	Magnesium hydroxide[b]
J	Tablet	1	2	Calcium carbonate, glycine
J-L	Liquid	1	...	Calcium carbonate, glycine[b]
K	Tablet	2	3	Sodium bicarbonate, calcium carbonate, bismuth subcarbonate, magnesium carbonate, magnesium oxide, papain
L	Tablet	6	...	Sodium bicarbonate, charcoal, ginger, capsicum.
M	Tablet	1	2	Dihydroxy aluminum-sodium carbonate
N	Tablet	1	4	Calcium carbonate, magnesium carbonate, magnesium trisilicate
O	Tablet	1	4	Aluminum hydroxide, magnesium oxide, sodium lauryl sulfate
P	Tablet	1	2	Aluminum hydroxide, calcium carbonate, magnesium peroxide

[a] Expressed as number of tablets in the case of tablets; number of teaspoonfuls (5 ml.) for liquids or granules. [b] No equivalency between whole dosage units of liquid and tablet forms is available.

ferent test fluid has been proposed for each of the evaluative procedures reported in the literature (6). In 1953, Brindle (5) found that the results obtained from *in vitro* evaluations of antacids depended upon the presence or absence of pepsin and peptone in the test fluid. Others have stated (14, 15) that such materials have little effect on the results. Recently, Beekman (16) and Newey (17) have confirmed Brindle's observations and have shown that pepsin and peptone exhibit a marked inhibitory effect on the *in vitro* activity of dried aluminum hydroxide gel. The effect of polypeptides has not, however, been evaluated with other antacids which are formulated with calcium or magnesium compounds or with effervescent antacids. Furthermore, the mechanism of such an inhibition has never been elucidated.

The objectives of this study were therefore (*a*) to compare the evaluation of the basic types of marketed antacids and the different dosage forms of individual antacid preparations, and (*b*) to investigate the role of polypeptides on *in vitro* antacid activity.

EXPERIMENTAL

Volume of Test Fluid.—According to Fuchs (12) the human stomach contains the equivalent of approximately 50 ml. of free 0.1 *N* HCl shortly after a meal. He also concludes that an additional 240 ml. of 0.1 *N* HCl is secreted within 2 hours thereafter. Based on these observations it was decided to employ 50 ml. of 0.1 *N* HCl as the starting volume in all neutralization determinations. When buffering capacities of an antacid were evaluated, 0.1 *N* HCl was added to this volume at the rate of 2 ml./minute.

TABLE II.—INDIVIDUAL ANTACID COMPONENTS EVALUATED

Code Designation	Material	Quantity Evaluated
Q	Sodium bicarbonate U.S.P.	1 Gm.
R	Sodium citrate U.S.P.	1 Gm.
S	Dried aluminum hydroxide gel U.S.P.	1 Gm.
S-L	Aluminum hydroxide gel, 10%	10 ml.
T	Magnesium trisilicate U.S.P.	1 Gm.
T-L	Magnesium trisilicate gel, 10%	10 ml.
U	Calcium carbonate U.S.P.	1 Gm.
U-L	Calcium carbonate gel, 10%	10 ml.
V	Magnesium oxide U.S.P.	1 Gm.
W	Magnesium carbonate U.S.P.	1 Gm.

TABLE III.—RATES OF NEUTRALIZATION AND MAXIMUM pH ATTAINED BY MINIMUM RECOMMENDED DOSAGES OF COMMERCIAL ANTACIDS

Code Designation	Minutes Required to Attain pH 4	Max. pH Reached
A	< 0.5	6.7
B	< 0.5	5.3
C	< 0.5	9.1
D	2 (<1)[a]	6.4
D-L	< 0.5	4.9
E	2 (1)	7.7
E-L	2	5.0
F	...	3.8
F-L	...	3.9
G	...	3.9
G-L	8	4.4
H	17 (1.5)	7.4
H-L	13	6.0
I	< 2 (<0.5)	8.9
I-L	< 0.5	8.9
J	< 2 (<0.5)	7.6
J-L	< 1	7.7
K	< 0.5	8.6
L	1 (<0.5)	9.2
M	...	3.8
N	22 (4)	7.8
O	25 (3)	5.2
P	4 (4)	7.4

[a] Figures in parentheses indicate values for crushed tablets.

