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Differential Binding to Sepharose-Con A of B_{12}-Binding Proteins from Human Gastric Juice (38629)

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(Introduced by A. L. Jones)

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Normal human gastric juice (NHGJ) contains a glycoprotein which combines with vitamin B_{12} in the diet and is essential for adequate B_{12} absorption in humans (1). There is evidence that more than one B_{12} binding protein exists in gastric juice, but that only one of these is the biologically active intrinsic factor (2). Human intrinsic factor has been recently purified by conventional means (3) and by affinity chromatography utilizing B_{12} derivatives coupled to inert matrices (4, 5). The former method is too time consuming to be effectively used in a clinical assay for intrinsic factor and the affinity systems suffer in that all vitamin B_{12} binding proteins will adsorb to the columns. We thus wanted to examine other potential means for purifying intrinsic factor from other proteins, especially from other B_{12} binding proteins in gastric juice.

Methods and Materials. Gastric juice was taken from healthy male and female volunteers with no preference for age and from patients with pernicious anemia as determined by the Schilling test. No patient had juvenile pernicious anemia. Gastric samples were aspirated over a 30 min to 1 hr period directly into ice-cold, pH 7 buffer to minimize digestion of proteins. In some cases the stomach was flushed with 50 ml of buffer and the gastric juice and wash pooled. All samples were taken before the morning meal following an overnight fast. Aliquots from each gastric sample were used for analyses.

The binding capacity of gastric juice for B_{12} was determined by slowly adding Co^{57}-B_{12} (S.A. 1 μCi/μg) to a sample of gastric juice in an Amicon Microultrafiltration chamber fitted with a PM-30 membrane, and counting aliquots of the filtrate with a Packard gamma scintillation spectrometer. Electrophoresis on cellulose acetate was used to separate the B_{12} binding proteins. Gastric samples and column effluents were incubated with an appropriate amount of Co^{57}-B_{12} (86 μCi/μg) for 15 min at 25° followed by dialysis against water and lyophilization. Samples were applied to cellulose acetate strips and run at pH 8.5 and at pH 4.5. The system which proved to be best for separating B_{12} binding proteins consisted of running the strips for 4 hr at 0.9 ma/strip in a 50 m*M* sodium acetate-50 m*M* glycine buffer, pH 4.5. The strips were cut at intervals from the origin and each strip counted in a gamma counter. A strip containing Co^{57}-B_{12} alone run simultaneously served as a standard.

Concanavalin A (ConA) purified by the method of Agrawal and Goldstein (6) was covalently coupled to Sepharose by the procedure of Lloyd (7). Gastric juice which had been incubated with Co^{57}-B_{12} was passed through a column containing 2 ml of moist Sepharose-ConA which had been washed with phosphate-buffered saline (0.15 *M* sodium chloride, 10 m*M* phosphate, pH 7.0). The column was washed with the same buffer to remove any unbound substances and then eluted with 100 m*M* α-methyl-D-mannoside in the same buffer. The material not adsorbing to the Sepharose-ConA was designated "pass", and the eluted fraction, "eluate".

Results and Discussion. Preliminary examination showed that 0.5 ml of NHGJ bound 1.5 ng of B_{12}. When samples of NHGJ were saturated with B_{12}, passed through a column containing Sepharose-ConA and the column eluted with α-methyl-D-mannoside the results in Table I were obtained. The amount of B_{12}-binding protein in several gastric juice samples which bound to Sepharose-ConA varied from 22% to

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TABLE I. INTERACTION OF B_{12}-BINDING PROTEIN WITH SEPHAROSE-CON A

NHGJ Sample	Pass	Eluate
	%[a]	
1	73	23
2	71	28
3	41	58
4	76	22
5	69	22
6	35	54

[a] Expressed as a percent of the amount applied to the column.

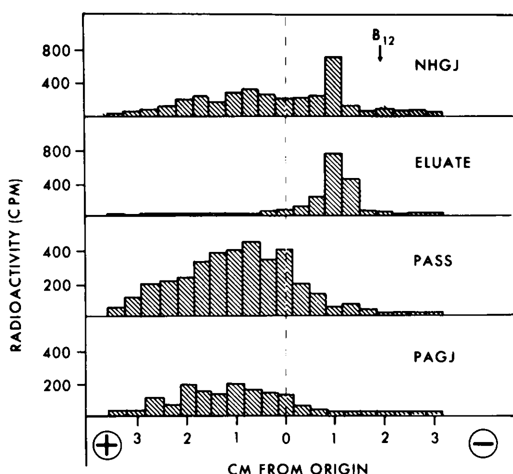

FIG. 1. Electrophoretic profile of B_{12}-binding proteins. Arrow indicates mobility of unbound B_{12}. NHGJ-normal human gastric juice, PAGJ-pernicious anemia gastric juice. Conditions of electrophoresis are those described in the text.

58% suggesting that the relative amount of this protein differed among the donors. All of the gastric samples were taken following an overnight fast. When the "pass" and "eluate" fractions were examined by cellulose acetate strip electrophoresis, patterns typical of those shown in Fig. 1 were obtained. Although the "pass" fraction appeared to be rather heterogeneous, the eluted fraction migrated as a single species toward the cathode at pH 4.5, in agreement with the direction of mobility of intrinsic factor reported by Kakei and Glass (8). When gastric samples from patients with pernicious anemia (PAGJ) were saturated with Co^{57}-B_{12} and examined by electrophoresis, the peak corresponding to the eluate fraction was missing as shown in Fig. 1. When pernicious anemia gastric juice was passed through a Sepharose-ConA column over 95% of the protein-bound B_{12} passed through the column.

The data presented above indicated that among perhaps several B_{12} binding proteins in human gastric juice, only one major species, i.e. intrinsic factor, binds to Sepharose-ConA. The differential binding of various B_{12} binding proteins in gastric juice suggests a method to distinguish between intrinsic factor and other protein which form complexes with B_{12} but are not biologically active. Sepharose-ConA beads might be utilized in a clinical method for the diagnosis of pernicious anemia.

Summary. Human gastric juice was found to contain at least two vitamin B_{12} binding substances. One of the proteins which formed a complex with B_{12} was found to bind to a column containing Sepharose-ConA. Since the protein which bound to Sepharose-ConA was absent in the gastric juice of pernicious anemia patients it was concluded that this protein was intrinsic factor. The ability of intrinsic factor to bind to Sepharose-ConA offers a potential means by which intrinsic factor could be separated from other B_{12} binding proteins in gastric juice. The ConA binding properties of intrinsic factor might be exploited in the development of a diagnostic test for pernicious anemia.

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