Evaluation of Antacid Activity for Metal Complexes of Tris(hydroxymethyl)aminomethane (THAM)

By NANCY CHEN and GABRIEL G. NAHAS

An ideal antacid should buffer in the physiological pH range of 3 to 5, and should have a high neutralizing capacity per unit weight. Also, its initial effect should be rapid and the ideal pH range should be maintained for a relatively prolonged period. The organic aluminum and bismuth salts of a weak organic base, tris(hydroxymethyl)aminomethane (THAM) were prepared and their relative buffer capacities were compared. THAM-gluconatodihydroxo-aluminate was found to be an effective soluble buffer antacid compound.

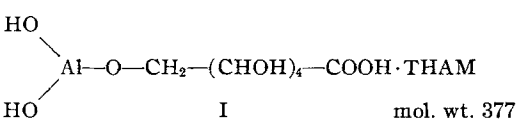
THE EXACT evaluation of antacids for *in vivo* titration is extremely difficult because of the many biological parameters involved. Therefore, *in vitro* methods must be used and these do not take into account individual physiological variations (*e.g.*, variations in composition of gastric juice, rate of secretion, rate of discharge from the stomach, etc.). However, such *in vitro* studies also present methodological difficulties. Hefferren *et al.* (1) compared the results obtained with different procedures and found that there were variations characteristic of a particular antacid and also of the method. Regardless of the procedure used, these *in vitro* studies indicate that an ideal antacid should buffer in the physiological pH range of 3 to 5, and should have a high neutralizing capacity per unit weight. Also, the initial effect should be rapid and the ideal pH range should be maintained for a relatively prolonged period.

The weak organic base, tris(hydroxymethyl)-aminomethane (THAM), is an unsatisfactory antacid because it fails to meet these criteria. Therefore, organic aluminum and bismuth salts of THAM (pH 7.0) were prepared and their relative buffering capacities were compared potentiometrically.

METHODS

Chemical and Physical Properties of Complexes and their Preparations (2).—The complexes with THAM were prepared from the aluminum and bismuth salts of several α-hydroxy-carboxylic acids (citric, gluconic, salicylic, *p*-aminosalicylic acids). Equivalent amounts of THAM were added to a suspension of the salt or to a solution obtained by heating the suspension to 70–80°. The complex was isolated by concentrating this aqueous solution to dryness *in vacuo*. Preparation of these compounds was performed in cooperation with Charra (2) as follows.

Aluminum–Gluconic Acid–THAM (1:1:1).—Gluconic acid (43.6 Gm. of 45% solution, 0.1 mole) is diluted in 200 ml. of water. In this solution, THAM (12.1 Gm., 0.1 mole) is dissolved, and the solution of aluminum isopropylate (20.4 Gm., 0.1 mole) in 100 ml. of isopropanol is added. Only a slight cloudiness appears. The mixture is stirred for 2 hr. at room temperature. It is then filtered and concentrated *in vacuo*. The solid residue is dried to constant weight in a vacuum desiccator. The analysis of the obtained product corresponds to formula I.

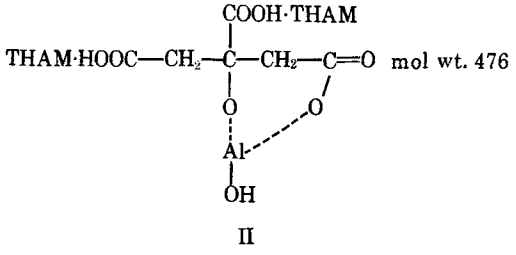


I mol. wt. 377

The pH of a 5% solution of this complex is 7.2.

Aluminum–Citric Acid–THAM (1:1:2).—Potassium alum [$Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24\ H_2O$, 47.45 Gm., 0.05 mole] is dissolved in 350 ml. of lukewarm water. The solution is alkalized with ammonia. The precipitate of aluminum hydroxide is dried, filtered, and washed until there are no more $SO_4^=$ ions in the filtrate.

