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STUDIES ON THE NATURE OF THE INTRINSIC FACTOR OF CASTLE*

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In the years immediately following the demonstration by Minot and Murphy (1926) of the therapeutic efficacy of orally administered liver in pernicious anaemia, Castle and his associates (Castle, 1929 ; Castle and Townsend, 1929 ; Castle, Townsend, and Heath, 1930a ; Castle, Heath, and Strauss, 1931 ; Castle and Ham, 1936 ; Castle, Heath, Strauss, and Heinle, 1937), in a series of brilliant investigations, concluded that there was in normal gastric juice from human beings a substance (intrinsic factor) which acted on a dietary substance (extrinsic factor) to produce a material required for the normal maturation of erythrocytes. Patients having pernicious anaemia lacked intrinsic factor.

Since these publications first appeared an intensive search has been made to isolate and to identify substances essential to haematopoiesis and to define the relationships between them. In recent years two pure compounds possessing marked haematopoietic activity have been obtained from liver. The first, pteroylglutamic acid, was isolated in 1943 (Pfiffner *et al.*, 1943) and synthesized in 1945 (Angier *et al.*, 1946). The second, vitamin B_{12}, was isolated in crystalline form in 1948 (Rickes *et al.*, 1948 ; Smith and Parker, 1948). Both substances possessed the property of converting megaloblastic erythropoiesis to a normoblastic type of regeneration, but only one, vitamin B_{12}, when administered parenterally, was found to be fully as effective as extracts of liver in controlling all manifestations of pernicious anaemia. Pteroylglutamic acid stimulated haematopoiesis, but failed to prevent the development or progression of the neurodegenerative changes characteristic of this disorder.

Vitamin B_{12}, therefore, appeared to be either the anti-pernicious-anaemia component of liver or a substance closely allied to it. However, in contrast to the haematopoietic activity of orally administered extracts of liver, vitamin B_{12}, in doses known to be effective parenterally, failed to produce a haematopoietic response when it was administered by mouth to patients having pernicious anaemia in relapse, unless gastric juice or other known sources of intrinsic factor were given simultaneously with it. Thus it appeared that vitamin B_{12} functioned also as extrinsic factor (Berk *et al.*, 1948 ; Hall *et al.*, 1949). Confirmation of the extrinsic-factor activity of this substance subsequently was obtained by the demonstration that it was an active haematopoietic component of beef muscle (Gardner *et al.*, 1949 ; Morgan *et al.*, 1949 ; Wolf *et al.*, 1950).

Since Minot and Castle (1931) had shown that liver extracts given by intramuscular injection were from thirty to sixty times as active as when administered by the oral route, it appeared to be advisable to determine the therapeutic activity of vitamin B_{12} administered orally in the absence of intrinsic factor, in doses in excess of those known to be effective parenterally. Few data are available in the literature concerning the minimal effective dose of orally administered vitamin B_{12}. In most instances only small quantities have been employed. However, Spies and his associates (1949) presented evidence to show that approximately fifty times the minimal effective parenteral dose is required when vitamin B_{12} is administered by mouth. On the other hand, Meyer and his associates (1950) observed no haematopoietic response to the daily oral administration of 150 μg. to one patient and of 250 μg. to a second patient. These quantities represent doses of from 150 to 250 times the minimal effective parenteral dose.

Oral Administration of Vitamin B_{12} in the Absence of Intrinsic-factor Activity

Nineteen patients having pernicious anaemia in relapse received vitamin B_{12} alone by mouth. Sixteen of the 19 were given the vitamin daily in doses varying from 5 to 100 μg. for periods of from seven to thirteen days (average ten days). The haematopoietic responses elicited are summarized in Table I.** Among 10 of 11 patients who ingested 5 to 10 μg. of vitamin B_{12} daily, haematopoietic

TABLE I.—*Oral Administration of Vitamin B_{12} in Absence of Intrinsic Factor to 19 Patients Having Pernicious Anaemia in Relapse*

