TABLE I. Effect of Ultrasonic Energy on Movement of Cortisol through Pig Skin. (Values are expressed as μg/10 g of tissue.)

Sample	Control	Cortisol ointment	Difference
1	.26	1.45	+1.19
2	.45	.62	+ .17
3	.19	.89	+ .70
4	.29	1.93	+1.64
5	.08	.10	+ .02
6	.10	.17	+ .07
7	.29	.82	+ .53
8	.18	.68	+ .50
9	.31	.33	+ .02
10	.91	1.17	+ .26
Mean ± S.D.	.31 ±.24	.82 ± 1.14	+ .51 ±.54

tern as those exposed to sound plus ointment without cortisol.

Calculation of the quantity of cortisol recovered from skeletal muscle (Table I) showed that tissues exposed to sound but not to cortisol had an average of 0.31, whereas tissues exposed to sound plus cortisol had an average of 0.82 γ of cortisol per 10 g of tissue. Using the method of least squares the standard deviation of the difference was 0.54, ($p = <0.02$).

Summary. 1. Pig muscle contained an average of 0.31 γ of cortisol per 10 g when analyzed by McLaughlin's modification of Sweat's fluorescence method. 2. After inunction of an ointment containing cortisol followed by exposure to ultrasonic energy an average of 0.82 γ per 10 g of muscle was found.

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Serum Albumin and Gamma-Globulin in Normal Human Gastric Juice*† (27238)

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In 1934, one of us (FH) speculated on the possibility that the alkaline component of gastric juice includes a transudate of interstitial fluid, along with one or more mucinous secretions(1). It was not until 1953, however, that the first evidence to support this hypothesis was made available by the electrophoretic studies of Henning *et al.*(2), which revealed the presence of albumin in specimens of normal as well as pathological gastric juice from human subjects. In 1959 Citrin *et al.*(3) reported the occurrence of serum proteins in gastric juice of a patient with Menetrier's disease. The following year serum albumin was demonstrated in this laboratory(4) to be an important constituent of anacid gastric mucinous secretions obtained from dogs equipped with Heidenhain pouches. Gullberg and Olhagen(5), by instilling buffer into the stomach to prevent proteolysis, found albumin in normal human gastric juice. Holman *et al.*(6) used I^{131}-labeled albumin and γ-globulin to study serum proteins in small bowel secretions, and mentioned incidentally that serum proteins were found in saliva and gastric juice from normal human subjects. The current study was undertaken to confirm the presence and quantitate the amounts of albumin and γ-globulin in normal human gastric juice obtained by nasogastric intubation, and to evaluate the possible contribution of saliva.

Materials and methods. Only subjects without evidence of gastrointestinal disease and with normal serum proteins were studied.

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Five subjects were studied with γ-globulin and 9 with labeled albumin. All subjects except one (G3) had free acid in the fasting gastric aspirate. After first blocking the thyroid with Lugol's solution, 100 μc of either I^{131}-labeled human serum albumin (Squibb), or I^{131}-labeled human serum γ-globulin (Squibb) was given intravenously. Fourteen hours later, after an overnight fast, a Levin tube was passed into the stomach and gastric contents were completely aspirated. The stomach was then lavaged 3 times with 30 ml of phosphate buffer (pH 7.2-7.4, Γ/2 0.2) and this material was discarded, following which a fourth portion was allowed to remain in the stomach for 15 minutes. The gastric contents were again completely aspirated, and immediately chilled in an ice bath and kept refrigerated until analyzed. Absence of blood (by guaiac reaction) and bile (by inspection) was ascertained and the pH measured electrometrically. All specimens with blood and all but one with bile (G 5) were discarded.

