THE IMMUNOLOGIC IDENTIFICATION AND QUANTITATION OF HUMAN INTRINSIC FACTOR IN GASTRIC SECRETIONS *

BY GRAHAM H. JEFFRIES AND MARVIN H. SLEISENGER WITH THE TECHNICAL
ASSISTANCE OF LLOYD L. BENJAMIN

(From the Department of Medicine, The New York Hospital-Cornell Medical Center,
New York, N. Y.)

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Intrinsic factor (IF) differs from other naturally occurring, vitamin B₁₂-binding substances in its ability to promote the intestinal absorption of vitamin B₁₂. Thus, in patients with pernicious anemia, a failure of IF secretion causes vitamin B₁₂ malabsorption, although other vitamin B₁₂-binding substances are present in both their saliva and gastric juice (1-4). The presence of IF in gastric secretions or gastric mucosal preparations can be established only on the basis of this biological property, the ability to promote vitamin B₁₂ absorption.

An antibody that combines with and inactivates human IF has been identified in the sera of some patients with pernicious anemia (5-7). If this antibody could be shown to combine specifically with human IF, a sensitive *in vitro* method to identify and to measure IF could be developed. Such an *in vitro* test would have both clinical application and usefulness as an investigative tool in studies on IF.

The experiments herein described were carried out to define the specificity of this interaction between naturally occurring IF antibody and human IF. Vitamin B₁₂-binding substances saturated with cobalt⁶⁰-labeled vitamin B₁₂ (Co⁶⁰B₁₂) were separated by electrophoresis of saliva and gastric secretions and were tested for their ability to combine with antihuman IF γ -globulin from pernicious anemia serum (7). A bound Co⁶⁰B₁₂ complex that combined with antihuman IF γ -globulin was present only in acid gastric juices or in the achlorhydric gastric juice from patients with normal vitamin B₁₂ absorption. Co⁶⁰B₁₂-binding fractions from gastric juice of patients with pernicious anemia or from saliva did not react. On the basis of this specific immunological reaction

between antihuman IF γ -globulin and IF, an *in vitro* test for human IF is suggested.

METHODS

Patients and gastric juice collection. Studies were carried out on gastric secretions and saliva from patients with pernicious anemia, with atrophic gastritis but without pernicious anemia, and with normal gastric secretion of acid. The diagnosis of pernicious anemia had been established by demonstration of a) a macrocytic anemia with megaloblastic bone marrow, b) gastric achlorhydria on maximal histamine stimulation (8), and c) subnormal absorption of vitamin B₁₂ which was corrected by IF. Patients with atrophic gastritis without pernicious anemia exhibited achlorhydria on maximal histamine stimulation and atrophy of the fundal gastric mucosa with absent parietal and chief cells on gastric biopsy. Vitamin B₁₂ absorption measured by a modified Schilling test (9) was either normal or slightly decreased in these patients, and vitamin B₁₂ deficiency as evidenced by megaloblastic anemia was not present.

Gastric secretion was stimulated in fasting subjects by a subcutaneous injection of histamine phosphate (0.04 mg per kg) given 20 minutes after intramuscular chlorphenpyridamine maleate (20 mg). The fasting contents of the stomach were discarded, as were secretions contaminated by blood or bile. Gastric juice collected during 30 minutes, beginning 15 minutes after histamine injection, was used in this study. Secretions were immediately chilled to 4° C, and surface mucus and epithelial debris were removed by centrifugation at 2,500 *g* for 10 minutes. The pH of the gastric juice was measured electrometrically, and acid secretions were neutralized by adding 0.3 M borate buffer at pH 8.6 to prevent further peptic digestion of vitamin B₁₂-binding components.

Electrophoretic separation and quantitation of Co⁶⁰B₁₂-binding substances from saliva and gastric juice. Co⁶⁰B₁₂¹ (specific activity, 1 μ c per μ g of vitamin B₁₂) was added to 0.2-ml volumes of saliva or gastric juice (achlorhydric or neutralized) within 30 minutes of their collection. The concentration of added vitamin B₁₂ in saliva or gastric juice from patients with pernicious anemia varied between 240 and 560 μ g per ml of secretion. In neutralized gastric juice and the achlorhydric gastric juice from patients with normal vitamin B₁₂ absorption, the concentration

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¹ Obtained from Abbott Laboratories, Oak Ridge, Tenn.

varied between 480 and 560 μg vitamin B_{12} per ml of undiluted secretion. Each mixture was introduced into a transverse slot in a starch gel electrophoretic strip, and electrophoresis was carried out at 10°C for 5 hours at a constant voltage of 6 v per cm and at pH 8.6 (10). The distribution of radioactivity was measured by counting 1-cm segments of the electrophoretic strip in plastic tubes in a well-type scintillation counter.