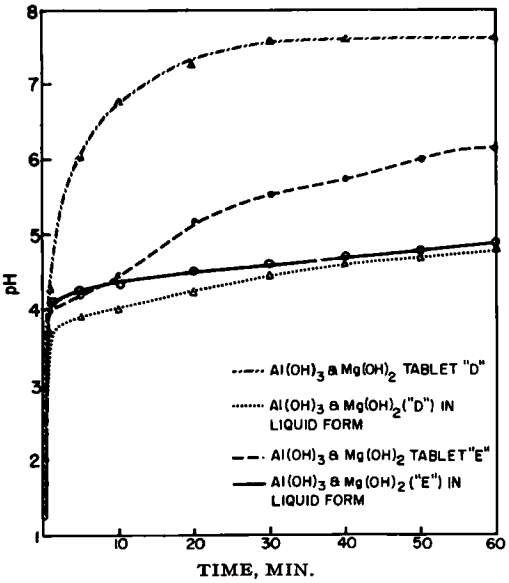

Fig. 1.—Rate of neutralization and maximum pH attained with minimum equivalent doses of two dosage forms of two commercial antacids in 50 ml. 0.1*N* HCl.

Range of Antacid Activity.—Lack of agreement exists regarding the pH range required for antacid effect. These have been reported as ranges of 2 or above, 3 or above, and from 4 to 5.5. Dale and Booth (13) have stated, "despite this apparent disagreement it is likely that all are correct since for certain clinical purposes a final pH range of from 4 to 6 may be desirable while for others a pH of 2 to 3 may suffice." These authors have advocated that antacids could be considered as being effective as long as the pH of the stomach contents remains above that of normal gastric fluid which is considered to be about 1.5 to 2. In view of these considerations, a return to a pH of 2 was considered as the end point of all buffering capacity determinations.

Selection of Antacids.—The commercial antacid products and the antacid components utilized in this study are tabulated and coded in Tables I and II. These were selected as being representative of the various types of antacids and the variety of mixtures of antacid materials currently employed.

Procedures

A. Rate of Neutralization.—The recommended minimum dose of a commercial antacid was added to a 400-ml. beaker containing 50 ml. of 0.1 *N* HCl. When the dosage form tested was a tablet, it was evaluated in both crushed and uncrushed form. The test mixture was maintained at a constant temperature of 37 ± 0.5° by immersing the beaker in a constant temperature water bath. Stirring was effected by an overhead stirrer. Stirring was maintained at a constant rate throughout the investigation and provided sufficient agitation to preclude the sedimentation of insoluble material during each evaluation.

The pH changes were recorded with respect to time utilizing a Beckman zeromatic pH meter with an accuracy of ±0.05 pH unit. Readings were taken at regular intervals until the pH remained constant for a period of 15 minutes. This procedure, although not indicative of *in vivo* conditions, nevertheless presents data on neutralization rates of the antacids and gives a clear picture of the speed of neutralization and maximum pH achieved.

B. Buffering Capacity.—Each commercially avail-

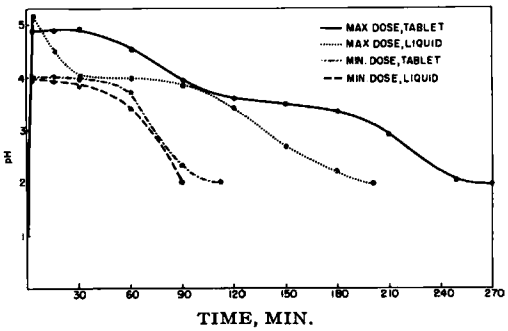

Fig. 2.—Differences in buffering capacities of minimum and maximum equivalent doses of two dosage forms of a commercial antacid containing aluminum and magnesium hydroxide, "D."

