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The Intraluminal Transport of Vitamin B_{12} and the Exocrine Pancreatic Insufficiency (40457)

YVES PARMENTIER, GEORGE MARCOULLIS,[1] AND JEAN-PIERRE NICOLAS

Laboratoire de Biochimie Medicale, Universite de Nancy I UER Alimentation et Nutrition BP 1080, 54019 Nancy Cedex - France

Unexplained impairment of vitamin B_{12} (B_{12}) absorption sometimes occurs (1–5) in exocrine pancreatic insufficiency (EPI). This discrepancy was not found to be directly related to parameters of the exocrine pancreatic function (6). Toskes and his co-workers observed that gastric juice treated with immobilized pancreatic enzymes can restore B_{12} absorption in EPI (7). More recently, Allen and his co-workers (8) demonstrated that these enzymes can degrade *in vitro* the R type B_{12}-binding proteins (R-binders) (also called non-IF, cobalophilin, etc.) (9) but not intrinsic factor (IF), and postulated that the B_{12}-malabsorption in EPI is due to the presence of unabsorbable macromolecular formations of B_{12} with undegraded R-binders (8). This could be true, however, only if the R-binders from gastric juice, saliva and bile are actually being degraded *in vivo* by pancreatic enzymes. To test the validity of this hypothesis, therefore, the best approach would be simply the demonstration that the R-binders do not survive the passage down to the ileum under physiological conditions. Such studies are reported here for the first time and fully support the suggestion made by Allen *et al.*

Materials and methods. Tissue and fluid preparations. Gastric and intestinal juices obtained by introducing naso–gastric and duodeno–jejunal tubes into the gastric succus and in the intestine below the Treitz angle, respectively, were collected from 96 fasting humans and depepsinized by pH adjustments (e.g., see Ref. 10). Drs. Katz and Cooper (Montreal, Canada) kindly provided the gastric juice of the patient reported by Katz *et al.* (11) known to contain abnormal IF which does not bind to the IF-receptors (12, 13). Sera were prepared from blood collected from pernicious anemia patients having circulating anti-IF antibodies. Rabbit anti-human R-binder (anti-R-binder) sera were produced using granulocyte preparations as antigen (10). Specific IF-receptor was solubilized from fresh porcine ileal mucosal scrapings as described (10).

Radioactive compounds. Cyano (^{57}Co) cobalamin ((^{57}Co) B_{12}) (215 Ci/g) was purchased from Philips-Duphar, Petten, Holland. (^{58}Co) B_{12}, (^{125}I) iodide and (^{3}H) water were purchased from the Radiochemical Centre, Amersham, Bucks, U.K. (^{125}I) iodide labeling by the method of Greenwood *et al.* with chloramine-T (14) was used to label purified marker proteins (10) of human origin; purified (15) immunoglobulin-G (IgG) and serum albumin (HSA) (Kabis, Stockholm, Sweden).

Other procedures. Sephadex G-200 filtration was carried out at +4°, in columns dimensioned 2.5 cm × 100 cm and equilibrated with 50 m*M* Tris-HCl buffer pH 7.4 containing 0.05% (v/v) Triton X-100, 0.9% NaCl and 0.02% NaN_3. The flow was 12–15 ml/hr and 1.5–2 ml fractions were collected. Molecular sizes from gel filtration data were computed by the method of Andrews (16). Unsaturated B_{12}-binding capacity ($UB_{12}BC$) was measured using hemoglobin coated charcoal (17).

Results. The mean $UB_{12}BC$ of intestinal juices was found to be lower than in the gastric juices by 60%. Aliquots from pooled gastric and intestinal juices were saturated with cyanocobalamins labeled with ^{57}Co and ^{58}Co, respectively, and filtered separately with ^{125}I-labeled marker proteins (10) (see Figs. 1A and B) through Sephadex G200 (10). The ^{57}Co-radioactivity resolved (see Fig. 1A) into two protein peaks having estimated (16) molecular sizes of 120,000 and 57,000 daltons, respectively, and corresponding to R-binder

