necessarily reflect the histologic activity of the hemopoietic tissues. The decrease in the red blood counts during the first 7 days is similar. The Rbc's of the glutathione treated mice do not fall as low thereafter and recover more rapidly.

The testicular injury by histologic criteria and testicular weight is identical in both groups over a 21 day period during which there is no evidence of regeneration in either.

It appears that glutathione by an unknown manner protects the mechanisms which accelerate the regeneration of the hemopoietic tissues but does not prevent the cellular destruction. This concept is not proved. Evidence collected elsewhere lends support. Trowall(11) has shown that cysteine does not protect lymph node cultures. Hennessey (12) by a precise technic has shown that neither glutathione nor cysteine protect against the radiation inhibition of iron uptake by the erythropoietic tissue.

Conclusions. 1. By the criteria used there is no significant difference in the destructive effects of irradiation on normal mice or mice treated with large doses of glutathione before irradiation. 2. A striking difference is seen in the rate of regeneration of the hemopoietic tissues.

11. Trowall, O., personal communication.
12. Hennessey, T. G., personal communication.

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Acid-Binding Capacity of Dialyzed Mucin Fractions from Human Gastric Juice.* (18503)

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(Introduced by D. P. Barr.)

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The mechanisms responsible for variations in gastric acidity are still incompletely understood. Hollander(1) has shown that hydrochloric acid is secreted in constant concentration at 0.17 normal, but at a variable rate. It has generally been assumed that variations in gastric acidity are attributable not only to dilution but to the partial neutralization of the hydrochloric acid by the constituents of the gastric mucus, although full data on the buffering power of human gastric mucin are not available. By the method of Glass and Boyd(2) the chief constituents of the gastric mucin can be determined separately. These authors found that a mucous substance which they called mucoprotein is secreted by the glandular cells, and that another constituent of the dissolved mucin, which they called mucoproteose, is derived from the visible mucus fraction which in turn is secreted by the lining columnar epithelium. Which of the mucous substances or their degradation products is chiefly concerned with neutralizing hydrochloric acid is still not known and there is little agreement to be found in the literature concerning the buffering role of gastric mucus. Some workers have attributed a marked buffering effect to the mucous substances(3), others, a moderate effect(4), and

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1. Hollander, F., *Am. J. Physiol.*, 1931, v98, 551; *J. Biol. Chem.*, 1934, v104, 33.

2. Glass, G. B. Jerzy, Boyd, L. J., Heisler, A., and Drekter, I. J., *Bull. N. Y. Med. Coll., Flower and Fifth Ave. Hosps.*, 1948, v11, 8; Glass, G. B. Jerzy, and Boyd, L. J., *Ibid.*, 1949, v12, 1; *Gastroenterology*, 1949, v12, 821, 835, 849.

3. Bolton, C., and Goodhard, G. W., *J. Physiol.*, 1931, v73, 115; *ibid.*, 1933, v77, 287; Leriche, R., 40e Congrès de Chirurgie, 1931, Octobre; Helmer, O. M., *Am. J. Physiol.*, 1934, v110, 28; Grant, R., *Am. J. Physiol.*, 1942, v135, 496.

others, a negligible effect(5). The discrepancies in results seem most probably to be related to differences in methods of preparing the mucous substrate for testing, and perhaps also to the fact that these substances were derived variously from animals and humans and some may have contained contaminating substances from the saliva and regurgitated bile.

The present investigation concerns an inquiry into the buffering capacity of mucous substances extracted and refined from the stomach of Tom, a fistulous human subject with a completely occluded esophagus which precludes contamination of the gastric juice with salivary and nasopharyngeal mucus(6).

It has been shown earlier that the gastric juice of Tom is poor in mucoprotein. Therefore, no attempt was made to extract sufficient mucoprotein for the testing procedure from his gastric juice. Instead, pooled gastric juice rich in mucoprotein was collected 40 to 60 minutes after insulin stimulation from five patients with active duodenal ulcer(7).

Material and methods. The material tested was prepared as follows:

A. *Visible mucus from the surface epithelium.* The gastric content of Tom was collected under fasting conditions directly from his fistulous opening. The visible mucus fraction was separated by centrifugation from the supernatant fluid of the gastric juice, pooled and refrigerated under 0.05 normal hydrochloric acid. Fifteen cc of this material were placed in a Visking bag and dialyzed against flowing tap-water at 6°C for 48 hours. The material was then homogenized by shaking and again dialyzed in a parchment dialyzing shell against distilled water for another 48 hours at 6°C. The material was not considered suitable for use until its specific conductance was smaller than 0.0001 reciprocal ohms. Five samples of Tom's visible gastric mucus were prepared in this fashion. The content of mucoid substance (dry weight) in tested samples ranged from 16.0 to 50.0 mg.