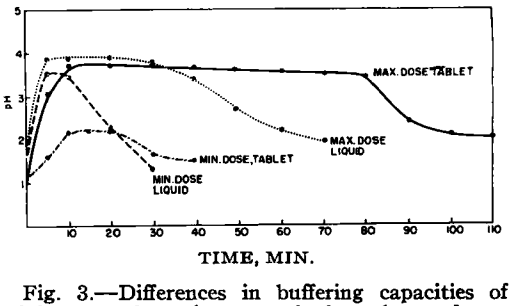

Fig. 3.—Differences in buffering capacities of minimum and maximum equivalent doses of two dosage forms of a commercial antacid containing aluminum hydroxide, "F."

TABLE IV.—BUFFERING CAPACITIES OF COMMERCIAL ANTACIDS

Code Designation	Min. Dose: Max. pH	Min. Dose: ml. 0.1 *N* HCl Neutralized	Max. Dose: Max. pH	Max. Dose: ml. 0.1 *N* HCl Neutralized
A	6.4	290	7.6	530
B	5.3	930	...	...
C	8.0	330	8.3	690
D	4.0	310	4.9	580
D-L	4.0	250	5.1	450
E	4.0	190	5.8	390
E-L	4.3	310	4.6	610
F	2.3	130	3.8	270
F-L	3.8	110	4.0	190
G	3.4	130	4.0	280
G-L	3.9	170	4.0	320
H	2.2	160	6.3	290
H-L	2.8	100	4.7	410
I	8.9	250	8.9	490
I-L	8.9	170	8.9	510
J	4.2	130	7.3	230
J-L	6.6	210	...	...
K	7.8	250	7.7	390
L	8.9	250	...	...
M	3.6	90	3.7	150
N	1.8	80	7.6	280
O	2.3	120	5.4	510
P	2.5	120	6.2	220

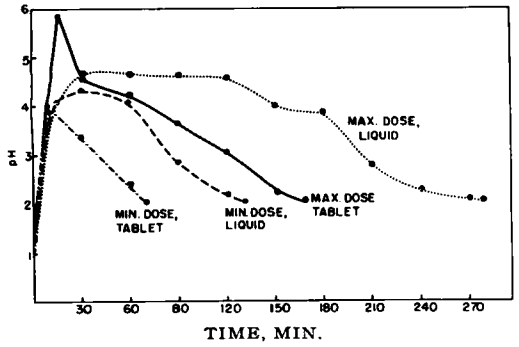

Fig. 4.—Differences in buffering capacities of minimum and maximum equivalent doses of two dosage forms of a commercial antacid containing magnesium and aluminum hydroxide, "E."

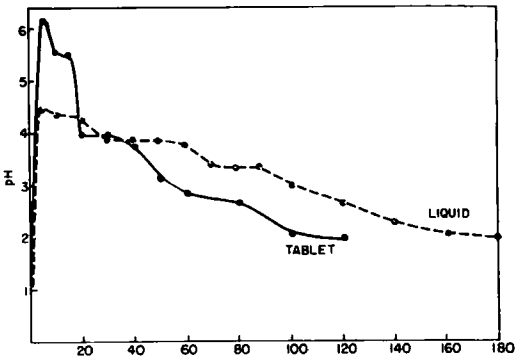

Fig. 5.—Difference in buffering capacity of the maximum equivalent doses of two dosage forms of a commercial antacid containing magnesium and aluminum hydroxide, "H."

able antacid product was evaluated as described in *Procedure A* by adding either the minimum or the maximum dose recommended by the manufacturer. Immediately after adding the dose, additional 0.1 *N* HCl was added to the reaction mixture at the rate of 2 ml. per minute until the pH returned to a base value of 2. This procedure indicated the total amount of acid consumed by the antacid under consideration as well as the time-pH relationship. An additional aspect of this phase of the study was the evaluation of the individual component chemicals currently utilized in antacid preparations.