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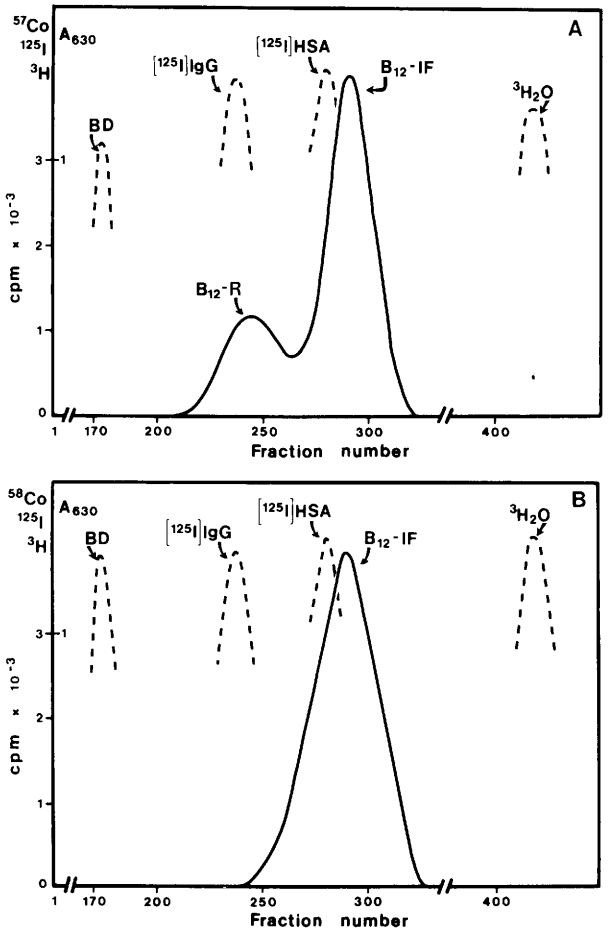
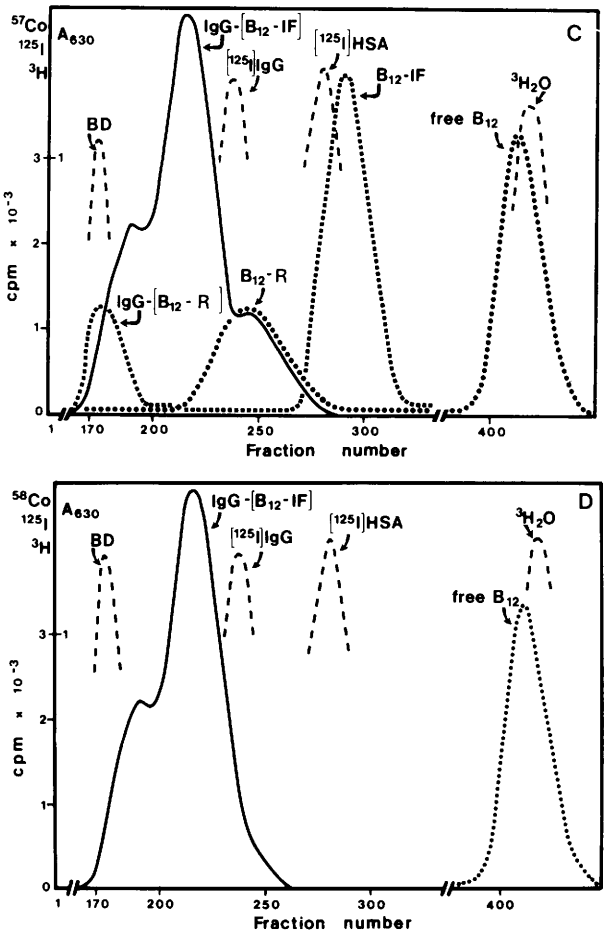


FIG. 1. Sephadex G200 radiochromatograms of gastric juice (Fig. 1A and 1C) and intestinal juice (Fig. 1B and 1D) saturated with ^{57}Co- and ^{58}Co-vitamin B_{12} (B_{12}), respectively. The lines ____ represent the elution patterns observed when the gastric (1A) and the intestinal juices (1B) were mixed with the radiovitamins (see above) and filtered as such or when similar samples have been preincubated with binding anti-F antibodies and then filtered (Figs. 1C and 1D, lines ____), or when the fluids have been preincubated with blocking anti-IF antibody prior to saturation with radiovitamins and then filtered (Figs. 1C and 1D, lines · · · · ·). When gastric or intestinal juices were first dialyzed against guanidine–HCl and then renatured by dialysis against 0.1 *M* phosphate buffer, pH 7.4, in the presence of radiovitamins and then filtered, the patterns observed were as when crude extracts were used (Figs. 1A and 1B, lines ____). When a rabbit anti-R-binder serum was used, only the B_{12}-R-binder (B_{12}-R) peak (1C) of gastric juice changed and eluted into the Vo (Fig. 1C, line ■■■■). ^{125}I-labeled human immunoglobulin-G (^{125}I (IgG)) and serum albumin (^{125}I (HSA)) used as marker proteins (-----) while Blue Dextran 2000 (BD) and ^{3}H-water ($^{3}H_2O$) were used to estimate the totally excluded volume (Vo) and that accessible to the solvent. IgG-(B_{12}-IF); immunocomplexes of B_{12}-IF. IgG(B_{12}-R); immunocomplexes of B_{12}-R.

