dentin that was being calcified during the immediate effect of the first injection and a secondary hypercalcified stripe in the dentin that was being calcified subsequently. The extent of the secondary stripe varied with the number and unitage of the doses injected and the duration of the experiment.

Two animals that were given 12 daily injections of 10 units each of parathyroid hormone showed a normal histologic picture.

The absence of reaction in the 3 animals that were given inactivated hormone preparation gives conclusive proof that the effects observed were due to the parathyroid hormone and not to any foreign protein impurities.

It is believed that the experimental pattern in the dentin represents its response to the changes in the calcium and phosphorous metabolism induced by the injections of the parathyroid hormone. The dentin reaction can be explained best by the theory that the parathyroid hormone controls a fraction of the serum calcium. The findings suggest the feasibility of a method of standardization of parathyroid hormone based on the changes shown to occur in the dentin.

7195 P

A Method for the Assay of Commercial Gastric Mucin.

RICHMOND K. ANDERSON, SAMUEL J. FOGELSON AND CHESTER J. FARMER.

From the Departments of Experimental Surgery and Biochemistry, Northwestern University Medical School.

Following the introduction by Fogelson[1] of gastric mucin therapy for gastro-duodenal ulcerative disease, a method for the assay of commercial preparations became desirable.

The commercial gastric mucin is prepared by following the customary procedure for commercial pepsin, in which the mucin dough is separated from the pepsin by precipitation at its iso-electric point. It is then purified by repeated washing with 70% alcohol,[2] mixed with required base, and diluted with peptone to decrease the viscosity to a practical state for administration to patients. Although it is the opinion of Hurst[3] that "mucus differs from other proteins in the

[1] Fogelson, S. J., *J. Am. Med. Assn.*, 1931, **96**, 673.

[2] Ivy, A. C., and Jones, K. K., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 218.

[3] Hurst, A. F., *Brit. Med. J.*, 1933, **3784**, 89.

extreme slowness with which it undergoes peptic digestion . . . ," nitrogen and reduction values to be presented for commercial mucin suggest the possibility of a partial digestion, when compared with values obtained for gastric mucin by Webster and Komarov.[4] This phase of the subject is being investigated.

Commercial samples were found to vary greatly as to viscosity, acid combining power, sulphur, nitrogen, and mucoitin content. Methods were therefore sought by which mucin could be separated from the commercial diluents and contaminants before assay. For this purpose we have used an additional peptic digestion followed by precipitation with 70% alcohol. By weighing the precipitated material, comparative values for the mucin content of the samples may be obtained. Our data shows this procedure to give a product with characteristic N_2 content and reduction values. Details of the procedure are as follows: Dissolve 1 gm. commercial mucin and 0.1 gm. pepsin (Wilson's 10,000 units/gram) together in 25 ml. of 0.25% (0.068 N) HCl. This strength acid was found to give a pH approximating 2 with most samples. Digest in the incubator at 37°C. over night, then pipette 10 cc. aliquot samples into weighed 50 cc. centrifuge tubes, and add 28 ml. of 95% alcohol. The contents of the tubes are thoroughly mixed and centrifuged at high speed until the precipitate is well packed (5-10 min.). The supernatant fluid is then decanted, the tubes placed in a vacuum oven at 40-50 mm. pressure, and dried at a temperature not exceeding 50°C. Constant weight is obtained with approximately 6 hours' drying. If it is desirable to decrease the time of drying, the precipitate may be washed with higher concentrations of alcohol and lastly with ether, before being placed in the oven.

TABLE I.
Percentage Precipitation, Nitrogen, and Reduction Values for Crude and Purified Commercial Mucin.

	Crude Commercial Mucin			Purified Product	
Sample	% Ppt. after Purifying Process*	N. %	Reduction % as glucose	N. %	Reduction % as glucose
1	27.1	13.3	14.7	7.7	35.1
2†	69.0	8.0	28.2	7.2	33.8
3	41.4	12.2	18.9	7.2	37.9
4	30.6	12.6	13.4	7.5	35.6
5	58.8	9.3	22.3	7.4	33.8
6	39.2	11.2	16.8	7.2	37.9

*Percentage of crude sample precipitable by 70% alcohol after peptic digestion.

† Sample washed with 70% alcohol by manufacturer and submitted without addition of diluent.

[4] Webster, D. R., and Komarov, S. A., *J. Biol. Chem.*, 1932, **96**, 133.

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 2½ to 3 hrs., using an air condenser.

For estimation of reducing substances, Somogyi's modification[5] 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 McMeekin[6] is employed. Usually 1 cc. of the hydrolyzed sample is used for this determination.

7196 P

Gastric Mucin Secretion in Gastro-duodenal Ulcerative Disease.

RICHMOND K. ANDERSON, SAMUEL J. FOGELSON AND CHESTER J. FARMER.

From the Departments of Experimental Surgery and Biochemistry, Northwestern University Medical School.

It was shown[1] 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.