The aluminum hydroxide is suspended in 100 ml. of water. Hydrated citric acid (10.5 Gm., 0.05 mole) dissolved in 50 ml. of water is added and the mixture stirred for 1 hr. at room temperature. THAM (12.1 Gm., 0.1 mole) dissolved in 50 ml. of water is then added and the mixture heated for 1 hr. in a water-bath at 50°. The dissolution of aluminum hydroxide is now virtually complete. The solution obtained is filtered and concentrated *in vacuo* while stirring with a magnetic stirrer and heating in an oil bath at 80° at the maximum. The residue is made into paste with 50 ml. of absolute ethanol. It is dried and washed with 25 ml. of ethanol. The residue is then dried to constant weight in a vacuum desiccator. Analytical results of the product show that it corresponds to formula II.



II mol wt. 476

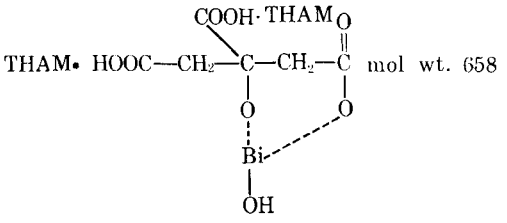
The pH of a 5% solution of this complex is 7.8.

Bismuth–Citric Acid–THAM (1:1:2).—Bismuth citrate (19.9 Gm., 0.05 mole) is suspended in 50 ml. of water. THAM (12.1 Gm., 0.1 mole) dissolved in 30 ml. of water is added. The mixture is heated for 1 hr. at 50°, while stirring, in order to obtain a complete dissolution. It is concentrated *in vacuo* while stirring magnetically. The white crystalline residue is dried to constant weight in a vacuum desiccator. The analysis of the product corresponds to formula III.

Received June 29, 1966, from the Department of Anesthesiology, College of Physicians and Surgeons, Columbia University, New York, N. Y. 10032.

Accepted for publication October 6, 1966.

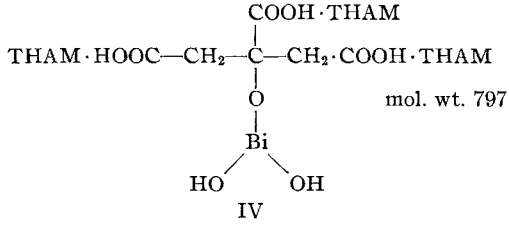
This work was supported in part by grant GM-09069-04 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md., and Army contract DA-49-193MD-2265.



III

The pH of a 5% solution of this complex is 6.70.

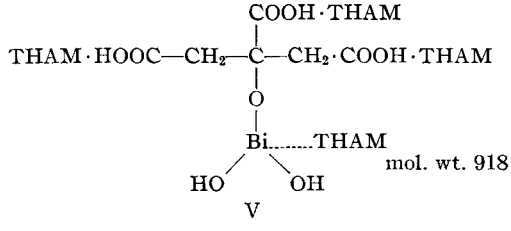
Bismuth–Citric Acid–THAM Complex (1:1:3).—The product is prepared according to the above described method, 0.15 mole of THAM being used. The analysis of the product corresponds to formula IV.



IV

The pH of a 5% solution of this complex is 7.55.

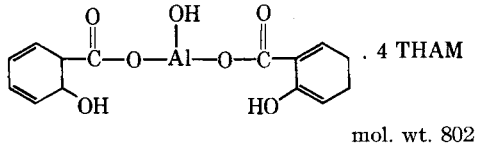
Bismuth–Citric Acid–THAM (1:1:4).—The product prepared by the method described above corresponds to formula V.



V

The pH of a 5% solution of this complex is 7.85.

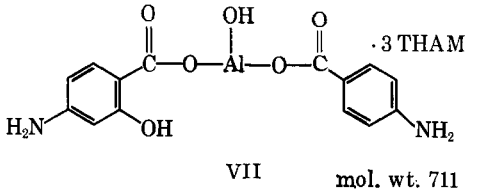
Aluminum–Salicylic Acid–THAM (1:2:4).—The product corresponds to formula VI.



VI

The pH of a 5% solution of this complex is 7.80.

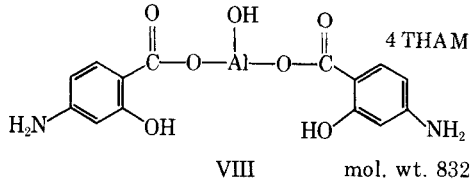
Aluminum–PAS Acid–THAM (1:2:3).—The analysis of the product corresponds to formula VII.



