Effects of Gastric Juice Fractions on Uptake of Labeled Vitamin B_{12} By Rat Liver Slices. (24930)

THEDA DRISCOLL, JANE FAULKNER, BETTY CARROLL AND P. C. JOHNSON
Radioisotope Service, Vet. Admin. Hospital, Dept. of Medicine, University of Oklahoma School of Medicine

Miller and Hunter(1) have shown that hog intrinsic factor concentrates increase uptake of cobalt^{60} labeled Vit. B_{12} by a rat liver slice. This uptake is concentration dependent with low concentrations increasing uptake and high concentrations inhibiting uptake. Addition of other proteins without known intrinsic factor activity fails to enhance uptake. Recently we found that at all concentrations tested, human gastric juice inhibited uptake of Co^{60} Vit. B_{12} by rat liver slice(2). Herbert found that this inhibitory effect of human gastric juice could be overcome by use of sequential incubation technic(3). He stated that this may be an *in vitro* test of intrinsic factor activity since gastric juice from a pernicious anemia patient failed to show this uptake. Latner, in purification of hog pyloric mucosa was able to separate a fraction that inhibited Vit. B_{12} absorption from a fraction with intrinsic factor activity(4). Using the method of Richmond, we chromatographed individual human gastric contents on IRC resin column(5). We found 3 Co^{60} Vit. B_{12} binding peaks in this effluent. Since the effluent of column of Richmond *et al.* contained intrinsic factor(6,7), we thought it would be of interest to determine if individually these fractions which bind Vit. B_{12} are capable of enhancing uptake of Co^{60} Vit. B_{12} by the rat liver slice and to determine if any of these fractions caused inhibition of Vit. B_{12} uptake produced by human gastric juice.

Technic. Gastric contents were obtained from 2 fasting subjects after stimulation of gastric secretion by 10 units of regular insulin administered intravenously. Patient No. 1 had a duodenal ulcer and 8.7% Co^{60} Vit. B_{12} liver uptake (normal>4.7%)(8). Patient No. 2 had no signs of anemia and his gastric contents contained acid and pepsin. He was receiving therapy for chronic urticaria. The fasting gastric contents of these 2 patients were chromatographed from pH 3.1 to 6.1 using 5 buffers pH 3.1, 3.9, 4.5, 5.4 and 6.1 on an IRC-50 cation exchange resin column(5). In this procedure 30 mg of lyophilized, dialyzed and neutralized gastric contents were incubated with 0.3 μc Co^{60} Vit. B_{12} after which they were applied to the column. Radioactivity of the effluent was determined before and after dialysis(2). It is reported on graphs as net counts/minute/ml (ncpm) of effluent over detector background counts/minute. All samples were counted for at least 10,000 net counts. In this analysis (Fig. 1) gastric juice from patient No. 1 produced 5 more or less well defined protein peaks in the effluent. These protein peaks were measured using optical density at 280 mμ. Peak 1, effluent pH 3.1, contains practically all the polysaccharide present in gastric juice. This included 'A' substance. Peak 2A, effluent pH 3.7 to 3.9 and 3, effluent pH 4.2 to 4.4, contain proteolytic activity by the hemoglobin method for measuring peptic activity(9). Peak 2B, effluent pH 3.9, and 4, effluent pH 4.6 to 4.8, contains protein substances of unknown physiologic significance. Since presence of peptic activity may change binding ability and other characteristics of gastric juice, pepsin was inactivated by bringing its pH to 10 for 30 minutes prior to adding Co^{60} Vit. B_{12} to the gastric juice of Patient 2(10). Slices prepared from chilled liver of rats sacrificed by decapitation were immersed in Hastings buffer containing .015 μg of Vit. B_{12} and .015 μc of Co^{60} Vit. B_{12}. Varied amounts by weight of dialyzed, lyophilized human gastric juice fractions were then added. The system was gased with 5% CO_2 and 95% oxygen, incubation was at 37°C for 60 minutes. After incubation the slices were washed with buffer by immersing in fresh buffer. The amount of radioactive B_{12} remaining was measured in scintillation well counter. Uptake of Vit. B_{12} was corrected for weight of tissue and expressed as % difference from con-