C. Effect of Test Fluid Composition on Antacid Activity.—Commercially available antacids and antacid component chemicals were evaluated as described in *Procedure B* except that the test fluid used was modified as follows:

Test Solution 1.—Pepsin 0.32% and sodium chloride 0.2% dissolved in 0.1 *N* HCl was used. This is U.S.P. XVI artificial gastric fluid.

Test Solution 2.—Pepsin 0.32%, sodium chloride 0.2%, and peptone[1] 0.15% dissolved in 0.1 *N* HCl was used. The concentration of peptone approximates the amount of this material found in human gastric fluid (5).

These solutions were used in additional modified procedures which were designed to study the effect of polypeptides on rate of neutralization. The method outlined in *Procedure A* was employed but *Test Solution 2* was used instead of 0.1 *N* HCl.

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

RESULTS AND DISCUSSION

The onset of action of an antacid is a critical feature of its efficiency. This action is demonstrated by the rate of neutralization manifested by the product. The results listed in Table III indicate that in almost all cases antacid effect is observed within 5 minutes. For comparison, the time required to reach a level of pH 4 for each antacid is reported. It is evident from the data presented in Table III that there is little difference in onset of action between the liquid form of an antacid product compared to a chewable tablet.

TABLE V.—BUFFERING CAPACITIES OF INDIVIDUAL ANTACID COMPONENTS

Code Designation	Max. pH Attained	0.1 *N* HCl Neutralized, ml.
Q	8.2	130
R	4.1	110
S	3.8	330
S-L	3.8	310
T	5.9	110
T-L	5.8	100
U	7.6	230
U-L	7.4	210
V	9.6	...
W	8.5	210

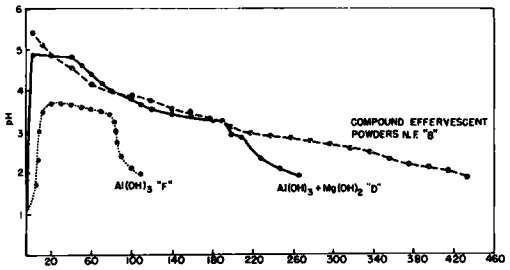

Fig. 6.—Buffering capacities of representatives of three different types of antacid preparations.

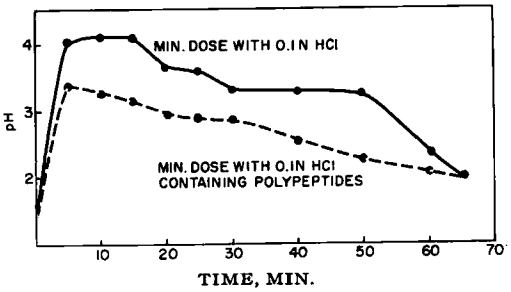

Fig. 7.—Polypeptide inhibition on buffering activity of a commercial antacid tablet containing aluminum and magnesium hydroxide, "E."

Moreover, the liquid forms of products D and E seem to be less basic than the equivalent amounts of the same components in tablet form. Figure 1 indicates that in both products D and E a lower maximum pH is attained with the liquid form. This may be attributable to the nonactive components of the suspension or the tablet which may combine with the antacid material to yield a compound of lower alkalinity upon neutralization or which may physically impede the availability of the active components. Viscosity increasing agents used to stabilize suspensions or binding agents used in tablets may possibly demonstrate this effect. This phenomenon is more clearly observed in Figs. 2 and 3 which indicate buffering capacity of antacids D and F. In each case the tablets consume a significantly greater quantity of acid compared to the liquid form.

In Fig. 3, even though the minimum dose of the liquid preparation is a superior antacid (as indicated by the area under the curve to a base line at pH 2), the equivalent dose in tablet form neutralizes

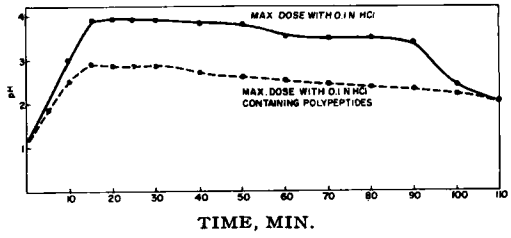

Fig. 8.—Polypeptide inhibition on buffering activity of a commercial antacid tablet containing aluminum hydroxide, "F."