Case	B_{12} Given Daily (μg.)	Days Observed	Erythrocytes (Millions per c.mm.)		Maximal Reticulocyte Response		
			Onset	End	% Observed	Day Observed	Expected*
1	5	7	1·52	1·63	4·9	2	22·3
2	5	12	2·64	2·05	3·5	1	7·5
3	5	10	1·89	1·27	—	—	15·6
4	5	9	2·50	2·84	2·3	9	8·4
5	5	10	2·53	2·81	—	—	8·4
6	5	9	2·40	2·02	2·7	5	9·4
7	10	9	1·64	1·95	—	—	20·4
8	10	13	2·32	2·74	6·1	10	10·5
9	10	9	2·30	2·36	3·6	2	10·5
10	10	9	2·39	2·31	—	—	9·4
11	10	9	2·08	1·90	—	—	12·9
12	25	11	2·63	3·43	5·2	12	7·5
13	25†	6	1·73	1·91	—	—	18·7
14	75†	7	1·47	1·50	8·0	5	22·3
	150†	9	1·50	1·60	4·9	9	22·3
15	1,000†‡	12	2·33	2·74	3·8	9	10·5
16	50	13	2·31	2·39	10·2	13	10·5
17§	100‡	13	1·65	2·10	7·1	6	20·4
18	100	8	2·01	2·29	5·7	7	14·1
19	100	10	1·35	2·17	13·0	8	26·5

* Sturgis. † Single oral dose. ‡ Vitamin B_{12} concentrate. § Received one meal of cooked liver by error on second day of therapy.

*To be read at the First International Congress of Internal Medicine, Paris, September 11–14, 1950.

**Detailed data concerning the haematopoietic responses noted in these 19 patients have been published elsewhere.

responses either were not observed or they were of such minor degree that they were listed as being insignificant. One patient who received 10 μg. a day showed a significant although suboptimal response. Of the remaining five patients who received daily doses of vitamin B_{12} three were given 100 μg. daily, one 50 μg. daily, and one 25 μg. daily. It is of interest that the patient who ingested 25 μg. a day showed an optimal haematopoietic response, whereas two patients who received 100 μg. daily manifested distinctly suboptimal responses. The patient who was given 50 μg daily and one who was given 100 μg. a day had good but suboptimal responses.

Three of the 19 patients were given single oral doses of vitamin B_{12}, one receiving 25 μg., one 75 μg. on one occasion and 150 μg. seven days later, and one 1,000 μg. No haematopoietic response was observed in the patient ingesting 25 μg. of the vitamin, and the other two patients had suboptimal responses.

Studies on the Nature of Intrinsic Factor

Earlier studies on the nature of intrinsic factor in the gastric juice of human beings have demonstrated that the activity of the material is destroyed by heating to a temperature of 40° C. for three days, of 70° to 80° C. for thirty minutes, and by boiling for five minutes (Castle, Heath, and Strauss, 1931). Intrinsic factor may be separated from the enzymes pepsin and renin by precipitation with casein without losing its activity (Castle, Townsend, and Heath, 1930b ; Helmer *et al.*, 1934) and it is not destroyed by a degree of alkalinity (*p*H 9.8 for thirty minutes) which will inactivate pepsin and pepsinogen (Ungley and Moffett, 1936).

Intrinsic factor does not pass through a semi-permeable membrane which permits the passage of extrinsic factor. It is partially soluble in 80% alcohol and 80% acetone and is precipitated on saturation with ammonium sulphate (Helmer and Fouts, 1937 ; Goldhamer and Kyer, 1938).

The observations of Fouts, Helmer, and Zerfas (1935) on the intrinsic-factor activity of a powdered liver extract are of current interest in view of the isolation of vitamin B_{12} from liver. In 1935 these authors reported that the intrinsic factor contained in from 50 to 100 ml. of normal gastric juice seemed to be the minimal amount necessary to produce an optimal response when incubated with an extract derived from 100 g. of liver and administered by mouth daily to a patient with pernicious anaemia. Some response was obtained from as little as 10 ml. of the juice. The vitamin B_{12} content of the liver extracts employed in these studies is not known, but it seems probable that the daily dose supplied at least 10 μg.

In December, 1948, Berk and his associates demonstrated that vitamin B_{12} functions as food or extrinsic factor when administered by mouth simultaneously with gastric or intrinsic factor to patients having pernicious anaemia in relapse. Normal gastric juice from human beings was used as a source of intrinsic factor. They confirmed the observation of Castle and Ham (1936) that a haematopoietic response was not elicited if an interval of twelve hours elapsed between administrations of sources of extrinsic and intrinsic factors.