B. *Dissolved mucoproteose.* After separation of the visible mucus fraction from the pooled gastric juice of Tom, the remaining supernatant fluid was mixed with one-half volume of 10% trichloracetic acid. After it was allowed to stand for 15 minutes, the mixture was centrifuged and the supernatant fluid was decanted from the precipitate. One and one-half volumes of acetone were then added to the supernatant fluid. This mixture was left for one hour in an incubator at 40°C. The resulting precipitate was then collected by centrifugation, was washed with acetone-water and then pure acetone, and was finally redissolved in 0.1 normal sodium hydroxide. To this solution was added 1½ volumes of 0.1 normal hydrochloric acid and 2½ volumes of distilled water. This mixture was allowed to stand until a precipitate settled out and then centrifuged. The supernatant fluid was separated, mixed with two volumes of acetone and left for 24 hours at 37°C. The resulting precipitate from this operation was collected, washed with acetone-water and dialyzed as under "A." The final material recovered was then divided into two parts, each of them containing about 50 mg of dry weight substance. Each was titrated as below and subjected to analysis for mucoproteose by the method of Glass and Boyd (2).

C. *Dissolved mucoprotein.* The separation of mucoprotein from the pooled gastric juice of 5 patients with duodenal ulcer was carried out as described under "B" until the stage of precipitation by hydrochloric acid. The precipitate thus obtained was washed with acetone-water and pure acetone and then dialyzed as under "A." The recovered material was then divided into two parts, each of them containing about 50 mg of dry weight substance. Each was titrated as outlined be-

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6. Wolf, S., and Wolff, H. G., Human gastric function; An experimental study of a man and his stomach; Oxford University Press, 1947, 2nd Ed., New York.

7. Glass, G. B. Jerzy, and Boyd, L. J., *Bull. N. Y. Acad. Med.*, 1949, v25, 459; *Gastroenterology*, 1950, v15, 438; Glass, G. B. Jerzy, Pugh, B. L., and Wolf, S., *J. Appl. Physiol.*, 1950, v2, 571.

low and each was analyzed for the presence of mucoprotein by the method of Glass and Boyd(2).

Testing procedure. The dialyzed material was suspended and homogenized in 5 to 6 cc of redistilled water before titration. Each of the fractions was then titrated electrometrically with 0.1 normal and 0.5 normal hydrochloric acid. The titration was accomplished using the Beckman Research Model Potentiometer with glass electrode and the Koch Automatic Microburette, calibrated in 0.01 cc and equipped with a ground glass tip for delivery of the smallest possible drop. Control titration curves were obtained using redistilled water in volume equal to the dissolved mucous substances. All titrations covered a pH range from approximately 6.0 to 1.6. Following titration, the material was carefully collected and re-analyzed for its content of visible mucus, mucoproteose or dissolved mucoprotein respectively. After construction of the titration curves, the control curves obtained with the distilled water were deducted, thus establishing the buffering capacity of each tested material per gram of dry mucous substance at pH 2, 2.5 and 3. The acid binding capacity below this range

TABLE I. Results of Electrometric Titration of Dialyzed Samples of Surface Epithelium Mucus, Dissolved Mucoproteose, and Dissolved Mucoprotein from the Gastric Juice of Man.

Material tested	Acid binding capacity of 1 g of mucous substance tested in cc 1/10 N HCl		
	pH 3.0	pH 2.5	pH 2.0
A. Dialyzed pooled gastric surface epithelium mucus from Tom			
Sample #1	2.51	3.14	4.70
" 2	5.40	6.24	7.55
" 3	5.00	5.80	6.60
" 4	3.12	3.69	4.40
" 5			5.77
Avg with stand. error	4.01 ± .71	4.72 ± .76	5.80 ± .39
B. Dialyzed pooled gastric dissolved mucoproteose from Tom			
Sample #1	3.25	4.10	5.28
" 2	3.75	4.73	5.92
Avg	3.51	4.42	5.60
C. Dialyzed pooled gastric dissolved mucoprotein from 5 individuals			
Sample #1	3.18	3.73	4.32
" 2		3.56	4.27
Avg	3.18	3.66	4.31

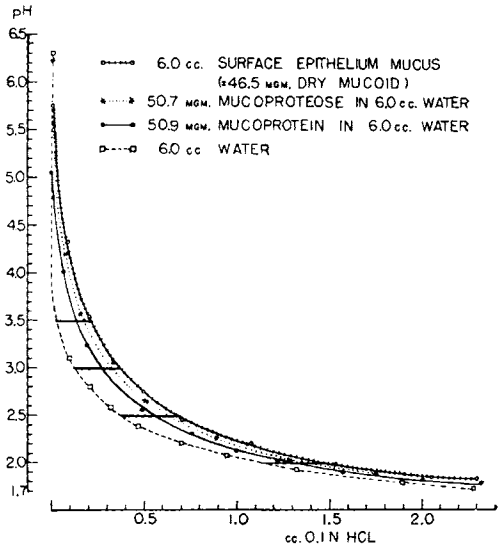

FIG. 1.