Anodally migrating, bound $\text{Co}^{60}\text{B}_{12}$ in saliva or gastric juice was separated from the cathodally migrating, unbound (free) $\text{Co}^{60}\text{B}_{12}$ (10). The content of vitamin B_{12} -binding substances in each secretion, expressed as μg of vitamin B_{12} bound by 1 ml of secretion, was calculated from the equation: *vitamin B_{12} -binding capacity (μg per ml) = [anodally migrating radioactivity (cpm)/total radioactivity recovered on the electrophoretic strip (cpm)] $\times$ concentration of vitamin B_{12} added to the secretion (μg B_{12} per ml of undiluted secretion).* The possibility that radioactive breakdown products of $\text{Co}^{60}\text{B}_{12}$ migrated anodally and contaminated the bound $\text{Co}^{60}\text{B}_{12}$ fractions was excluded by starch gel electrophoresis of $\text{Co}^{60}\text{B}_{12}$ alone. Radioactivity was confined to cathodal segments.

Interaction of $\text{Co}^{60}\text{B}_{12}$ -binding fractions from gastric juices and saliva with antihuman IF γ -globulin. Gamma globulin was prepared by starch gel electrophoresis of sera from normal subjects and from a patient with pernicious anemia. The latter serum had been shown to

TABLE I
The concentration of added $\text{Co}^{60}\text{B}_{12}$ bound to intrinsic factor in normal, neutralized gastric juices

Sample no.	Total B_{12} -binding capacity	Percentage of bound B_{12} combining with antibody		IF- $\text{Co}^{60}\text{B}_{12}$ content of secretion	
		μg B_{12} /ml gastric juice		μg bound B_{12} /ml gastric juice	
		A	B	A	B
1	290	14.4	15.7	41.7	45.5
2	134	34.8	36.2	46.6	48.5
3	130	37.3	39.0	48.8	50.3
4	109	33.5	33.7	36.5	36.7
5	228	75.6	73.0	172.0	166.4
6	163	40.5	44.0	66.0	71.8
7	123	26.8	25.5	33.0	31.0
8	113	47.5	47.9	53.6	54.2
9	154	64.1	59.0	99.0	91.0
10	243	49.1	48.8	115.0	114.3
11	105	26.8	27.7	28.3	29.3

contain γ -globulin which combined with and inactivated a partially purified preparation of $\text{Co}^{60}\text{B}_{12}$ -labeled human IF. The γ -globulin in 1 ml of this serum combined with 58 μg of $\text{Co}^{60}\text{B}_{12}$ bound to IF [(7), Table I, Patient 1]. Electrophoresis of 0.4-ml volumes of sera was carried out for 5 hours at pH 8.6 and a constant voltage of 6 v

FIG. 1. THE CONCENTRATION OF VITAMIN B_{12} -BINDING SUBSTANCES IN SALIVA AND GASTRIC SECRETIONS. A. Total concentration of bound $\text{Co}^{60}\text{B}_{12}$. B. Quantity of IF-bound B_{12} as estimated by antibody technique.

TABLE II
The concentration of vitamin B₁₂-binding substances in gastric juices and saliva