The findings of Berk and associates (1948) were confirmed by my associates and me (Hall *et al.*, 1949) in February, 1949. Berkefeld-filtered pooled gastric juice obtained from patients who had either duodenal ulcer or functional disturbances of the gastro-intestinal tract was employed. The haematopoietic activity of the simultaneous oral administration of 5 to 10 μg. of vitamin B_{12} and 100 to 150 ml. of Berkefeld-filtered pooled gastric juice of human beings is shown in Table II.

TABLE II.—*Oral Administration of Vitamin B_{12} and Pooled Filtered Gastric Juice of Human Beings*

Case	Period	Daily Oral Therapy		Days Observed	Erythrocytes (Millions per c.mm.)		Maximal Reticulocyte Response	
		B_{12} (μg.)	Source of Intrinsic Factor		Onset	End	%	Day
20	1	—	150 ml. of gastric juice	6	1·43	1·50	—	—
	2	5	,, ,,	18	1·50	2·92	31·7	10
21	1	5	,, ,,	12	1·63	2·40	16·5	9
15	1	1,000*	—	12	2·33	2·74	3·8	9
	2	10	100 ml. of gastric juice	11	2·74	2·71	11·8	7

* Single dose of vitamin B_{12} concentrate.

Since, as has previously been shown by others, intrinsic factor passes readily through a Berkefeld filter, the risk of the transmission of bacterial infections, such as tuberculosis, may be avoided when pooled gastric juice is administered to patients. It also makes possible the collection of relatively large quantities of gastric juice of normal or high acidity in the routine gastric laboratory for the purpose of studying the effects of various physical and chemical agents on intrinsic factor. A preliminary survey of the results of some of these studies has been reported (Campbell *et al.*, 1949).

Some of the earlier observations on the properties of intrinsic factor, derived either from gastric juice of human beings or from the stomach and intestines of swine, have been confirmed in our studies on the effectiveness of orally administered vitamin B_{12} when given in conjunction with various derivatives of gastric juice or gastro-intestinal substances to patients having pernicious anaemia in relapse.

Gastric juice heated to a temperature of 63° C. for thirty minutes was ineffective as an intrinsic factor when given to a patient with pernicious anaemia in a daily dose of 150 ml. together with 5 μg. of vitamin B_{12}. The patient subsequently responded well to 75 ml. of unheated gastric juice daily, with 5 μg. of vitamin B_{12} (Case 2, Table III).

TABLE III.—*Oral Administration of Vitamin B_{12} and Pooled Filtered Gastric Juice of Human Beings: Effect of Heat on Intrinsic Factor*

Case	Period	Daily Oral Therapy*		Days Observed	Erythrocytes (Millions per c.mm.)		Maximal Reticulocyte Response	
		B_{12} (μg.)	Intrinsic Factor		Onset	End	%	Day
2	1	5	150 ml. of gastric juice previously heated to 63° C. for 30 min.	12	2·64	2·05	—	—
	2	5	75 ml. of unheated gastric juice	13	2·05	3·38	11·3	11
3	1	Mixture of 5μg. of B_{12} + 100 ml. of gastric juice stored at 25° C. for 24 hours, then heated to 70° for 1 hour		10	1·89	1·27	—	—
	2	Unheated mixture of 5 μg. of B_{12} + 100 ml. of gastric juice stored at 25° C. for 24 hours		14	1·27	1·91	5·2	14
	3	Single intramuscular injection of 20 μg. of B_{12}. Same dose repeated 3 and 5 days later		8	1·91	2·64	—	—

* Unless otherwise specified.

Since a temperature of 63° C. is required to destroy most viruses, the administration of pooled gastric juice of human beings which possesses intrinsic-factor activity could result in the transmission of a virus infection, such as infectious hepatitis.

Vitamin B_{12}, unlike intrinsic factor, is not destroyed by relatively high temperatures. A thermostable combination of intrinsic factor and vitamin B_{12} was not obtained by allowing a mixture of the vitamin and neutralized gastric juice to stand for twenty-four hours at a temperature of 25° C. before heating to 70° C. for one hour. Such a mixture, containing 100 ml. of gastric juice and 5 μg. of vitamin B_{12}, administered daily to a patient having pernicious anaemia in relapse failed to produce a therapeutic response. During a second period an unheated mixture of the two substances after standing for twenty-four hours at 25° C. was administered orally each day. While the reticulocytosis was suboptimal, there was a significant rise in the erythrocyte count, and the subsequent administration of vitamin B_{12} intramuscularly did not elicit a second reticulocyte response (Case 3, Table III). It was concluded, therefore, that either vitamin B_{12} and intrinsic factor present in human gastric juice did not combine chemically, or if chemical union did occur the product of the interaction was also thermolabile.