The pH of these aspirates usually ranged between 7.0 and 7.2, but a few specimens were pH 6.8. In these latter cases, additional buffer was immediately added to bring the pH to 7.0. In most experiments, 2 or 3 successive 15-minute collections were made. In the globulin experiments, the gastric aspirates were pooled and analyzed *in toto*; in the albumin studies, they were analyzed separately. In Experiment G 2, the second collection was made after injection of 0.5 mg histamine acid phosphate, and the 2 specimens from this experiment were analyzed separately. In Exp. G 3, only one fasting specimen was studied (without buffer, pH 7.2), because subsequent specimens all were guaiac positive. In Exp. G 4 and G 5, the phosphate buffer was mixed with phenol red to use the indicator dilution method, developed in this laboratory(7) to estimate the relative volumes of gastric juice and buffer solution comprising the gastric aspirate. Since the color of the phenol red interfered with the recognition of bile, this technic was not used in later experiments.

During each experiment, the subject was carefully instructed not to swallow saliva, and the saliva was collected continuously by constant suction with a dental saliva collector. Just before the end of each experiment, venous blood was collected and the serum separated for subsequent analysis.

Total serum protein was determined by the biuret method of Weichselbaum(8). Serum was electrophoresed on paper strips in veronal buffer (pH 8.6, Γ/2 0.075). The strips were stained with amido black, and percentage of total protein as albumin or γ-globulin evaluated by elution and photometric determination of dye intensity. Total I^{131} activity and protein-bound I^{131} (PBI^{131}) activity of the serum were determined by counting in a well-type scintillation counter.

Aliquots of the gastric aspirates were analyzed for total protein by a modified biuret reaction(9) and for total I^{131} activity. Large aliquots were dialyzed extensively to remove non-protein-bound I^{131}, then concentrated by dialysis against polyvinylpyrrolidone, and finally lyophilized. The lyophilized material was resuspended in water and the protein precipitated by alkaline zinc sulfate. The precipitate was washed several times with distilled water to remove any remaining non-PBI^{131}, and the PBI^{131} radioactivity was determined on the precipitate.

Analyses of saliva were performed as with gastric juice.

Calculations. From the serum total protein and the electrophoretic analysis, the concentration of albumin or of γ-globulin in the serum was calculated. The albumin (A) or γ-globulin (G) content per ml of gastric or salivary aspirate was calculated by the following equation:

$$\text{mg A or G/ml aspirate} = \text{PBI}^{131}\text{ counts/ml aspirate} \times \frac{\text{mg A or G/ml serum}}{\text{PBI}^{131}\text{ counts/ml serum}}$$

An estimate of the 24-hour gastric output of albumin or γ-globulin was calculated as the product of the concentration in the gastric aspirate, the mean volume recovered per 15 minutes, and 96 (the number of 15-minute periods in 24 hours). The percentage of albumin or γ-globulin in total protein of the gastric aspirate or saliva was derived from the appropriate concentration estimate and the total protein concentration.

The term "gastric aspirate" is employed in

TABLE I. Albumin in Gastric and Salivary Specimens.

Exp. No.	Conc. in aspirate (μg/ml)	% of total protein in aspirate	Gastric output in 24 hr (mg)	Conc. in saliva† (μg/ml)	% of total protein in saliva
A 1	451	28	671	490	21
	436	26	690		
A 2	* 29	13	253	35	3.2
	* 58	13	390		
A 3	197	17	397	Not detectable	
A 4	124	36	357		
	82	19	268	85	4.2
	122	28	365		
A 5	26	3	54	62	11
	30	16	72		
A 6	* 17	2.3	21	24	2.8
	21	7	41		
A 7	113	37	444	Not detectable	
	23	7	60		
A 8	23	14	80		
	80	35	273	Not detectable	
	107	31	318		
A 9	144	14	200	173	15
	60	15	122		
	55	16	143		

* Gastric aspirate pH = 6.8.
† Removed by continuous oral aspiration.

preference to "gastric secretion" because in all but one experiment, the gastric aspirate included buffer solution as a diluent of the gastric juice. In addition, the possibility always exists of slight contamination of the specimens by swallowed saliva or regurgitated duodenal contents.