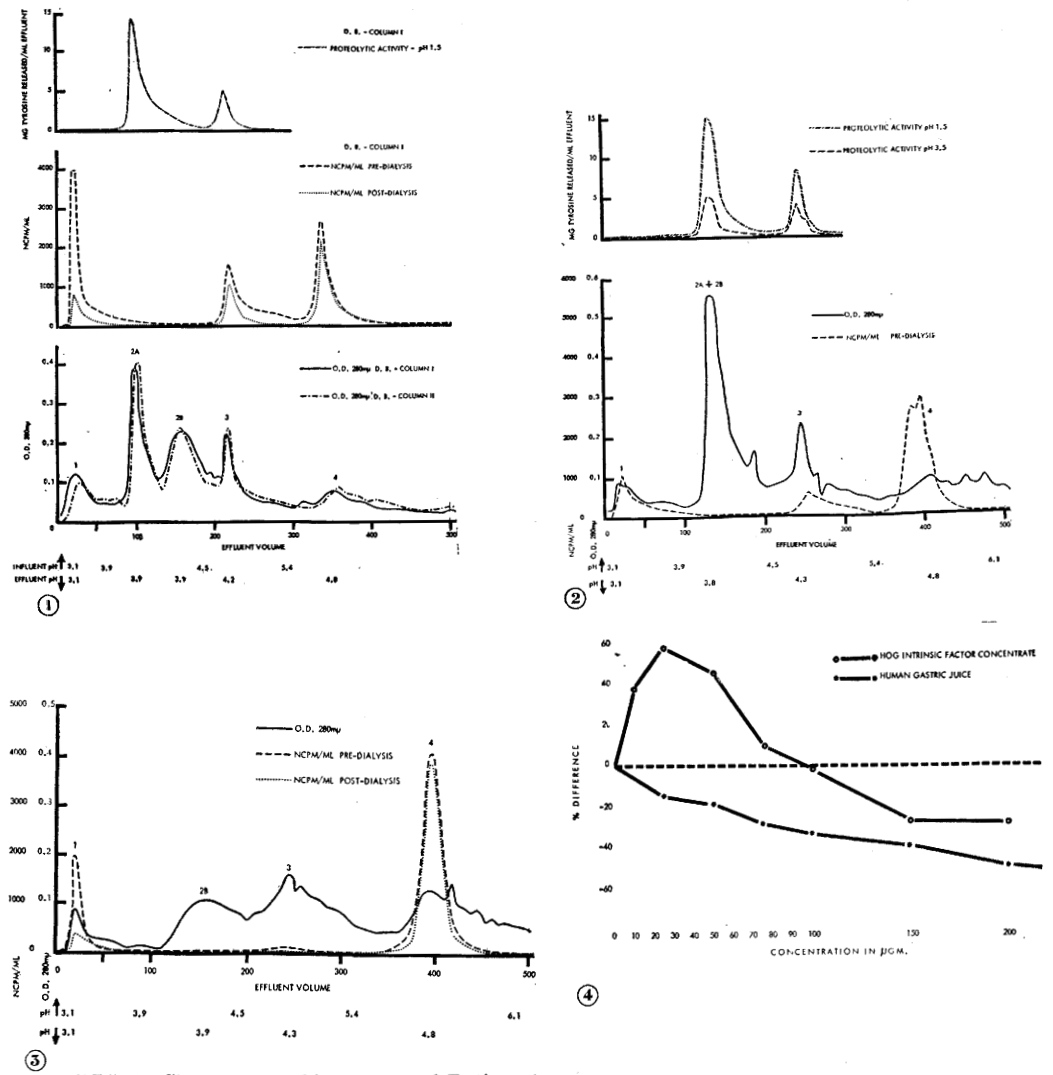

FIG. 1. Chromatographic pattern of Patient 1.
FIG. 2. Chromatographic pattern of Patient 2.
FIG. 3. Chromatographic pattern of Patient 2 after inactivation of proteolytic activity.
FIG. 4. Effect of hog intrinsic factor concentrate and human gastric juice on rat liver slice.

trol value. Uptakes less than the control are indicated by negative numbers. In 35 determinations, mean control uptake was 485 ± 19 (mean ± standard error of mean) net counts/minute/100 mg liver.

Results. Fig. 1 shows resin column separation of gastric contents of Patient 1. The top curve is peptic activity. The middle curve shows that Co^{60} labeled Vit. B_{12} appears in 3 of the 5 protein peaks and that a good portion of radioactivity is nondialyzable and presumably bound to the protein. The bottom 2 curves show the protein pattern obtained by chromatographing gastric contents on 2 separate occasions. Good agreement is noted.