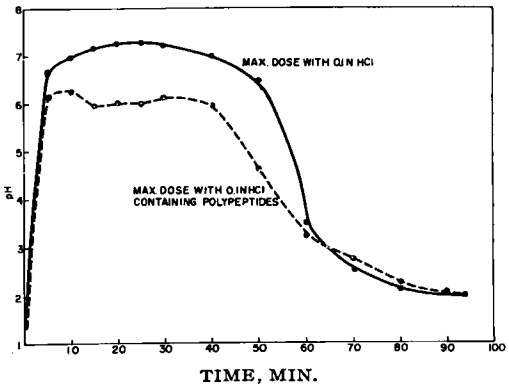

Fig. 9.—Polypeptide inhibition on buffering activity of a commercial antacid tablet containing calcium carbonate and glycine, "J."

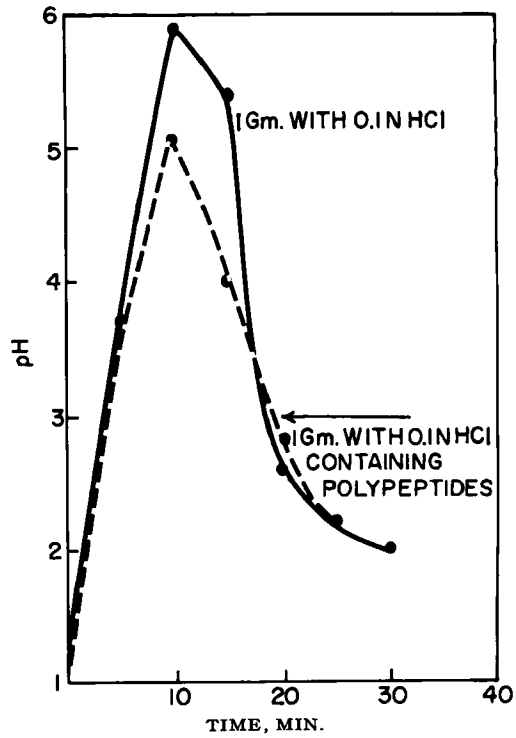

Fig. 10.—Polypeptide inhibition on buffering activity of magnesium trisilicate.

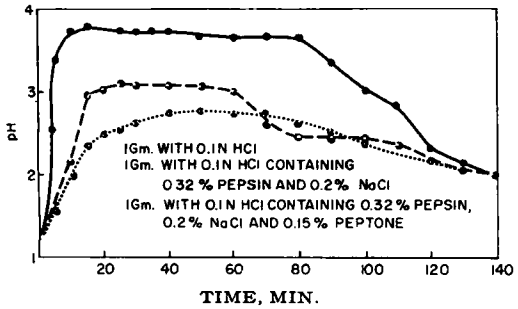

Fig. 11.—Polypeptide inhibition on buffering activity of dried aluminum hydroxide gel U.S.P. The legends identify the respective curves beginning at the top.

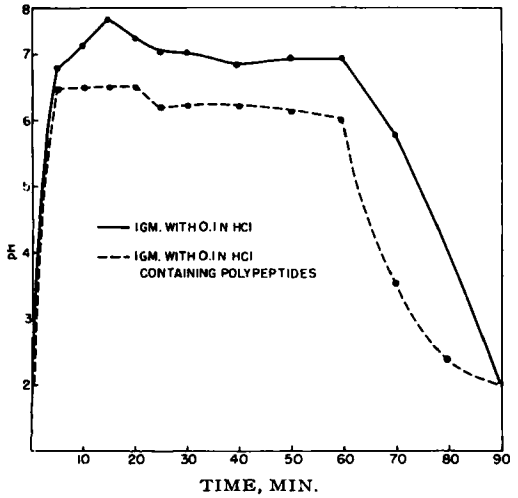

Fig. 12.—Polypeptide inhibition on buffering activity of calcium carbonate.

a greater amount of acid. If the assumption is made that a similar picture exists *in vivo*, then it is conceivable that the tablet form would be severely limited in treatment of peptic ulcer where inactivation of gastric pepsin activity due to elevated pH is desired. In such cases, the liquid form would manifest a greater degree of therapeutic activity.