Pooled Berkefeld-filtered gastric juice from human beings, collected and stored for three months at a *p*H of 1.7 and a temperature of 5° C., retained intrinsic-factor activity although it appeared to be less active than the fresh material. The results of the daily oral administration of 5 μg. of vitamin B_{12} and 100 ml. of stored gastric juice to two patients having pernicious anaemia in relapse are shown in Table IV. One patient (Case 22, period 2)

TABLE IV.—*Oral Administration of Vitamin B_{12} and Pooled Filtered Human Gastric Juice: Presence of Intrinsic Factor After Storage for Three Months at pH 1.7 and 5°C.*

Case	Period	Daily Oral Therapy*		Days Observed	Erythrocytes (Millions per c.mm.)		Maximal Reticulocyte Response	
		B_{12} (μg.)	Intrinsic Factor		Onset	End	%	Day
22	1	—	100 ml. of fresh gastric juice	10	2·91	2·78	5·8	2
	2	5	100 ml. of gastric juice stored 3 months at *p*H 1·7 and 5° C., then neutralized to *p*H 7	11	2·78	2·66	7·0	10
	3	5	100 ml. of fresh gastric juice	6	2·66	3·18	6·3	6
23	1	—	100 ml. of fresh gastric juice	7	1·25	1·31	3·3	7
	2	5	100 ml. of gastric juice stored 3 months at *p*H 1·7 and 5° C., then neutralized to *p*H 7	12	1·31	2·20	13·5	7
	3	10 μg. of B_{12} administered intramuscularly daily for 5 days, then weekly		21	2·31	3·56	—	—

* Unless otherwise specified.

manifested a reticulocyte peak equal to the estimated maximal response calculated for the initial level of erythrocytes, but there was no rise in the erythrocyte count during administration of the mixture. Since secondary reticulocytosis was demonstrated during period 3, when the patient received vitamin B_{12} and fresh gastric juice, the haematopoietic response to the oral administration of the vitamin and the stored gastric juice probably was suboptimal. The other patient (Case 23), on the other hand, showed a significant haematopoietic response that approached an optimal figure.

At low temperatures the enzymic action of pepsin is largely inhibited, and previously reported observations by Castle and Ham have indicated that loss of the intrinsic-factor activity of gastric juice on standing may be due to inactivation by the native pepsin contained in the juice.

A 95% solution of ethyl alcohol apparently destroys intrinsic factor, inasmuch as no significant haematopoietic activity could be demonstrated in either the precipitate or the soluble fraction of gastric juice when these fractions were administered with vitamin B_{12} successively to a patient having pernicious anaemia in relapse (Case 24, Table V).

TABLE V.—*Oral Administration of Vitamin B_{12} and Precipitates of Gastric Juice of Human Beings*

Case	Period	Daily Oral Therapy		Days Observed	Erythrocytes (Millions per c.mm.)		Maximal Reticulocyte Response	
		B_{12} (μg.)	Intrinsic Factor		Onset	End	%	Day
24	1	5	Cold alcohol precipitate derived from 150 ml. of gastric juice	10	2·44	2·22	4·5	7
	2	5	Alcohol-soluble fraction derived from 150 ml. of gastric juice	9	2·22	2·10	5·7	5
	3	5	75 ml. of gastric juice	11	2·10	2·87	9·8	8

In November, 1949, Ternberg and Eakin reported that normal gastric juice contained a heat-labile non-dialysable substance which combined with vitamin B_{12} in such a manner as to make the vitamin unavailable for the growth of certain bacteria (*Bacterium coli, Lactobacillus lactis* Dorner, *Lactobacillus leichmannii*). The application of heat to the complex thus formed liberated vitamin B_{12}, whereupon the vitamin again became available for microbial growth. Heated gastric juice did not contain a principle capable of combining with vitamin B_{12}. Ternberg and Eakin (1949) postulated that the heat-labile factor in gastric juice, which they called "apoerythein," either was intrinsic factor itself or an important component of this factor. Commercial preparations of gastric mucosa of swine made for therapeutic use were found to contain a principle which seemed to be analogous to the apoerythein in gastric juice.