Results. The results of the albumin studies are summarized in Table I. Twenty specimens of gastric aspirate from 9 subjects were analyzed.‖ In 3 of these, the pH was 6.8, so that additional buffer was added immediately. The calculated concentration of albumin ranged from 17 to 451 μg/ml of gastric aspirate; values for albumin as per cent of total protein in the entire aspirate ranged from 2.3 to 37%. The calculated minimum 24-hour gastric output ranged from 21 to 690 mg. In the 9 saliva specimens, 3 had no detectable protein-bound radioactivity despite significant total protein content; in the remaining 6 specimens, albumin concentration ranged from 24 to 490 μg/ml, corresponding to 2.8 to 21% of salivary total protein.

In the γ-globulin experiments, 6 specimens of gastric aspirate from 5 subjects were analyzed. Additional buffer had been added to one post-histamine specimen and to one specimen of unstimulated secretion, to raise the pH from 6.8 to 7.0. The concentration of γ-globulin ranged from 8.7 to 95 μg/ml, corresponding to 0.8 to 7.0% of total protein of the aspirate. In Exp. G 4 and G 5 with phenol red, the pooled gastric specimens were estimated to contain 41 and 40% gastric juice respectively. The calculated minimum 24-hour gastric output ranged from 33 to 384 mg. The saliva was analyzed in only 4 of these experiments. Protein-bound radioactivity was detected in only 2 of these 4 specimens; they contained 306 and 75 μg of γ-globulin per ml of saliva, constituting 22 and 6% of the salivary total protein respectively.

Discussion. Shortly before the completion of this study, Wetterfors *et al.*(10) reported on the use of RISA to quantitate the content of serum albumin in gastric juice, utilizing intragastric buffering with phosphate to prevent proteolysis. They emphasized again the necessity for such buffering, since incubation of RISA with acid gastric juice *in vitro* resulted in rapid loss of protein-bound radioactivity. They also demonstrated that intra-

TABLE II. Gamma-Globulin in Gastric and Salivary Specimens.

Exp. No.	Conc. in aspirate (μg/ml)	% of total protein in aspirate	Gastric output in 24 hr (mg)	Conc. in saliva† (μg/ml)	% of total protein in saliva
G 1	59	4.0	71	Not determined	
G 2	95	7.0	384	306	22
	‡*33	3.7	127		
G 3	§60	3.3	86	75	6
G 4	‖ 8.7	.8	33	Not detectable	
G 5	¶42	6.8	97	"	"

* Gastric aspirate pH = 6.8.
† Removed by continuous oral aspiration.
‡ Post histamine.
§ No buffer used.
‖ Using phenol red, 41% of aspirate is gastric juice.
¶ Using phenol red, 40% of aspirate is gastric juice.

‖ All gastric aspirates gave positive precipitin reactions with rabbit antiserum to human serum albumin.

venous injection of inorganic I^{131} did not result in the appearance of significant amounts of PBI^{131} in the gastric aspirate. Their estimates of 24-hour gastric output of albumin were somewhat higher than those found in our study. When the combined trichloracetic acid and phosphotungstic acid precipitates were used for such estimates, their outputs were generally 0.9 to 1.3 g/day, although in a few cases they were as low as 0.2 g/day. When their calculations of radioactivity included the protein-bound radioactivity and also that not precipitable with silver nitrate, the estimates were raised to 3 to 4 g/day. In our studies, only non-dialyzable radioactivity which was precipitable with alkaline zinc sulfate was employed for such calculations. Although we agree that some of the total radioactivity in the gastric aspirate was probably associated with products of partial proteolysis of albumin in the stomach, we had no way of reliably estimating the amount, so made no correction for this in calculating the outputs. Other factors may also be responsible for the differences. In the current study, smaller volumes of buffer were instilled intragastrically (30 ml as opposed to 100-200 ml as used by Wetterfors *et al.*) so that losses *via* the pylorus may have contributed a greater proportional error in the volumes recovered. In addition, the continuous collection of saliva throughout each experiment in the current study minimized the possible salivary contribution of protein to the gastric aspirate.

