trary, the species which are more resistant to protamine show absence of red cell agglutination by this agent.

On the basis of these findings, the intravenous use of large doses of protamine in clinical practice would seem not to be without danger.

Summary. 1. Intravenous administration of the protamine, salmine sulfate, to untreated guinea pigs and rats produced death at dose levels of 6-12 mg/100 g. Intraperitoneal injection delayed these effects. 2. The addition of salmine sulfate to whole blood or plasma resulted in the formation of a flocculent protein precipitate. Addition to whole blood caused hemagglutination in the human, dog, cat, and rat. 3. The addition of salmine sulfate to whole blood perfusing isolated organs caused cessation of flow. Addition of comparable amounts to Ringer's fluid and serum perfusing organs was without effect upon the rate of flow. 4. These experiments suggest the possibility that protamines exert toxic effects through embolic vascular phenomena.

13780

Studies on Peptic Inhibition.

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This report is a study of peptic activity as affected by the various substances used clinically in the treatment of gastro-duodenal ulcerative disease as well as of pepsin inactivators. Our specific objective was to learn whether these substances act directly on the pepsin or indirectly through the medium of a pH change.

Hammarsten[1] and Helmar, *et al.*,[2] showed that peptic activity decreases as the pH increases from 1 to 7, being completely inactivated at pH 7. Recently Komarov[3] and Schiffrin[4] reported that various aluminum and magnesium compounds inhibit peptic activity at the optimum pH range, from 1 to 2. Their use of Mett tubes to determine peptic activity plus their procedure of centrifuging

1 Hammarsten, O., *Hoppe Seyler's Z.*, 1922, **121**, 261.

2 Helmer, O. M., Fonts, P. J., and Zerfas, L. G., *Am. J. Dig. Dis.*, 1934, **1**, 120.

3 Komarov, S. A., and Komarov, O., *Am. J. Dig. Dis.*, 1940, **7**, 166.

4 Schiffrin, M. J., and Komarov, S. A., *Am. J. Dig. Dis.*, 1941, **8**, 215.

out the undissolved material made this method unsatisfactory for our use. The Mett tube method possesses the disadvantage of a slow rate of digestion as well as the difficulty in obtaining a reproducible substrate. Centrifugation removes the adsorbed pepsin but does not prove its inactivation.

Methods. We found the Anson-Mirsky[5, 6] hemoglobin method as modified by Beazell[7] reliable and accurate. The source of pepsin was either a solution of granular pepsin* in N/10 HCl or gastric juice collected from a Pavlov pouch dog.

Our procedure was as follows: In one-half of our experiments the substances studied were incubated with gastric juice for 15 minutes at 37°C, determining the pH before and after incubation. Then an aliquot portion of the incubated material was added to 5 cc of a 4% hemoglobin substrate. This was incubated for 15 minutes at 37°C and the undigested hemoglobin precipitated with 10 cc of 4% trichloracetic acid. The amount of digestion was determined by measuring the tyrosine, colorimetrically by Folin's method, in an aliquot portion of the filtrate.

In the second group of experiments exactly the same procedure was followed except that before incubation the substances used were brought to a pH between 1 and 2 with HCl. The substances tested were aluminum hydroxide, aluminum phosphate, magnesium trisilicate, and Mucin.

In addition to these therapeutically used substances, Dr. H. B. Bull of the Department of Physiological Chemistry, suggested using a protein denaturant.[8] This denaturant was a commercial sample of sodium lauryl sulfate.[9, 10, 11] It is a surface active agent which has the property of denaturing proteins in very small concentrations.

Results. Unbuffered aluminum hydroxide, aluminum phosphate, magnesium trisilicate, and Mucin inhibited peptic activity. From Graphs I, III, V, and VII it can be seen (curves A) that as the concentration increased the peptic activity decreased. However, these same graphs show that as the concentration increased the pH increased (curves B), the two curves paralleling each other. The one exception was sodium lauryl sulfate where the pH remained inde-

5 Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 1932, **16**, 59.

6 Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 1938, **22**, 79.

7 Beazell, J. M., Schmidt, C. R., Ivy, A. C., and Monaghon, J. G., *Am. J. Dig. Dis.*, 1938, **5**, 661.

* 1/10,000 spongy pepsin (3 × U.S.P.) Generously supplied by Armour & Co.