Fig. 2 shows chromatographic separation of gastric contents of Patient 2. Proteolytic activity measured at pH 1.5 and 3.5 and presence of Co^{60} Vit. B_{12} in the effluent are also shown. In the effluent of this column peaks 2A and 2B overlap. The Co^{60} Vit. B_{12} binding peaks of this fractionation were combined prior to dialysis. Percentages of radioactive cobalt incubated with gastric juice and which

TABLE I. Percent Difference from Control Uptake Produced by Protein Peaks of Human Gastric Juice

Protein peak #	1			2A	2B	3			4		
Patient #	1	2	2 (treated)	1	1	1	2	2 (treated)	1	2	2 (treated)
μg applied											
25	8		48						0		
	19		32						0		
50	25	60	30			0			0		
	33	45	26			0			0		
75						0		26	17		
						0		32	22		
100	19	28	-6	0	3				32		14
	26	31	-5	4	0				35		2
150						24	17	35	55		
						22	21	32	78		
200	-22	-8		0	0	10			28		
	-20	-4		7	0	13			15		
						11					
300						0	55	56			
						0	66	56			
400				16	0					10	41
				11	0					0	27
500		-44		37	28					37	80
		-38		33	20					35	90
600				28	4		29				
					0		15				
700				22	0						
				20	0						
1000										46	
										48	
1500							-45				
							-35				
2500										14	
										3	

appeared in each binding peak were as follows: Peak 1, 14%, Peak 3, 16% and Peak 4, 64%. The remaining 6% was retained by the resin column. These 3 peaks were dialyzed to determine percentage of radioactivity which remained. Of radioactivity found in each peak, 86% remained in Peak 1, 35% in Peak 2 and 100% in Peak 4. Peak 4 is thus by far the strongest binding peak.

Fig. 3 illustrates the effect of inactivating proteolytic activity prior to chromatography. Proteolytic Peak 2A has disappeared while the other proteolytic Peak 3 remains and may have increased slightly. Peaks 1 and 4 remain undisturbed. Of radioactivity incubated with this gastric juice 21% appeared with Peak 1, 2% with Peak 3 and 75% with Peak 4. After dialysis 13% of radioactivity of Peak 1 remained, 12% of Peak 3 and 79% of Peak 4. Again Peak 4 is the strongest binding peak compared both to amount of radioactivity appearing in effluent and ability to make this radioactivity nondialyzable.

Fig. 4 shows the effect of varying concentrations of hog intrinsic factor and gastric contents of Patient 1 on uptake of Vit. B_{12} by the rat liver slice. Small concentrations of hog intrinsic factor* increased uptake of Vit. B_{12} and as the amount was increased, uptake diminished and finally reached a point below control value. Human gastric juice on the other hand showed no enhancement of uptake. Inhibition was present at lowest concentrations, more marked as concentration of gastric juice was increased. This was also true when the entire effluent from column was lyophil-

* W. L. Williams, Am. Cyanamid Co., Pearl River, N. Y.

ized and dialyzed and placed on the liver slice in similar concentrations.

Table I shows results obtained when these dialyzed and lyophilized chromatographic fractions were redissolved and added to rat liver slice. Unlike intact gastric juice, each of these protein peaks was capable of increasing uptake of Co^{60} labeled Vit. B_{12} by the rat liver slice. Like hog intrinsic factor, there is an optimal concentration point for each peak. Destroying proteolytic activity did not seem to affect the results. The peaks that bound Vit. B_{12} were somewhat more active on a μg basis than the other 2 peaks which did not bind Vit. B_{12}.

The areas between protein peaks were tested by rat liver slice technic. Control values were produced in all except the area beyond Peak 4 which showed a slight increase in uptake and inhibition at higher doses. We interpreted this to mean that there was trailing off of activity of Peak 4. Recombination of the effluent of Patient 1 produced only inhibition of the rat liver slice uptake similar to that shown in Fig. 4.

Discussion. We have shown that 5 fractions of human gastric juice are capable of enhancing uptake of Vit. B_{12} by rat liver slice at low concentrations. One fraction contains almost all polysaccharides present in gastric juice and 2 of these fractions contain proteolytic enzyme activity. Three fractions bound Vit. B_{12} as measured by dialysis. This increase in uptake was noted in gastric juice which only produced inhibition of Co^{60} Vit. B_{12} uptake at similar concentrations prior to fractionation and after recombination of the entire effluent. This suggests that each protein peak is capable of producing increased uptake by the rat liver slice when used by itself but, combined with others, inhibition occurs. The cause of this is unknown. That this difference is not due to physical chemical changes produced by the column during chromatography is shown by the fact that recombined effluent acted in a way similar to that of the gastric juice prior to chromatography. One might surmise that these fractions interact with each other producing inhibition when added together. The uptake noted with sequential incubation of human gastric juice could be explained in this way (3), since washing off the gastric juice prior to addition of Vit. B_{12} might leave one of these fractions intact on the liver cells while washing the others from the slice.