Subsequently, Eakin (unpublished data) reported that apoerythein possessed all of the known chemical properties of intrinsic factor. Its concentration was found to be greater in the gastric juice of normal subjects than in that of patients having pernicious anaemia. Eakin estimated that 25 μg. of vitamin B_{12} could be bound by the apoerythein contained in 1.5 litres of gastric juice. This amount of juice is an approximation of the volume secreted by a normal person in twenty-four hours. Apoerythein was found in the lining of the gastro-intestinal tract of all vertebrates studied. Its presence was not demonstrable in other tissues.

In a collaborative study with Dr. Frank Bethell and his associates of the University of Michigan, my associates and I (unpublished data) reported recently the enhancement of haematopoietic activity of orally administered vitamin B_{12} when preparations of stomach and duodenum of swine were given with the vitamin. The results of the simultaneous administration of vitamin B_{12} and extracts of gastric mucosa of swine to two patients with pernicious anaemia in relapse are shown in Table VI. The lyophilized preparation had greater activity than the non-lyophilized material. However, of the extracts employed by us, greatest intrinsic-factor activity was found in the lyophilized non-dialysable fraction of a dilute hydrochloric acid extract of duodenal mucosa. A dilute acid extract of ground whole duodenum, prepared at a *p*H of 3 to 4, was found to be moderately effective when given daily in a dose of 2 g. of desiccated material together with 5 μg. of vitamin B_{12}. Desiccated duodenal mucosa without extraction or fractionation also possessed considerable therapeutic

TABLE VI.—*Oral Administration of Vitamin B_{12} and Extracts of Gastric Mucosa of Swine*

Case	Period	Daily Oral Therapy* Time	Material Given	Days Observed	Erythrocytes (Millions per c.mm.) Onset	End	Maximal Reticulocyte Response %	Day
25	1	10 p.m.	5·4 g. of non-lyophilized extract of hog gastric mucosa	7	2·01	1·89	3·9	3
	2	10 p.m.	5 μg. of B_{12} + 5·4 g. of non-lyophilized extract of hog gastric mucosa	13	1·89	1·30	7·2	11
	3	10 p.m.	5 μg. of B_{12} + 75 ml. of pooled human gastric juice	11	1·30	1·32	8·6	5
	4		5 μg. of B_{12} intramuscularly	9	1·32	2·57	20·5	4
26	1	10 a.m. 10 p.m.	10 μg. of B_{12} 4 g. of lyophilized extract of hog gastric mucosa	9	1·64	1·95	—	—
	2	10 p.m.	10 μg. of B_{12} + 4 g. of lyophilized extract of hog gastric mucosa	15	1·95	2·04	11·3	10
	3		5 μg. of B_{12} intramuscularly	7	2·04	2·37	—	—

* Unless otherwise specified.

activity when administered by mouth in a dose of 2 to 4 g. together with 5 μg. of vitamin B_{12}.

Bethell and his associates have assayed the vitamin-B_{12}-binding power of these materials and of the gastric juice of normal human beings, according to the method of Ternberg and Eakin, employing the test organism *Lactobacillus leichmannii*. The materials, after sterilization by passage through a Berkefeld filter without heating, were added to the culture medium. The highest binding activity of the duodenal materials tested was shown by the water-soluble non-dialysable fraction of duodenal mucosa. The acid extract of ground whole duodenum possessed about half and the desiccated unfractionated duodenal mucosa about an eighth of the activity of the water-soluble non-dialysable material.

The property of binding vitamin B_{12} shown by derivatives of the lining of the gastro-intestinal tract, which inhibits the characteristic stimulation of microbial growth exerted by the vitamin, should serve as a useful adjunct to the concentration and purification of intrinsic factor. Nevertheless, it seems probable that natural substances other than intrinsic factor may possess the property of combining with vitamin B_{12} in a similar manner. Thus