More recently, Glass and Ishimori(11) used paper electrophoresis to detect and quantitate serum albumin in human gastric juice. They did not use intragastric buffering but they detected albumin in anacid juice from 40 of 400 patients, with and without gastric disease. In 32 of the 40, histamine fast achlorhydria was present, and so are not considered here. The remaining 8 had acid after histamine. In these 8, albumin was present in a concentration of 1.1 to 9.8 mg per 100 ml of gastric juice. If the 24-hour volume of gastric juice in these patients is estimated at 2500 ml, this would indicate a daily output of 28 to 245 mg of albumin in the gastric juice. In several gastric juice specimens containing serum albumin, these workers found some material on paper electrophoresis which behaved like γ-globulin. By implication, they apparently did not find this material in many other specimens. They also found this material in several anacid juices where no serum albumin could be detected.

In our experiments, we did not study the simultaneous presence of albumin and globulin in any of the specimens. However, we found significant amounts of either albumin or γ-globulin, depending on the labeled protein administered, in every specimen of gastric aspirate analyzed. In the albumin experiments, where individual collections were analyzed separately, successive specimens were in excellent agreement in some subjects, but varied markedly in others. Where the differences were great, possible explanations include incomplete prevention of proteolysis, variation in gastric emptying, and actual physiologic differences during the successive periods.

The estimates of daily gastric outputs of the plasma proteins are undoubtedly too low and should be considered only as minimal values. One reason for this is partial proteolysis; another is the use of periodic rather than continuous aspiration to effect intragastric buffering, which permits some loss of gastric contents through the pylorus.

The salivary findings were variable in both the albumin and globulin studies. In some of the patients, no protein-bound activity was detected at all, whereas in others a significant amount was found. We have no explanation for this variability. However, there was no apparent difference in the protein-bound activity of the gastric aspirates as between the patients with high salivary activity and those with none. Consequently, saliva cannot constitute the only, or even the major, source of the serum proteins found in the gastric aspirates.

The current study, as well as the findings of others, indicate that serum proteins are normal constituents of gastric juice, and are demonstrable even in acid secretion if proteolysis is avoided. The mechanism of their

appearance, however, remains to be elucidated.

Summary. 1. With the aid of an intragastric buffering technic, albumin and γ-globulin were detected in gastric aspirates of human subjects without gastric disease. 2. The range of albumin concentration in these gastric aspirates was 17-451 μg/ml whereas that for γ-globulin was 33-95 μg/ml. Estimates of the corresponding minimum 24-hour outputs for these 2 proteins were 21-690 mg and 33-384 mg respectively. 3. Saliva aspirated concomitantly with gastric contents also contained these plasma proteins, but not invariably. 4. It is unlikely that the gastric juice proteins derived in significant degree from saliva because of (a) the careful continuous removal of saliva by oral aspiration, and (b) presence of significant amounts of plasma proteins in gastric aspirates of individuals whose saliva did not contain detectable amounts of these proteins.

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Studies of Fibroblast-Like Cells from the Bone Marrow of Leukemic and Nonleukemic Children.* (27239)

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A number of investigators have cultivated human bone marrow cells *in vitro* as reviewed by Bloom(1), Reisner(2) and Lajtha(3). The technics employed have varied according to the purpose for which the cultures were to be used.

The purpose of the present study was to devise a technic for isolation and serial propagation of bone marrow cells from leukemic and nonleukemic children which would:

(1) Allow for cytologic studies of cells in long-term culture so that unmasking of latent viral agents could be observed.
(2) Provide cultures on which to perform hemadsorption studies to detect the presence of latent viral agents.
(3) Provide normal bone marrow cell cultures which could be used for attempts to isolate cytopathogenic agents from saline suspensions and phenol extracts of human leukemic tissues.

The present report describes a simple technic for cultivation of fibroblast-like cells from human bone marrow and the results of cytologic and hemadsorption studies upon such cells.

Materials and methods. Nutrient medium. The nutrient medium consisted of Eagle's medium(4) supplemented with 20% inactivated calf or human serum, 5% human cord serum, and 5% beef embryo extract.‡ Penicillin,

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‡ All ingredients were obtained from Microbiological Associates, Inc., Bethesda, Md.