Patient	Diagnosis	Total vitamin B ₁₂ -binding capacity of secretion	Concentration of vitamin B ₁₂ bound to intrinsic factor as estimated by antibody technique	Vitamin B ₁₂ absorption*
		mμg B ₁₂ /ml	mμg B ₁₂ /ml	%
A. Acid gastric juices				
1 F.E.	Myxedema, carcinoid syndrome	138	61	7.6
2 M.T.	Chronic cholecystitis	173	109	
3 W.B.	Sjögren's syndrome	170	32	
4 L.M.	D. latum infestation	231	49	15.0
5 J.S.	Blind loop syndrome	382	137	28.0
6 P.S.	Gluten enteropathy	398	189	
7 E.H.	Duodenal ulcer	150	61	
8 M.E.	Scleroderma	245	185	12.0
9 A.S.	Gastric ulcer	266	53	
10 M.M.	Gastric carcinoma	211	50	
11 F.C.	Duodenal ulcer	251	106	
12 L.J.	Gastric ulcer	117	49	
13 H.M.	Iron deficiency anemia	522	166	30.5
14 I.C.	Gastric ulcer	304	116	
B. Achlorhydric gastric juices from patients with atrophic gastritis but without pernicious anemia				
15 L.B.	Hypertension	319	119	14.1
16 T.C.	Refractory anemia	201	68	38.5
17 A.M.	Iron deficiency anemia	190	38	20.3
18 A.B.	Gastric polyp	235	98	23.4
19 M.R.	Gastric carcinoma	146	23	15.9
20 M.W.	Anxiety neurosis	230	26	8.5
C. Gastric juice from patients with pernicious anemia				
21 J.O.		156	0	0.5
22 M.W.		82	0	1.4
23 E.B.		111	0	1.6
24 E.K.		85	0	1.9
25 F.M.		207	0	6.8
26 J.F.		198	0	4.6
27 J.A.		163	0	0.7
28 W.L.		211	0	1.5
29 A.B.		203	0	0.6
30 B.K.		149	0	
31 E.B.		207	0	0.6
32 E.D.		220	5.0	1.7
33 B.H.		110	4.5	0.7
D. Saliva				
5 J.S.	Blind loop syndrome	145	0	
23 E.B.	Pernicious anemia	111	0	
24 E.K.	Pernicious anemia	38	0	
25 F.M.	Pernicious anemia	81	0	

* 48-hour urinary excretion of an oral dose of 0.2 μg of Co⁶⁰B₁₂. Normal value, the mean of 15 subjects, is 24.0%; range, 12.1 to 30.5%; SD, 6.44%.

per cm. The cathodal zone containing γ-globulin was cut from the starch gel strip; the fraction separated from normal serum has been referred to as normal γ-globulin, whereas the fraction separated from the pernicious anemia serum has been referred to as antihuman IF γ-globulin.

Starch gel segments containing the anodally migrating, bound Co⁶⁰B₁₂ fraction² from saliva or gastric juices were

² Segments 2 and 3 cm from the anode were tested initially and were shown to have a similar content of binding material that reacted with antihuman IF γ-globulin. Thereafter, the segment that contained maximal radioactivity was tested.

bisected within an hour of their electrophoretic separation. Each half-segment containing radioactive complex was crushed and mixed with starch gel segments containing normal and antihuman IF γ-globulin, respectively. The amount of bound Co⁶⁰B₁₂ that was mixed with the γ-globulin from 0.4-ml of serum did not exceed 10 mμg. These mixtures of crushed gel containing γ-globulin and bound Co⁶⁰B₁₂ were inserted into starch gel electrophoretic strips and were separated electrophoretically at pH 8.6 for 16 hours at a constant voltage of 5 v per cm. The distribution of radioactivity was again measured by counting 1-cm segments of the gel strips in plastic tubes in a well-type scintillation counter.

Interaction between bound $\text{Co}^{60}\text{B}_{12}$ and antihuman IF γ -globulin was indicated by retention of radioactivity in the application zone (7).

The concentration of $\text{Co}^{60}\text{B}_{12}$ bound to IF (IF- $\text{Co}^{60}\text{B}_{12}$) in individual secretions was calculated from the total content of bound vitamin B_{12} and from the proportion of radioactivity that was retained in the application zone during electrophoresis of the bound $\text{Co}^{60}\text{B}_{12}$ fraction with antihuman IF γ -globulin. This measure of IF would be accurate only when IF- $\text{Co}^{60}\text{B}_{12}$ in each test mixture was totally combined with antibody, i.e., in the presence of an excess of antihuman IF γ -globulin. Although it was shown in a previous study that antihuman IF γ -globulin from 0.4 ml of the pernicious anemia serum would combine with 23.2 μg of vitamin B_{12} bound to IF—antibody in 1 ml of this serum combined with 58.0 μg IF- $\text{Co}^{60}\text{B}_{12}$ [(7), Table I, Patient 1]—further experiments were