TABLE VII.—*Oral Administration of Vitamin B_{12} and Egg-white Lysozyme*

Case	Period	Daily Oral Therapy* B_{12} (μg.)		Days Observed	Erythrocytes (Millions per c.mm.) Onset	End	Maximal Reticulocyte Response %	Day
7	1†	10	100 mg. of egg-white lysozyme	13	2·32	2·74	6·1	10
	2	10	" "	14	2·74	2·61	3·0	8
	3	Complex formed by 10 μg. B_{12} + 200 mg. of egg-white lysozyme		11	2·61	2·71	—	—
	4	10	2·1 g. extract of hog duodenum	11	2·71	3·03	4·0	7
	5	10	150 ml. of gastric juice	11	3·03	3·68	—	—
	6	10 μg. of B_{12} intramuscularly		7	3·77	3·92	—	—
12	1	25		11	2·63	3·43	5·2	12
	2	Complex formed by 25 μg. of B_{12} + 500 mg. of egg-white lysozyme		16	3·43	3·27	—	—

* Unless otherwise specified.
† Vitamin B_{12} and egg-white lysozyme administered separately at an interval of twelve hours each day.

C. E. Meyer and his associates (1950) reported that lysozyme derived from egg white formed a complex with vitamin B_{12} which prevented stimulation of the growth of the test organism *Lactobacillus lactis* Dorner by the vitamin. Therapeutic trials on two patients with pernicious anaemia in relapse, employing daily oral doses of a complex of egg lysozyme and amounts of vitamin B_{12} up to 25 μg., have indicated that lysozyme has no significant potentiating effect on orally administered vitamin B_{12}. The results of these observations are shown in Table VII. Recently, two extracts of the gastric mucosa of swine, which showed a strong binding capacity for vitamin B_{12}, as determined by the Ternberg–Eakin method, were found to possess little or no intrinsic-factor activity when tested clinically on patients with pernicious anaemia in relapse (Table VIII).

TABLE VIII.—*Oral Administration of Vitamin B_{12} and Extracts of the Gastric Mucosa of Swine: Poor Haematopoietic Response from Extracts Showing a Strong Binding Capacity for the Vitamin*

Case	Period	Daily Oral Therapy* Time	Medication	Days Observed	Erythrocytes (Millions per c.mm.) Onset	End	Maximal Reticulocyte Response %	Day
9	1	10 a.m. 10 p.m.	10 μg. of B_{12} + 0·5 g. of gastric mucosa of swine	9	2·30	2·36	3·6	2
	2	10 p.m.	10 μg. of B_{12} + 0·5 g. of extract of gastric mucosa of swine	10	2·36	2·86	3·4	8
	3	10 p.m.	10 μg. of B_{12} + 1 g. of extract of gastric mucosa of swine	8	2·86	3·22	—	—
	4	10 p.m.	10 μg. of B_{12} + 150 ml. of pooled gastric juice of human beings	9	3·22	3·20	3·3	9
	5		10 μg. of B_{12} intramuscularly for 6 days	9	3·20	3·68	—	—
27	1	9 p.m.	5 μg. of B_{12}	9	2·40	2·55	2·7	5
	2	9 p.m.	5 μg. of B_{12} + 1·2 g. of extract of gastric mucosa of swine	11	2·55	3·00	2·0	4

* Medication administered orally unless otherwise specified.

Comment

Vitamin B_{12}, administered parenterally, is as effective as extracts of liver in the treatment of pernicious anaemia. However, when it is administered by mouth the presence of a potentiating agent is required for its effective utilization. In the absence of such an agent the haematopoietic responses to the oral administration of vitamin B_{12}, in doses up to 100 times the minimal effective parenteral dose, are variable and unpredictable.

Potentiation may be provided by known sources of intrinsic factor, such as the gastric juice of human beings and extracts of the stomach and intestines of swine. Extracts of stomach or duodenum of swine possess intrinsic-factor activity, the activity in duodenal mucosa of swine appearing to be greater than that contained in the entire wall of the stomach or duodenum. However, concentrated preparations that yield consistently effective and reliable therapeutic results when given in relatively small doses have not yet been produced. The barrier to the manufacture of dependable commercial products of this nature has been the destruction of a portion or all of the activity of the intrinsic factor during the process of concentrating gastric or duodenal substances. Until methods for preserving intrinsic-factor activity have been devised, patients with pernicious anaemia who are receiving preparations containing vitamin B_{12} by the oral route must be observed closely, and parenteral therapy substituted if the

haematopoietic and clinical response to oral therapy is not satisfactory.