On the other hand, when products E and H and their liquid equivalents, E-L and H-L, were evaluated under identical conditions, quite the opposite effects were noted. These results are depicted in Figs. 4 and 5 and indicate that the liquid showed a considerably greater neutralizing capacity compared to the equivalent dose in tablet form.

It is significant that products D and F contain aluminum hydroxide as the predominent component, while product E contains equivalent amounts of magnesium and aluminum hydroxides and product H contains magnesium trisilicate in a ratio of 2–1 to aluminum hydroxide. Auxiliary investigations are currently underway to discover whether these divergent results are due to individual product properties or the antacid components generally.

A comparison of the different types of products revealed that all the effervescent antacids tested possess a much greater buffering capacity than the noneffervescent antacids. These results are contained in Tables IV and V. Figure 6 indicates that compound effervescent powder N.F. (product B) neutralized almost twice the volume of 0.1 *N* HCl as product D. Product D is the most efficient noneffervescent compound with respect to neutralizing capacity. Of greater significance is that effervescent product B neutralizes almost four times the amount of 0.1 *N* HCl as does product F which contains aluminum hydroxide as the sole active component.

Effect of Polypeptides.—It has been demonstrated (16, 17) that polypeptides such as would be found in the stomach contents following ingestion of a meal have a decided inhibitory effect on antacid activity of aluminum hydroxide. To ascertain whether polypeptides such as pepsin and peptone exhibit a similar inhibitory effect on other antacid materials, the buffering capacities of a number of marketed antacid preparations of varying composition were evaluated in the presence of polypeptides. Figures 7–9 represent the results of these tests. These figures indicate that the inhibitory effect of polypeptides is not limited to aluminum hydroxide exclusively but is seen to occur with the other products tested which contain magnesium or calcium compounds.

Polypeptide inhibition was not observed in the

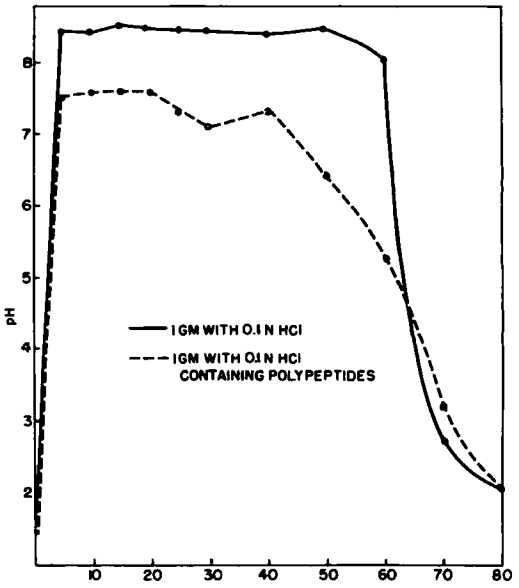

Fig. 13.—Polypeptide inhibition of buffering activity of magnesium carbonate.

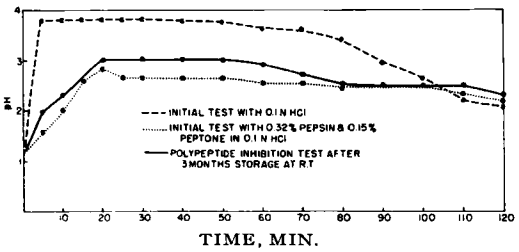

Fig. 14.—Effect of aging on polypeptide inhibition of the buffering capacity of a 10% w/v aluminum hydroxide aqueous suspension.