Whether enhancement of uptake Co^{60} Vit. B_{12} by the liver slice actually represents an assay for intrinsic factor cannot yet be stated. However, if it is, our result suggests that either several intrinsic factors exist or that intrinsic factor is capable of binding to the protein peaks and in this way it is spread out according to protein concentration in the effluent of the column.

Three of the 5 protein peaks obtained from a chromatography of human gastric juice make Vit. B_{12} nondialyzable. Destroying proteolytic activity prior to incubation with Vit. B_{12} changed the percentage of radioactivity appearing in the second proteolytic peak and decreased slightly the ability of the other 2 binding peaks to make Vit. B_{12} nondialyzable. This is different from the work of Grasbeck who found a decrease in binding and fewer binding peaks after proteolytic inactivation (10). However, his analysis by electrophoresis cannot be directly compared to the fractions obtained by the method used here.

Since we have found that inactivation of the proteolytic activity and presence or absence of Vit. B_{12} binding did not seem to influence materially the effect each fraction had on the liver slice, we could assume that binding of the vitamin to the gastric fraction or the presence of proteolytic activity are not important factors in producing the uptake by the rat liver slice.

Summary. 1. Gastric contents from 2 subjects without pernicious anemia have been chromatographed on an IRC resin column. Five more or less well defined protein peaks appeared in the effluent. Each peak was capable of enhancing uptake of Co^{60} Vit. B_{12} by the rat liver slice. 2. Prior to fractionation and after these gastric fractions have been reconstituted, these gastric juices produced inhibition of Co^{60} Vit. B_{12} uptake by the rat liver slice at all concentrations tested. 3. The effluent of these chromatographic separations of human gastric content produced 3 Co^{60} Vit. B_{12} binding peaks. Destroying proteolytic ac-

tivity of one of these gastric juices decreased the binding of one of these but did not change the results with the liver slice. 4. The enhanced uptake on the rat liver slice produced by these gastric juice fractions did not seem to be related to proteolytic activity.

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Serum Protein-Bound Carbohydrates and Lipids in Cholera. (24931)

SACHCHIDANANDA BANERJEE AND JAGADINDRA NATH BHADURI
Dept. of Physiology, Presidency College, Calcutta, India

Electrophoretic studies on plasma proteins in cholera showed(1) a diminution in albumin and α-globulin, increase in β-globulin which was further differentiated into β_1 and β_2 fractions and no change in γ-globulin. Plasma proteins are conjugated with carbohydrates and lipids. As distribution of these protein bound hexoses and lipids is likely to be disturbed in pathological conditions(2-8) it was of interest to study glycoproteins, mucoproteins and lipo-proteins in serum of cholera patients. Total lipid, phospholipid and cholesterol of serum were also determined. The above studies were also carried out in normal subjects for comparison.

Methods. Patients admitted into Nilratan Sircar Medical College Hospitals, Calcutta with typical symptoms of cholera were selected. As routine procedure, stool was cultured and cases whose stool showed presence of cholera vibrio were included in the report. Normal subjects were persons admitted in surgical ward of hospital for vaginal hydrocele or hernia. Examinations of blood, urine and stool were carried out to ascertain if subjects were otherwise normal. Blood was collected from anticubital vein in early morning, before breakfast and before any treatment, in centrifuge tubes and clear serum used for different estimations. Glycoprotein and mucoprotein were precipitated from aliquots of serum, dissolved in alkali, treated with orcinol-sulfuric acid and color compared with standard solution of galactose-mannose(9). For estimation of lipo-proteins(10) an aliquot of serum was treated with saturated solution of Sudan Black B in 95% ethanol, alcohol evaporated, centrifuged and supernatant stained serum used for electrophoresis. .04 cc of stained serum was applied to Whatman No. 1 strips, and electrophoretic separation carried out with barbiturate-barbituric acid buffer of pH 8.6 and ionic strength .05 for 8 hours, by using L.K.B. paper electrophoresis apparatus with current strength of 5 mA and 250 volts. Whatman strips were dried at 70°C for half hour, optical density curves of colored zones plotted using Photovolt Photoelectric Densitometer Model 425 on graph paper and areas of component section of graph were measured by planimetry. α,β lipo-proteins and "O" fraction were expressed as percentage of total lipids. Cholesterol was estimated by method of Sobel and Mayer(11), phospholipid by determining P of ether extract of serum(12) and multiplying value by 25(13) and total serum lipids by the method of Bragdon(14). Results are given in Table I.