Typical electrometric titration curves of surface epithelium mucus, mucoproteose, and mucoprotein from human gastric juice. Horizontal lines connecting the curves with the curve of the H_2O control indicate the acid-binding capacity of the mucous substances at a given pH in cc of 0.1 N HCl.

was not used for calculations because of possible error in the potentiometer at a low pH and errors due to the dilution.

Results. Visible mucus. The data obtained on the 5 pooled samples of visible gastric mucus from Tom's stomach are reported in Table I, and one of the typical titration curves is shown in Fig. 1. The average acid binding capacity of the dialyzed gastric visible mucus calculated with standard error was found to be 0.41 ± 0.07 mEq at pH 3, and 0.58 ± 0.06 mEq at pH 2 calculated per gram of dry substance of visible mucus, and 0.31 ± 0.05 mEq at pH 3, and 0.44 ± 0.045 mEq at pH 2 per 100 cc of native mucus.

In order to obtain an estimate of what part the visible gastric mucus might play in buffering gastric acid, the fasting content of Tom's stomach was collected for 20 consecutive days. The total volume, and free and total acidity were measured, and the content of visible mucus was determined volumetrically under standard conditions, that is, after centrifugation for 10 minutes at 3,000 r.p.m., and these quantities were multiplied by the values obtained above. It was found, as indicated in

TABLE II. Data on Fasting Gastric Secretion Recovered from Tom's Stomach.

Fasting gastric specimens from Tom	Avg value representing means with stand. error from 20 tests performed on as many different days
Volume in cc	19.4 ± 4.0
Conc. of HCl in mEq. per 1*	41.0 ± 2.4
Content of HCl in mEq.	.79 ± .17
Volume of visible mucus in cc	1.68 ± .05
Content of mucoid of visible mucus (dry wt) in mg†	12.7 ± .4
Acid binding capacity of visible mucus in collected‡ specimen at pH 2 in mEq.	.0074 ± .0002
% of HCl contained which may be buffered at pH 2 by visible mucus present in collected specimen	.93 ± .20

* Only acid containing specimens were considered.

† The dry wt of the mucoid substance calculated on basis of values reported previously(2) and which avg 0.757 g per 100 cc native mucus.

‡ Acid binding capacity of mucus calculated on basis of avg value reported in this paper, *i.e.*, 0.58 mEq. per 1 g dry mucoid substance.

Table II, that the visible gastric mucus recovered from the fasting gastric juice of Tom may not bind more than 1% of the free acid present in Tom's stomach under average fasting conditions.

Comment. It is clear from these experiments that the buffering capacity of dialyzed visible mucus is much less than that of undialyzed material from humans or animals (3,4). It seems likely that the buffering capacity of the undialyzed material depends chiefly on the presence in the mixture of bicarbonate radicals bound to mineral bases (5) and the dialyzable products of protein breakdown.

Dissolved gastric mucin. In Table I and Fig. 1 are represented the acid binding capacities of the 2 main components of the dissolved gastric mucin, mucoproteose and mucoprotein. The acid binding capacity of the dialyzed mucoproteose from Tom's stomach was found to be approximately 0.35 mEq at pH 3 and 0.56 mEq at pH 2, and that of mucoprotein 0.32 mEq at pH 3, and 0.43 mEq at pH 2, calculated per gram of dry substance. For purposes of comparison it may be noted that the acid binding capacity of commercially available crystallized pepsin (Armour) was found to be 0.42 mEq at pH 3 and 0.61 mEq at pH 2 per gram of dry substance.

In an attempt to calculate the role of these purified substances in the buffering of the gastric juice, the calculations listed in Table III were made. As a basis for these calculations, quantitative data on the content of the various secretory constituents in the gastric juice were used, which have been reported separately elsewhere(8).