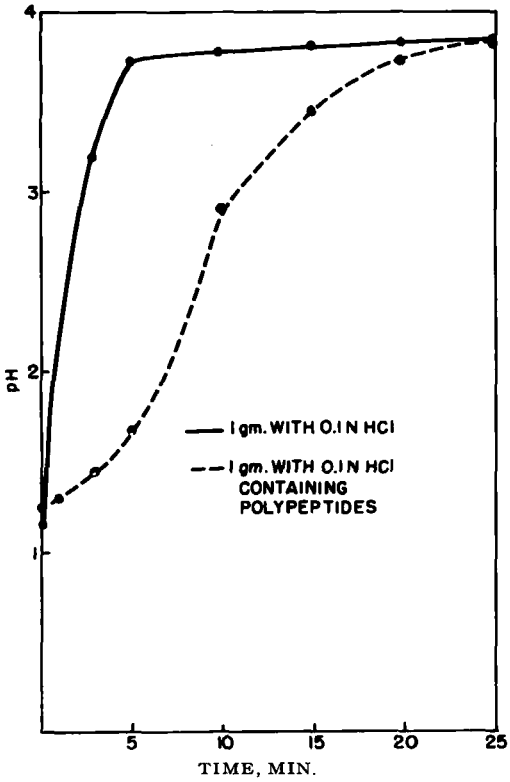

Fig. 15.—Polypeptide induced neutralization lag with dried aluminum hydroxide gel U.S.P.

liquid products tested regardless of composition. In addition, it was not demonstrated with the effervescent products tested. Increases in concentration of pepsin and peptone in the test solutions failed to cause deviations in the neutralization or buffering capacity curves of the effervescent antacids. This resistance to inhibition would seem to offer a marked advantage to the use of either the liquid form of an antacid or an effervescent antacid.

To complete the study of the inhibitory effect, a number of powdered antacid chemicals were selected and tested for buffering capacity in the presence of pepsin and peptones. Figures 10–13 demonstrate the pronounced depression in the curves for aluminum, magnesium, and calcium compounds.

Although there is a marked depression in the pH attained by an antacid in the presence of polypeptides, an important observation which has not been reported in the literature to date is that the total volume of HCl neutralized remains the same. In all cases the volume of hydrochloric acid required to return the pH to a level of 2 was the same regardless of whether pepsin and peptone were present. This indicates that the inhibition will be of little significance when considering maximum doses of calcium and magnesium compounds. Since both compounds elevate the pH well above 5 at these dosage levels, a slight decrease of 1 to 1.5 pH units will have little effect on their activity, considering that the same amount of hydrochloric acid is neutralized.

The inhibition effect, however, does become significant when considering the lower doses of these materials as well as dry aluminum compounds. It is therefore plausible to consider the possibility that the *in vivo* activity of these antacids in the presence of polypeptides will be markedly reduced or limited.

Mechanism of Inhibitory Effect.—Figure 14 depicts the results obtained when a 10% dispersion of dried aluminum hydroxide gel in distilled water was allowed to hydrate for a period of 3 months. The aging period served to decrease the polypeptide effect; these results are in agreement with those obtained with commercial liquid antacid products.

The neutralization lag was studied by subjecting 1 Gm. of dried aluminum hydroxide gel to *Test Solution 2*. Figure 15 indicates that the maximum pH level of the lower curve is the same as the upper curve. This fact together with those observed in all buffer capacity experiments, which showed the same amount of HCl neutralized with or without polypeptides, indicates that a kinetic involvement underlies these phenomena rather than some irreversible complex formation. Additional work is being conducted which will lead to a more complete elucidation of the mechanism involved.

SUMMARY

1. A comparative *in vitro* evaluation was conducted on effervescent and noneffervescent products and on liquid and solid dosage forms of the same product. It was demonstrated that the effervescent antacids were superior in buffering capacity to the noneffervescent types. Differences in efficiency were observed between solid and liquid dosage forms on an equivalent dosage basis.

2. The role of polypeptide inhibition in *in vitro* procedures was studied and produced data which indicate that a positive inhibitory effect may be observed with all solid noneffervescent antacids but not with liquid or effervescent forms.

3. The pronounced effect of polypeptides on many antacid preparations illustrates the vital role they exercise in *in vitro* evaluations and the necessity for their inclusion in all artificial test fluids.

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