carried out to prove that when bound $\text{Co}^{60}\text{B}_{12}$ fractions in amounts that did not exceed 10 μg were mixed with antihuman IF γ -globulin from 0.4-ml of serum, IF- $\text{Co}^{60}\text{B}_{12}$ in the test fractions was completely combined with antibody. Bound $\text{Co}^{60}\text{B}_{12}$ fractions separated from normal neutralized gastric juices were added at two concentrations, one (B) twice the other (A), to antihuman IF γ -globulin from 0.4-ml of serum. The maximal amount of added bound $\text{Co}^{60}\text{B}_{12}$ did not exceed 10 μg . At both concentrations, a similar proportion of each bound $\text{Co}^{60}\text{B}_{12}$ fraction combined with antibody, indicating that the latter was present in excess. Table I lists the vitamin B_{12} -binding capacity of eleven, normal, neutralized gastric juices together with the percentage of bound $\text{Co}^{60}\text{B}_{12}$ that combined with antihuman IF γ -globulin and the IF- $\text{Co}^{60}\text{B}_{12}$ content of each secretion calculated from the former values.

FIG. 2. STARCH GEL ELECTROPHORETIC SEPARATION OF BOUND $\text{Co}^{60}\text{B}_{12}$ IN NORMAL GASTRIC JUICE AND ITS INTERACTION WITH NORMAL AND ANTIHUMAN IF γ -GLOBULIN. A. Starch gel electrophoresis of neutralized gastric juice (from Patient 8, M.E.), containing added $\text{Co}^{60}\text{B}_{12}$, 500 μg vitamin B_{12} per ml of undiluted secretion. The distribution of cathodally migrating, free $\text{Co}^{60}\text{B}_{12}$ and anodally migrating, bound $\text{Co}^{60}\text{B}_{12}$ is plotted. The hatched zone indicates the segment of the electrophoretic strip containing bound $\text{Co}^{60}\text{B}_{12}$ that was tested for its interaction with normal and antihuman IF γ -globulin. Electrophoresis was done at 10° C for 5 hours at 6 v per cm and at pH 8.6. B. Bound $\text{Co}^{60}\text{B}_{12}$ from electrophoresis A was divided and mixed with normal and antihuman IF γ -globulin. Starch gel electrophoresis of these mixtures was carried out at 10° C for 16 hours at 5 v per cm and at pH 8.6. The distribution of radioactivity on the respective electrophoretic strips is plotted.

FIG. 3. BOUND $\text{Co}^{60}\text{B}_{12}$ IN GASTRIC JUICE FROM A PATIENT WITH ATROPHIC GASTRITIS, BUT WITHOUT PERNICIOUS ANEMIA. A. Starch gel electrophoresis of gastric juice from Patient 20, M. W. Concentration of added $\text{Co}^{60}\text{B}_{12}$ was 515 μg per ml of gastric juice. Electrophoresis was done at 10°C for 5 hours at 6 v per cm and at pH 8.6. The distribution of $\text{Co}^{60}\text{B}_{12}$ is plotted, and the fraction of bound $\text{Co}^{60}\text{B}_{12}$ that was tested for its interaction with normal and antihuman IF γ -globulin is indicated by cross-hatching. B. Bound $\text{Co}^{60}\text{B}_{12}$ from electrophoresis A was mixed with normal and antihuman IF γ -globulin. Starch gel electrophoresis of these mixtures was carried out at 10°C for 16 hours at 5 v per cm and at pH 8.6. The distribution of radioactivity on each electrophoretic strip is plotted.

RESULTS

Electrophoretic separation and quantitation of $\text{Co}^{60}\text{B}_{12}$ -binding substances in saliva and gastric juices. When saliva, achlorhydric gastric juice, or normal, neutralized gastric juice mixed with $\text{Co}^{60}\text{B}_{12}$ was subjected to starch gel electrophoresis, bound $\text{Co}^{60}\text{B}_{12}$ migrated anodally and was separated from unbound (free) $\text{Co}^{60}\text{B}_{12}$. $\text{Co}^{60}\text{B}_{12}$ complexes from saliva, achlorhydric gastric juice, and normal, neutralized gastric juice exhibited slight differences in anodal electrophoretic mobility (Figures 2-5).

The quantity of vitamin B_{12} -binding substances in different secretions, calculated from the concentration of added vitamin B_{12} and from the proportion of anodally migrating radioactivity, is expressed in Table II and Figure 1, A columns. It is apparent from these data that the total binding

capacity of gastric juices did not distinguish patients with pernicious anemia from those with normal gastric secretion, or from those with achlorhydria and normal vitamin B_{12} absorption.