(16%) and IF (84%) (vide infra), respectively. The ^{58}Co-radioactivity eluted as a single well defined peak having a molecular size of 57,000 daltons, corresponding (vide infra) to IF (100%) (Fig. 1B). Similar experiments were carried out using aliquots of the pooled gastric and intestinal juices which have been predialyzed against 7.5 *M* guanidine–HCl, buffered at pH 7.4 and renatured with 0.1 *M* phosphate–buffer, pH 7.4, in the presence of (^{57}Co) B_{12} and (^{58}Co) B_{12} (18), respectively. The ^{57}Co- and ^{58}Co-radioactivities behaved in gel filtration as before, respectively, (Fig. 1A and B), except that the intestinal juice could bind 16% more (^{58}Co) B_{12} than before dialysis. Again, no (^{58}Co) B_{12} complexed to



FIGS. 1C–D

the intestinal juice R-binder was observed, thus indicating that all endogenous B_{12} in this fluid was bound to IF. Subsequently, by incubating before gel filtration each one of the four above described preparations (pooled intestinal and gastric juices treated and untreated with guanidine-HCl) with specific rabbit anti-R-binder (10) or with various sera from pernicious anemia patients, the following observations were made: The anti-R-binder serum removed into the Vo of Sephadex G200, 16% of the ^{57}Co-radioactivities, but none of the ^{58}Co-radioactivities (Fig. 1C and 1D). A pernicious anemia serum containing mainly binding type anti-IF antibodies removed into the region between Vo and immunoglobulin-G, 86% of the ^{57}Co-radioactivities (Fig. 1C) and the entire ^{58}Co-radioactivities (Fig. 1D). Preincubation of the crude and the corresponding guanidine-treated preparations of pooled gastric and intestinal juices with blocking type antibodies from a pernicious anemia serum prior to adding (^{57}Co) B_{12} and (^{58}Co) B_{12}, respectively, resulted in the disappearance of the (^{57}Co) B_{12}-IF peak of the gastric juice (accounting for 86% of the total $UB_{12}BC$) as well as the analogous ^{58}Co-radioactive peak of intestinal juice (accounting for 100% of the total $UB_{12}BC$) (Fig. 1C and 1D). The residual free (^{57}Co) B_{12} and (^{58}Co) B_{12} eluted in their entirety near the total volume of the columns. Equivalent amounts of IF from pooled ^{57}Co- and ^{58}Co-radioactive fractions collected from the 57,000 dalton peaks (i.e. gastric juice (^{57}Co) B_{12}-IF and intestinal juice (^{58}Co) B_{12}-IF, respectively) were incubated simultaneously in the presence of 2 mmoles/liter Ca^{++}

at pH 7.4 with 2 ml porcine ileal mucosal IF-receptor extract (10) and filtered through Sephadex G200 columns (10) containing also 2 mmoles/liter calcium. Equal relative ^{57}Co- and ^{58}Co-radioactivities were totally excluded (in the Vo) indicating that the intestinal IF possesses the same biological capacity as the gastric IF to bind the solubilized IF-receptor (data not shown). That the totally excluded cobalt-radioactive complexes represent specific complexes between the human IFs and the porcine IF-receptor was shown by the fact that no radioactive fractions in the Vo were observed when human abnormal IF (11) incapable of binding to the solubilized porcine IF-receptor (13) was used.