VII

The pH of a 5% solution of this complex is 7.4.

Aluminum–PAS Acid–THAM (1:2:4).—The analysis of the product corresponds to formula VIII.



VIII

The pH of a 5% solution of this complex is 7.5.

Antacid Activity Test.—The method of Gore, Martin, and Taylor (3) was used to determine antacid activity. An arbitrarily selected amount of the complex (calculated exactly to be 20% in excess of the neutralization value) (Table I) was added to 200 ml. of 0.015 *N* hydrochloric acid and the mixture was titrated in a double-walled vessel at 37 ± 0.1°. The pH was measured with a Knick model 35 pH meter and Beckman glass electrodes. Beckman standard buffer solutions at pH 4.00 and 7.00 were used to calibrate the meter. The pH was recorded for 30 min. To simulate the continuous gastric secretion of HCl, hydrochloric acid was then added to the mixture by means of a buret at the rate of 6 meq./hr. until an end point of pH 3.0 was reached.

RESULTS AND DISCUSSION

The relative buffering capacities of the various substances are given in Table II and illustrated in Figs. 1 and 2. A quantity (0.15%) of pepsin, peptone, and sodium chloride can be added to the hydrochloric acid as suggested by Brindle (4), Clemow and Lowry (5), and Grossmith (6). However, the use of such a "mock" gastric juice has little effect and comparable results are obtained with hydrochloric acid alone. In addition, there is some disagreement on the degree of acidity that would be most representative of *in vivo* conditions. However, most investigators recommend a pH of 3.0 to 5.0 and agree that carrying neutralization far beyond this range is undesirable.

TABLE I.—COMPOSITION OF COMPLEXES USED

Complexes	Unit Dose, Gm.
Aluminum–gluconic acid–THAM (1:1:1)	2.3
Aluminum–PAS acid–THAM (1:2:3)	2.5
Aluminum–PAS acid–THAM (1:2:4)	2.8
Aluminum–salicylic acid–THAM (1:2:4)	2.9
Bismuth–citric acid–THAM (1:1:4)	2.0
Bismuth–citric acid–THAM (1:1:3)	1.4
Aluminum–citric acid–THAM (1:1:2)	1.7
Bismuth–citric acid–THAM (1:1:2)	2.2

TABLE II.—BUFFERING CAPACITY

Complexes	0.1 *N* HCl ml. Added
Aluminum–gluconic acid–THAM (1:1:1)	117
Aluminum–PAS acid–THAM (1:2:3)	130
Aluminum–PAS acid–THAM (1:2:4)	140
Aluminum–salicylic acid–THAM (1:2:4)	140
Bismuth–citric acid–THAM (1:1:4)	60
Bismuth–citric acid–THAM (1:1:3)	38
Aluminum–citric acid–THAM (1:1:2)	31
Bismuth–citric acid–THAM (1:1:2)	27

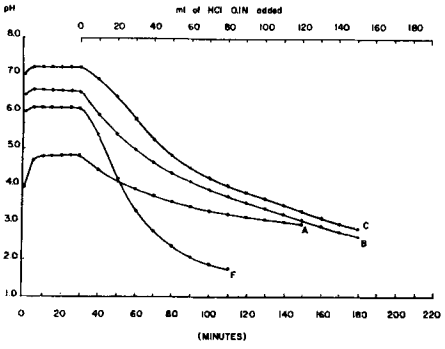


Fig. 1.—Acid consuming activity of four metal complexes of THAM. Key: A, aluminum–gluconic acid–THAM (1:1:1); B, aluminum–PAS acid–THAM (1:2:3); C, aluminum–PAS acid–THAM (1:2:4); F, bismuth–citric acid–THAM (1:1:3).