8 Bull, H. B., and Neurath, H., *J. Biol. Chem.*, 1937, **118**, 163.

9 *J. Ind. and Eng. Chem.*, 1941, **33**, 17.

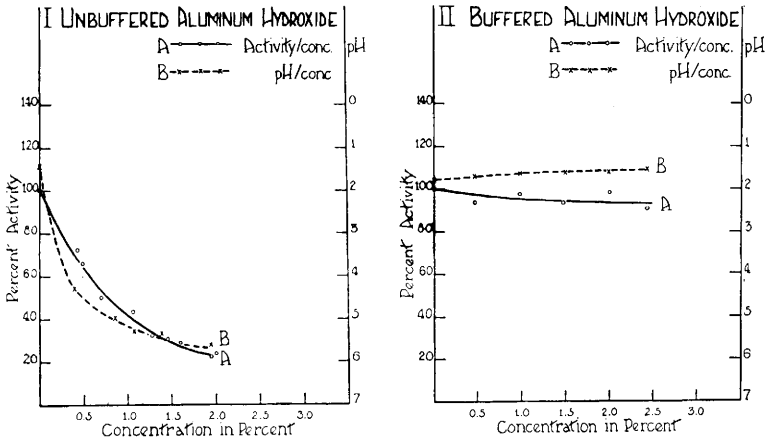
10 Degering, *J. Chem. Educ.*, 1941, **18**, 102.

11 Blair, C. M., *J. Chem. Educ.*, 1941, **18**, 246.

pendent of the concentration (Graph IX), but peptic activity was markedly inhibited with as small a concentration as 0.2%.

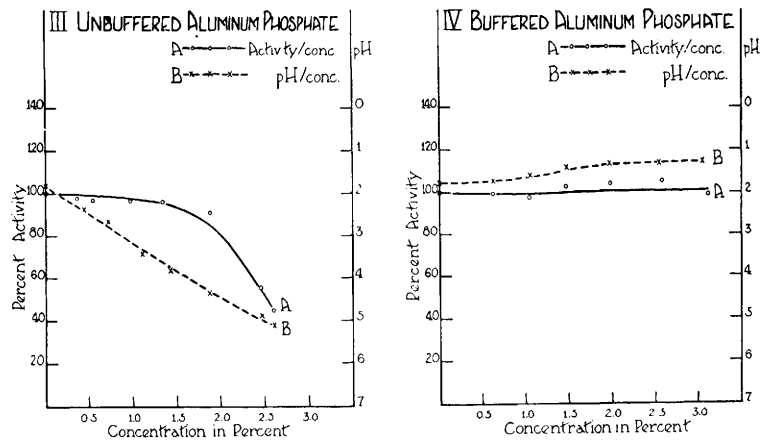
In the other half of the experiments where the pH was kept constant by buffering with HCl (Graphs II, IV, VI, VIII, curves B) the peptic activity (curves A) remained practically unchanged.

Discussion. With the exception of sodium lauryl sulfate peptic inhibition occurred only when the concentration was sufficient to markedly alter the pH, as is shown by the absence of inhibition when the pH is kept constant. With sodium lauryl sulfate, however, we found that pepsin inactivation occurred with no pH change.



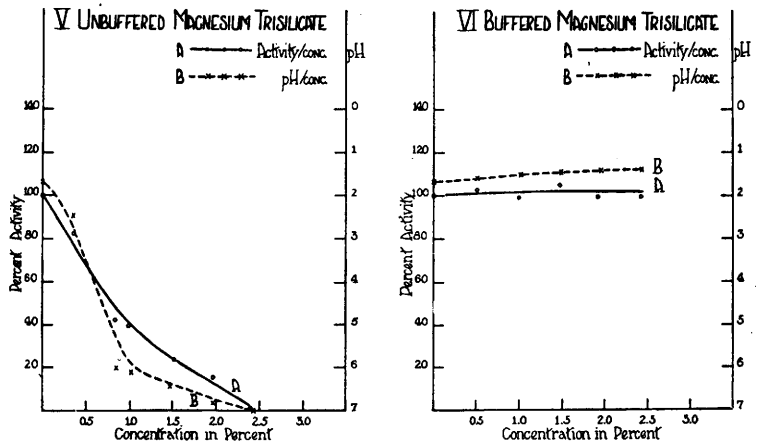
GRAPHS I AND II.