It would appear that the solution to effective oral therapy is dependent on a clearer understanding of the factors governing absorption in the gastro-intestinal tract. The demonstration by Ternberg and Eakin that vitamin B_{12} may be "bound" by a substance or substances in normal gastric juice of human beings and in the lining of the gastro-intestinal tract of vertebrates, thereby rendering the vitamin no longer available for the growth of certain bacteria, is important, even though present evidence suggests that this method cannot be used solely as an *in vitro* test for intrinsic-factor activity.

The assumption seems warranted that the role of intrinsic factor must be concerned with absorption of vitamin B_{12} by (1) altering the chemical or physical nature of the compound so that it more readily traverses the intestinal barrier; (2) protecting the vitamin from destruction by secretions of the digestive tract; or (3) preventing the removal of the vitamin from the contents of the upper part of the gastro-intestinal tract by micro-organisms. Little direct evidence has been brought forward in support of any of these hypotheses.

With respect to the possible removal of vitamin B_{12} by bacteria within the gastro-intestinal tract of patients with pernicious anaemia it is pertinent to recall some conclusions to which Davidson (1928) was led by his studies on the gastro-intestinal flora in pernicious anaemia. In 1928 Davidson stated: "A great numerical increase of organisms, normal inhabitants of the bowel—e.g., *Bact. coli*, streptococci, and especially *Cl. welchii*—was found in the gastro-intestinal contents of cases of pernicious anaemia. . . . The great quantitative increase of bacteria, especially at levels of the small intestine which in normal persons are relatively bacteria-free, may be a factor of great aetiological importance." Dick (1941) reported his finding of the almost constant presence of bacteria of the colon and lactis-aerogenes group in the fasting gastric contents of patients with pernicious anaemia, and suggested that there was a profound derangement of the whole gastro-intestinal tract in persons with this disease, which permitted the return of colon bacilli from the colon to the stomach. Recently Davis and Mingoli (personal communication) have shown that *Bact. coli* have an avidity for vitamin B_{12}, and Lichtman, Ginsbert, and Watson (unpublished data) have reported that the daily oral administration of 3 g. of "aureomycin" and 3 μg. of vitamin B_{12} to patients having pernicious anaemia in relapse is followed by haematopoietic responses. Since aureomycin would be expected to reduce greatly the number of coliform organisms in the gastro-intestinal tract, vitamin B_{12} may thereby be made more available for absorption. Studies along this line would appear to be a promising field of investigation.

Summary and Conclusions

In pernicious anaemia, orally administered vitamin B_{12} requires for its effective utilization the presence of a potentiating agent (intrinsic factor of Castle). In the absence of this factor the haematopoietic responses obtained from the oral administration of the vitamin, in doses up to 100 times the minimal effective parenteral dose, are variable and unpredictable. Potentiation may be provided by known sources of intrinsic factor, such as gastric juice of normal or elevated acidity from human beings and extracts of the stomach and intestines of swine. Concentrated preparations that yield consistently effective and reliable therapeutic results, however, have not yet been produced, owing to the ready destructibility of intrinsic factor and the consequent loss of a portion or all of the intrinsic-factor activity during the process of concentrating gastric or duodenal substances. It is believed that the studies herein reported support the concept of the enzymic nature of intrinsic factor.

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The World Health Organization reports a striking reduction in the malaria rate among the rural population of the Mymensingh District of East Bengal (Pakistan), where a W.H.O. malaria control demonstration team for the past year has been carrying out residual spraying operations with D.D.T. Dr. G. Gramiccia (Italy), the leader of the team, states that not a single case of new infection has been detected in people living in the area which has been covered since this time last year in the spraying operations. There has been a reduction in the rate of enlarged spleen from 74.5% to 21.2% of the children of the area. Incidence of malaria parasites in the blood has been reduced from 22% in May, 1949, to 1.3% in May, 1950. Meanwhile, in the surrounding unsprayed areas the percentages of enlarged spleen and malaria parasites in the blood of children tested have been increasing. In 1949 protection against malaria was provided by this team's operations to about 35,000 people. More than 22,500 separate rooms were sprayed. Dr. Gramiccia added that these encouraging results have had a profound effect on the population, who had taken a rather diffident and sometimes openly hostile attitude at the first. Now they not only are appreciative but earnestly request the continuation of the spraying and the extension of the programme to neighbouring regions.