These calculations indicate that the mucous substances dissolved in the gastric juice may participate to some extent in the buffering of the gastric acid exclusive of their content of electrolytes, amino acids, peptides, and other dialyzable substances. In each instance, the magnitude of the final buffering effect would depend mainly upon the rate of acid secretion prevailing at the time. In individuals who may secrete very little acid, approximately 10% of the acid under fasting conditions is capable of neutralization by the dialyzed mucous substances. In more active stomachs, however, despite the greater output of mucous substances, the percentage of acid which the dialyzed mucous fractions can neutralize under fasting conditions is much lower, and about only one-half of the above value. Following stimulation of hydrochloric acid secretion by insulin, still less acid can be neutralized (3.9 ± 1.4%), although secretion of the total mucous substances may be greatly enhanced. After histamine stimulation which does not cause a significant increase in secretion of gastric mucin a still smaller percentage of total gastric acid secreted can be buffered by mucin fractions dissolved in the gastric juice (not more than 1.2 ± 0.4%).

Summary and conclusions. Electrometric titration of dialyzed visible gastric mucus recovered directly from the stomach of a man with a large gastric fistula and an occluded esophagus revealed an acid binding capacity, if calculated per gram of the dry substance, far smaller than that reported for

8. Glass, G. B. Jerzy, and Boyd, L. J., *Gastroenterology,* 1950, v16, 697.

TABLE III. Data on Gastric Secretion Collected Under Fasting Conditions and After Stimulation with Insulin and Histamine and Calculated on the Basis of Secretory Patterns Reported Elsewhere(8). All values are calculated as means with standard errors, and are based on number of tests indicated below.

	Fasting gastric juice collected twice at intervals of 20 min.		Gastric juice collected within 1 hr after i.v. inj. of 16 U insulin		Gastric juice collected within 1 hr after inj. of 1 mg histamine	
Groups of individuals tested	Controls	Duodenal ulcer	Controls	Duodenal ulcer	Controls	Duodenal ulcer
No. of individuals tested	35	27	9	18	12	7
Vol. in cc	20 ± 3	34 ± 5	60 ± 10	128 ± 12	78 ± 10	123 ± 16
HCl						
Conc. in mEq./l	15 ± 3	30 ± 4	34 ± 6	64 ± 5	60 ± 8	89 ± 8
Content in mEq.	.3 ± .1	1 ± .2	2.2 ± .7	8.2 ± 1.5	4.7 ± 1.3	10.9 ± 2.1
Dissolved mucoprotein						
Conc. in mg/100 cc	48 ± 8	66 ± 9	90 ± 22	136 ± 12		
Content in mg	12 ± 2	23 ± 4	59 ± 17	174 ± 33		
Acid-binding capacity in mEq.*	.005 ± .001	.010 ± .002	.025 ± .007	.074 ± .014		
Dissolved mucoproteose						
Conc. in mg/100 cc	237 ± 26	156 ± 28	233 ± 35	144 ± 18		
Content in mg	46 ± 6	65 ± 19	107 ± 30	183 ± 35		
Acid-binding capacity in mEq.*	.026 ± .003	.036 ± .011	.060 ± .016	.102 ± .019		
Total dissolved mucin						
Conc. in mg/100 cc	285 ± 27	222 ± 29	323 ± 41.3	280 ± 21.6	136 ± 28	151 ± 17
Content in mg	58 ± 6.3	88 ± 19.4	166 ± 34.5	357 ± 47.9	106 ± 11	184 ± 22
Acid-binding capacity in mEq.*	.031 ± .003	.046 ± .012	.085 ± .017	.176 ± .024	.053 ± .006	.092 ± .011
% of HCl contained in collected gastric juice which may be buffered by total dissolved mucin at pH 2	10.3 ± 3.5	4.6 ± 1.3	3.9 ± 1.4	2.1 ± .5	1.2 ± .4	.8 ± .2

* The acid-binding capacities were calculated on the basis of avg buffering values of the dissolved mucoprotein and mucoproteose reported in this paper.

undialyzed human or animal mucus; the acid binding capacity for mucoproteose and mucoprotein dissolved in gastric juice was even smaller than that for visible mucus. This suggests that dialyzable materials including mineral bases are chiefly responsible for the alkalinity of gastric mucus and the buffering of the HCl of the stomach. The total buffering capacity of mucous substances dissolved in gastric juice ranges from 10.3 ± 3.5 to 1.2 ± 0.4% of the free acid present, depending upon the rate of acid and mucin secretion prevailing at the time. It is emphasized that this study has concerned dialyzed and refined mucous substances in the gastric juice. These experiments do not bear upon the ability of the visible gastric mucus which invests the lining of the mucous membrane of the stomach to protect the underlying columnar epithelial cells by physical means (9).

9. Wolf, S., and Wolff, H. G., *Gastroenterology*, 1948, v10, 251.

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