Discussion. The results illustrated in Fig. 1 demonstrate that the $UB_{12}BC$ in intestinal juice is due to a protein immunologically, biologically and molecularly identical with the known gastric IF. In addition, the B_{12}-binding capacity found to be saturated with endogenous B_{12} in intestinal juice is also due to IF, because the (^{58}Co) B_{12} coupled to intestinal juice after dialysis against guanidine was found to be bound, in its entirety, to IF (see Fig. 1B and D). Thus, neither the unsaturated nor the saturated with endogenous B_{12} R-binders survive the passage through the upper gastrointestinal tract. To the contrary, the bulk of the saturated and the unsaturated with B_{12}, IF, seems to tolerate the enzymatic or low pH hydrolysis that occurs during this transport (19). It was recently reported that purified trypsin and chymotrypsin can induce similar changes in IF and R-binder mixtures that do or do not contain B_{12} (17, 20). It is therefore plausible to suggest that the R-binders from saliva, gastric juice and bile remain undegraded because of the reduction (6) in the output of exocrine pancreatic secretion in EPI. The R-binders however, do not possess the ability to attach to the ileal IF-receptors (21) and, therefore, the entire R-binder-bound vitamin does not gain entry into the enterocyte in EPI. Under physiological conditions, however, the R-binders were shown here to undergo complete degradation, while IF retains its physicochemical, biological and immunological integrity. On the other hand, the intestinal juice could bind 16% more (^{58}Co) B_{12} than before dialysis with guanidine-HCl, apparently because guanidine had removed (18) some protein bound B_{12}. However, this endogenous B_{12} does not represent dietary B_{12} (because the juices were collected from fasting subjects only) but rather B_{12} excreted with the bile (22). Thus, the reduction of $UB_{12}BC$ in intestinal juice relative to that in gastric juice is partly due to saturation with the bile B_{12} and partly to degradation of R-binders. It is, therefore, conceivable that the B_{12} initially bound to R-binders in bile and also in saliva and gastric juice split off and ultimately bind to free IF in intestinal juice which is known to facilitate the B_{12} absorption. This interpretation is in fact in great accord with the hypothesis reported by Allen and his co-workers (8) and is compatible with the findings of Toskes and his co-workers (vide supra). In addition, the high concentration of free IF in the upper gastrointestinal tract is a strong argument against the theoretical "consideration" that the entero–hepatic circulation of B_{12} is of little significance (22) in man; as the 2.1 nmol B_{12} secreted daily in bile (22) is considered to be free or bound to R-binders (23), and because the latter protein is subject to degradation (as shown here), it is evident that the bile bound B_{12} ultimately splits off from R-binders and subsequently is picked up by the free IF which was shown here to be present in high concentrations in the upper gastrointestinal tract.

Summary. The vitamin B_{12}-binding proteins saturated or unsaturated with endogenous vitamin B_{12} in human gastric and intestinal juice were characterized in terms of molecular size, reaction with specific anti-intrinsic factor and R-binder (also called non-IF, Cobalophilin, etc.) sera, and reaction with specific porcine intrinsic factor receptor. It is concluded that the intestinal juice contains only intrinsic factor whereas gastric juice contains in addition R-binders. It appears likely that the absence of R-binders in intestinal juice is due to degradation by pancreatic enzymes and this fully supports the suggestion that the vitamin B_{12} malabsorption in exocrine pancreatic insufficiency is due to the presence of undegraded R-binders. The presence of free and biologically active intrinsic factor in intestinal juice suggests that entero–hepatic circulation may play an important role in vitamin B_{12} homeostasis.

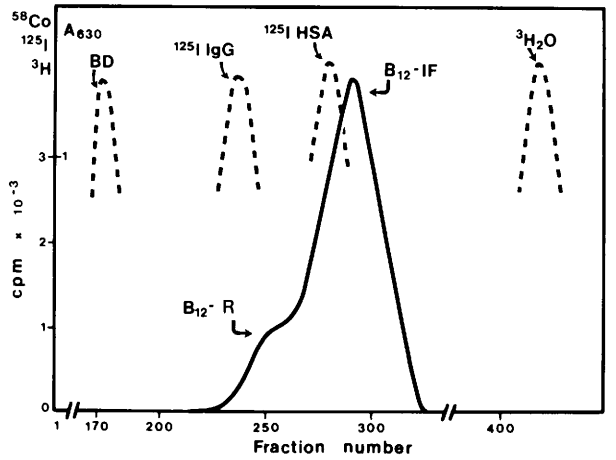


FIG. 2. Sephadex G200 radiochromatogram of (^{57}Co)B_{12} (line ——) coupled to the intestinal juice of a patient with exocrine pancreatic insufficiency. For explanation of the other curves see text under Fig. 1.

Note added in proof. While this paper has been in revision, we have progressed with the identification of B_{12}-binders in intestinal juice collected (see Materials and Methods) from a patient with proven EPI. Figure 2 illustrates the radiochromatogram through Sephadex G200 of (^{58}Co) B_{12} coupled to this intestinal juice. Contrary to the elution pattern observed when the intestinal juices from normal subjects were used, the protein bound ^{58}Co-radioactivity now delineated into two peaks corresponding by position to R-binder and IF (cf. Figs. 1B and 2). That R type B_{12}-binding proteins were present in the EPI intestinal juice was subsequently confirmed by specific radioimmunoassay (17) where the incubation of the juice with an excess of blocking type antibody from a pernicious anemia serum reduced the $UB_{12}BC$ of the juice from 16.0 ng B_{12}/ml to only 6.44 ng B_{12}/ml. Thus more than 40% of the $UB_{12}BC$ in the EPI intestinal juice was due to a non-IF binding protein, i.e., to R-binder. This observation further supports the suggestion (8, 19) that the R-binders in the upper gastrointestinal system do remain undegraded in EPI.

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