Increasing concentration of aluminum hydroxide decreases peptic activity only when the aluminum hydroxide is unbuffered.



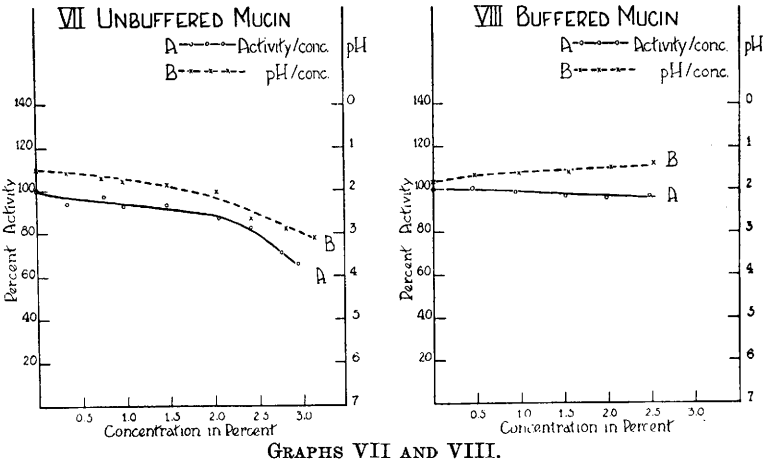
GRAPHS III AND IV.

Increasing concentration of aluminum phosphate decreases peptic activity only when the aluminum phosphate is unbuffered.



GRAPHS V AND VI.

Increasing concentration of magnesium trisilicate decreases peptic activity only when the magnesium trisilicate is unbuffered.



GRAPHS VII AND VIII.

Increasing concentration of gastric mucin decreases peptic activity only when the gastric mucin is unbuffered.

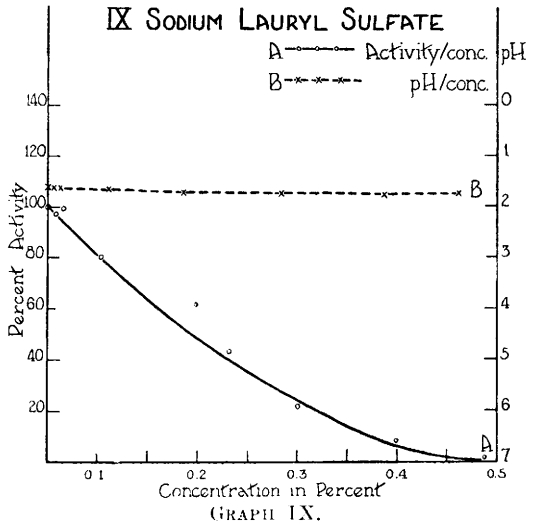
We have reported previously[12] that similar peptic inhibition by sodium lauryl sulfate occurs *in vivo* and will markedly increase the survival time of dogs with ulcers induced experimentally by the massive histamine injection method of Code, *et al.*[13, 14]

Conclusions. 1. Aluminum hydroxide, aluminum phosphate, mag-

12 Shoch, D., and Fogelson, S. J., *Quart. Bull. N. U. M. S.*, 1942, in press.

13 Walpole, S. H., Varco, R. L., Code, C. F., and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 619.

14 Varco, R. L., Code, C. F., Walpole, S. H., and Wangensteen, O. H., *Am. J. Physiol.*, 1941, **33**, 475.



GRAPH IX.

This shows how sodium lauryl sulfate decreases peptic activity without any alteration in pH. Note effectiveness of dilute concentrations.

nesium trisilicate, and mucin inhibited peptic activity by altering the pH, the percent of inhibition being directly related to change in pH. 2. Of these 4, magnesium trisilicate was the most effective inhibiting agent, exerting the greatest effect on the pH. 3. Of the substances used, sodium lauryl sulfate alone completely inhibited peptic activity without any alteration in pH.

13781

Forced Ovulation of Normal Ovarian Follicles in the Domestic Fowl.

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The induction of multiple ovulation following intravenous injection of a luteinizing preparation into hens pretreated with pregnant mares' serum administered subcutaneously for 6 to 100 days has been described.[1] The results set forth in this paper show that ovulation of normally maturing follicles can be forced by at least 17 hours. While the absolute prematurity of ovulation imposed on

[1] Fraps, R. M., and Riley, G. M., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 253.