Electrophoresis of bound $\text{Co}^{60}\text{B}_{12}$ fractions from saliva and gastric juices with normal and antihuman IF γ -globulin. The patterns of electrophoretic migration of bound $\text{Co}^{60}\text{B}_{12}$ complexes mixed with normal and antihuman IF γ -globulin are shown in Figures 2B through 5B. Bound $\text{Co}^{60}\text{B}_{12}$ prepared from normal, neutralized gastric juice showed an altered electrophoretic mobility in the presence of antihuman IF γ -globulin (Figure 2B). Radioactivity was retained in the application zone, indicating that IF- $\text{Co}^{60}\text{B}_{12}$ was combining with antibody (7). In the presence of normal γ -globulin, the bound $\text{Co}^{60}\text{B}_{12}$ fraction migrated anodally as a single radioactive zone (Figure 2B). Bound

$\text{Co}^{60}\text{B}_{12}$ separated from the gastric juice of patients with histamine-fast achlorhydria and normal vitamin B_{12} absorption combined in part with antihuman IF γ -globulin, but also contained a high proportion of unreactive, anodally migrating bound $\text{Co}^{60}\text{B}_{12}$ (Figure 3 and Table II).

Complexes from the gastric juice of pernicious anemia patients and from saliva did not react with antihuman IF γ -globulin. In the presence of both normal and antihuman IF γ -globulins, these radioactive complexes retained their anodal electrophoretic mobility (Figures 4 and 5).

The concentration IF- $\text{Co}^{60}\text{B}_{12}$ in individual secretions was calculated from the total content of bound vitamin B_{12} and from the proportion of radioactivity that was retained in the zone of application during electrophoresis with antihuman IF γ -globulin. These values are listed in Tables I and II and in Figure 1, B columns. An excess

of antibody (antihuman IF γ -globulin) ensured that all antigen (IF- $\text{Co}^{60}\text{B}_{12}$) present in the tested secretions was measured. The difference between the total vitamin B_{12} -binding capacity of each secretion and its IF- $\text{Co}^{60}\text{B}_{12}$ content is a measure of the content of non-IF-binding substances.

DISCUSSION

The measurement of the vitamin B_{12} -binding capacity of secretions depends on the partition of added vitamin B_{12} into bound and unbound fractions. In the experiments described, this separation was achieved by starch gel electrophoresis of native secretions without preliminary storage, concentration, or dialysis. This method had several advantages over the dialysis and paper electrophoretic techniques described by other workers (1, 2). A decrease in binding capacity, due either to denaturation of labile IF, or to dissociation of

FIG. 4. BOUND $\text{Co}^{60}\text{B}_{12}$ IN GASTRIC JUICE FROM A PATIENT WITH PERNICIOUS ANEMIA. A. Starch gel electrophoresis of gastric juice from Patient 22, M.W. The concentration of added $\text{Co}^{60}\text{B}_{12}$ was $515 \mu\text{g}$ per ml of gastric juice. Electrophoresis was done at 10°C for 5 hours at 6 v per cm and at pH 8.6. The distribution of $\text{Co}^{60}\text{B}_{12}$ is plotted, and the fraction of bound $\text{Co}^{60}\text{B}_{12}$ that was tested for its interaction with normal and antihuman IF γ -globulin is indicated by cross-hatching. B. Bound $\text{Co}^{60}\text{B}_{12}$ from electrophoresis A was mixed with normal and antihuman IF gamma globulin. Starch gel electrophoresis of these mixtures was carried out at 10°C for 16 hours at 5 v per cm and at pH 8.6. The distribution of radioactivity on each electrophoretic strip is plotted.

FIG. 5. BOUND $\text{Co}^{60}\text{B}_{12}$ IN SALIVA. A. Starch gel electrophoresis of saliva from Patient 5, J.S. The concentration of added $\text{Co}^{60}\text{B}_{12}$ was 265 μg per ml of saliva. Electrophoresis was done at 10°C for 5 hours at 6 v per cm and at pH 8.6. The distribution of $\text{Co}^{60}\text{B}_{12}$ is plotted, and the fraction of bound $\text{Co}^{60}\text{B}_{12}$ that was tested for its interaction with normal and antihuman IF γ -globulin is indicated by cross-hatching. B. Bound $\text{Co}^{60}\text{B}_{12}$ from electrophoresis A was mixed with normal and antihuman IF γ -globulin. Starch gel electrophoresis of these mixtures was carried out at 10°C for 16 hours at 5 v per cm and at pH 8.6. The distribution of radioactivity on each electrophoretic strip is plotted.

bound $\text{Co}^{60}\text{B}_{12}$ during exhaustive dialysis, was avoided; furthermore, the binding fractions prepared by starch gel electrophoresis were available for subsequent study.