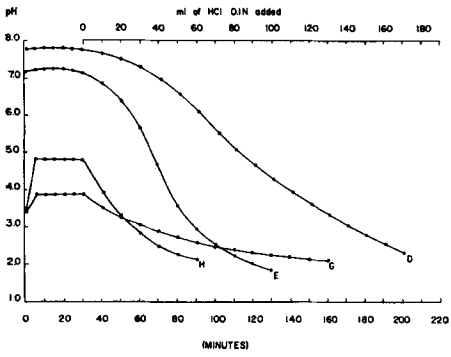


Fig. 2.—Acid consuming activity of four metal complexes of THAM. Key: D, aluminum–salicylic acid–THAM (1:2:4); E, bismuth–citric acid–THAM (1:1:4); G, aluminum–citric acid–THAM (1:1:2); H, bismuth–citric acid–THAM (1:1:2).

In the case of tris(hydroxymethyl)aminomethane–gluconatodihydroxo–aluminate (Al–gluconic acid–THAM, 1:1:1) the pH rose almost instantly to a value of 4.0 and after 5 min., reached a maximum pH of 5.0. The pH remained at about 5.0 for the next 30 min., during which time no additional acid was introduced. This suggests that *in vivo* an overdose of antacid would not increase the pH above 5.0. Moreover, when more acid was reintroduced, the pH did not fall below 3 until after approximately 12 meq. of HCl had been added also indicating that THAM–gluconatodihydroxoaluminate would be an effective soluble buffer antacid compound.

The complex of Bi–citric acid–THAM (1:1:1) had a smaller neutralizing capacity than Al–gluconic acid–THAM within the pH range 3 to 5 and the remaining antacids only have limited buffering capacities.

REFERENCES

(1) Hefferren, J. J., Schrotenboer, G., and Wolman, W., *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 564(1956).
(2) Charra, A., Laboratoire Roger Bellon, Neuilly-Paris, France, personal communication.
(3) Gore, D. N., Martin, B. K., and Taylor, M. P., *J. Pharm. Pharmacol.*, **5**, 686(1953).
(4) Brindle, H., *ibid.*, **5**, 692(1953).
(5) Clemow, P. R., and Lowry, J. W., *ibid.*, **6**, 610(1954).
(6) Grossmith, F., *ibid.*, **15**, 114(1963).

Catharanthus Alkaloids X. Isolation of Lanceine and Vinosidine from *Catharanthus lanceus* Roots

By RALPH N. BLOMSTER*, NORMAN R. FARNSWORTH, and DONALD J. ABRAHAM

In a continuing search for new biologically active entities in *Catharanthus lanceus*, additional work on the root (A) fraction has led to the isolation of two alkaloids. One of these, lanceine, was previously isolated from this plant by other workers. Vinosidine, however, although previously reported in a related species, is reported from this species for the first time.

EARLIER STUDIES on *Catharanthus lanceus*, as a part of a continued search for new and active antineoplastic principles from the various alkaloid fractions, and in an effort to elucidate, as completely as possible, the alkaloid composition of this plant, have resulted in the isolation of 12 crystalline alkaloids (1–4). Three of these, cathalanceine, pericyclivine, and periformyline proved to be new entities, with periformyline representing a new alkaloid of novel structure. Of the remaining nine, ajmalicine, yohimbine, and tetrahydroalstonine have been reported previously from *C. lanceus* roots by other workers (5–7). The additional six alkaloids have also been reported from the related *C. roseus* (8–12). Of these (leurosine, perivine, vindoline, pericalline, perimivine, and lochnerinine) leurosine is of major interest because of its high order of activity against the P-1534 leukemia, and because it was isolated initially from a *C. lanceus* crude alkaloid fraction that was devoid of activity against the P-1534 leukemia in DBA/2 mice (1).

This is a report on the isolation of two additional alkaloids, lanceine, previously reported from this plant by other investigators (6), and vinosidine, reported previously only from *C. roseus* (13).

Received April 26, 1966, from the Department of Pharmacognosy, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pa. 15213.
Accepted for publication September 26, 1966.
Presented to the Pharmacognosy and Natural Products Section, A.PH.A. Academy of Pharmaceutical Sciences, Dallas meeting, April 1966.
* Lederle Pharmacy Faculty Award Recipient, 1966.
This investigation was supported by research grants H-06162, CA-08228, CA-08509, and FR-5455-04 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md., and from the Thaw Fund, University of Pittsburgh, Pittsburgh, Pa.