

Samples of gastric mucin from various commercial sources when treated by the above procedure give closely agreeing values for nitrogen content and reduction after hydrolysis. Representative data are presented in Table I.

The hydrolyzed samples for analysis are prepared by weighing out 0.1 gm. of crude or purified mucin into a 125 ml. Erlenmeyer flask. To this 50 ml. of 2 N sulfuric acid is added, and the sample hydrolyzed in a boiling water bath for $2\frac{1}{2}$ to 3 hrs., using an air condenser.

For estimation of reducing substances, Somogyi's modification⁵ of the Shaffer-Hartmann method using the copper reagent No. 2 is employed. Two ml. aliquots of the acid digestion mixture are pipetted into the test tubes, 1 drop of phenolphthalein added, and the sample neutralized with normal KOH or NaOH. Five ml. of the copper reagent are then added and the analysis conducted as for blood sugar. Results are expressed as percentage reduction calculated as glucose.

For the estimation of nitrogen the method of Koch and McMeekin⁶ is employed. Usually 1 cc. of the hydrolyzed sample is used for this determination.

7196 P

Gastric Mucin Secretion in Gastro-duodenal Ulcerative Disease.

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It was shown¹ that commercial gastric mucin when subjected to peptic digestion and subsequent precipitation with alcohol at 70% concentration, yielded upon hydrolysis characteristic values for total nitrogen and total reducing substances. This suggested the use of a reduction method for the quantitation of mucin. Obviously before such a method can be applied to gastric juice, it is necessary to remove all other reducing substances, or to use a procedure for

⁵ Peters, J. P., and Van Slyke, D. D., *Quantitative Clin. Chemistry*, Baltimore, 1932, **2**, 469.

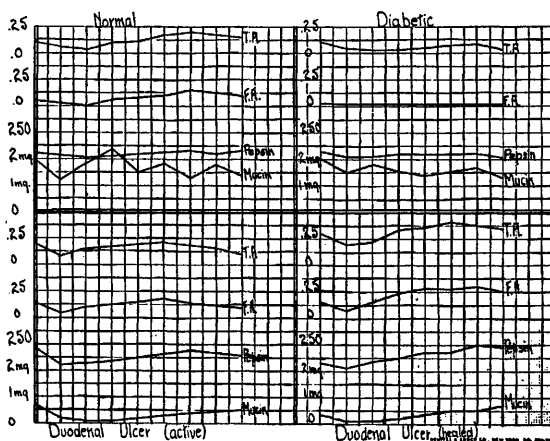
⁶ Koch, F. C., and McMeekin, T. L., *J. Am. Chem. Soc.*, 1924, **46**, 2066.

¹ Anderson, R. K., Fogelson, S. J., and Farmer, C. J., *Proc. Soc. Exp. Biol. and Med.*, 1934, **31**, 518.

obtaining gastric juice not subject to their presence. The former procedure would be capable of wider application and work is in progress with this end in view. Our present interest is in the variations in mucin content of gastric secretion, when juice is obtained from the fasting stomach by means of an alcohol test meal under conditions excluding other reducing substances.

In following the curve of mucin secretion, the stomach is emptied after a night's fast, basal secretory rate established, then 250 cc. of 7% ethyl alcohol is given and samples removed through a Rehfuß tube at 15 minute intervals for a period of 2 hours. Determinations of total and free acidity, and pepsin, are made. The mucin secretion is followed by the determination of total reducing substances according to the following method: The gastric sample (5 cc. if available) is diluted with an equal volume of 4 N H_2SO_4 . It is then hydrolyzed in a boiling water bath for $2\frac{1}{2}$ to 3 hours using an air condenser. After cooling, 2 cc. aliquot samples are pipetted into test tubes, the acidity neutralized to phenolphthalein with 1 N KOH or NaOH, and the reduction estimated by the Somogyi modification of the Shaffer-Hartmann method.² The results are expressed in milligrams of reducing substances (calculated as glucose) per cubic centimeter of gastric juice.

To illustrate values found, the accompanying graphs are appended.



These graphs illustrate the gastric secretory response in a normal individual, a diabetic with achlorhydia, a patient with an active duodenal ulcer, and one with a healed duodenal ulcer. Gastric con-

² Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Baltimore, 1932, 2, 469.

tents (after hydrolysis) in the normal and diabetic cases consistently show a reduction of 1 to 2 mg. per cubic centimeter, whereas both ulcer patients are consistently below 1 mg.

Although all curves of gastric mucin secretion from patients actively suffering from gastro-duodenal ulcerative disease have not been as classic as the ones reproduced here, they have been consistently of the same pattern and suggest a true quantitative mucin deficiency.

7197 P

Reactions of Hemoglobin and Hematin with Sodium in Liquid Ammonia.

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We have shown^{1, 2} that certain proteins and peptones are acidic in liquid ammonia and react with sodium with liberation of hydrogen. Proteins and peptones liberate approximately one-half of a molecular weight of hydrogen for each gram atom of nitrogen in the substance when an excess of sodium is used.

Hemoglobin and hematin react profoundly differently toward sodium in liquid ammonia than do the other proteins which we have studied. They liberate hydrogen continuously when excess sodium is present and in quantities disproportionate to the acidic groups present. Their behavior resembles the catalytic action of a rusty iron nail in decomposing the ammoniated sodium atom to give sodium ion, amide ion, and hydrogen.

The analytical procedure was essentially the same as that described previously. The substances were dried for 48 hours in an electric oven at 80°C. and then kept in a vacuum desiccator over sulphuric acid.

Hemoglobin is not appreciably soluble in liquid ammonia. It swells, softens, and the particles tend to coalesce. Within a few moments, the supernatant liquid is yellow, but slowly turns to a reddish color after 20 min. After standing for 4 hours, the solu-

¹ Miller, C. O., and Roberts, R. G., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29**, 535.

² Roberts, R. G., and Miller, C. O., *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 821.