Neither the total vitamin B_{12} -binding capacity of secretions, nor the electrophoretic mobility of their bound $\text{Co}^{60}\text{B}_{12}$ components indicated the presence of IF. Although the average vitamin B_{12} -binding capacity of acid secretions exceeded that of saliva or achlorhydric secretions (Figure 1), there was a wide range of values in each group, with considerable overlap. Similarly, although the electrophoretic mobility of bound $\text{Co}^{60}\text{B}_{12}$ in saliva and in gastric juice from patients with pernicious anemia exceeded that of bound $\text{Co}^{60}\text{B}_{12}$ in normal, neutralized gastric juice (Figures 2, 4, 5), this difference in electrophoretic mobility was not great enough to result in the separation of IF from other vitamin B_{12} -binders present in individual achlorhydric gastric juices of patients with normal vitamin B_{12} absorption (Figure 3).

Bound $\text{Co}^{60}\text{B}_{12}$ separated from gastric juices that contained IF—as indicated by their acidity, or

by normal Schilling tests in patients with atrophic gastritis—combined with antihuman IF γ -globulin. Bound $\text{Co}^{60}\text{B}_{12}$ fractions separated from the gastric juice of patients with pernicious anemia and from saliva were unreactive. These results establish that IF may be identified not only by its ability to potentiate vitamin B_{12} absorption, but also by its specific reaction with antihuman IF γ -globulin. This forms the basis of an *in vitro* test for IF and of a method for measuring the IF content of secretions.

A significant finding in this study was the relatively large amount of vitamin B_{12} -binding substance in normal, neutralized gastric juice that did not combine with antihuman IF γ -globulin. Although the IF activity of this fraction has not been tested *in vivo*, it is probable that this is a biologically inactive (non-IF), vitamin B_{12} -binding substance or substances.

Recently, Sullivan, Herbert, and Castle used a mucosal homogenate from guinea-pig ileum to identify IF *in vitro* (11). These workers showed that gastric juice with IF activity, as indicated by

vitamin B₁₂ absorption tests, potentiated the uptake of Co⁶⁰B₁₂ by the mucosal preparation. Gastric juice from pernicious anemia patients with complete vitamin B₁₂ malabsorption was inactive. In the future, the use of these *in vitro* techniques may be important in developing our understanding of IF physiology.

SUMMARY

1. The vitamin B₁₂-binding capacity of saliva and gastric secretions was measured by the partition of added cobalt⁶⁰-labeled vitamin B₁₂ (Co⁶⁰-B₁₂) into bound and unbound (free) fractions during starch gel electrophoresis.

2. The presence of intrinsic factor in secretions could not be established either on the basis of their total vitamin B₁₂-binding capacity, or on the electrophoretic mobility of their bound Co⁶⁰B₁₂ components.

3. The interaction of normal and antihuman intrinsic factor gamma globulin with bound Co⁶⁰B₁₂ fractions from saliva and gastric juices was studied by electrophoresis. Bound Co⁶⁰B₁₂ separated from gastric juices that contained intrinsic factor—as indicated by their acidity, or by normal Schilling tests in those patients with atrophic gastritis—combined with antihuman intrinsic factor γ -globulin, whereas bound Co⁶⁰B₁₂ fractions from other secretions were unreactive.

4. These studies demonstrate, therefore, that intrinsic factor can be identified not only by *in vivo* vitamin B₁₂ absorption tests, but also by its specific reaction *in vitro* with antihuman intrinsic factor γ -globulin. Thus, an *in vitro* test for intrinsic factor and a method of measuring specific intrinsic factor binding of vitamin B₁₂ are established.

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ADDENDUM

After this manuscript was submitted for publication, studies were carried out by Reisner, Wolff, McKay, and

Doyle (12) on two patients (Cases 1 and 2) with previously established, juvenile pernicious anemia. Their fasting and stimulated gastric juices were of normal acidity, pH 1 to 2. The vitamin B₁₂-binding substances present in the stimulated secretions in concentration of 118 and 124 μ g vitamin B₁₂ per ml, respectively, did not react with intrinsic factor antibody. Thus, the absence of intrinsic factor in these acid secretions was confirmed.

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