limit—and it may vary from case to case—then the figures marked (§) in the Table may be too low.

Discussion

The Table includes results obtained by other workers, whose names will not necessarily be repeated in the text. Daily doses of 5 μg. produced either no response or a small reticulocytosis without rise of red blood cells. In addition to the cases in the Table, Castle (private communication, 1950) has observed 10 others, in six of which there were detectable reticulocyte responses as follows:

Initial R.B.C. in mills./c.mm.	2·25	1·25	1·65	1·60	1·58	1·55
Reticulocytes %	4·3	3·1	14·1	4·0	2·7	11·3

Although not included in the Table, some cases from other sections are worth mentioning here. Negative or trivial responses were observed in two patients receiving daily doses of 10 μg. vitamin B_{12} with fresh milk and in three others given a single or split dose of 50 μg. with whey concentrate (Paper III). A single dose of 80 μg. was ineffective in Case 404 (Paper IV).

In reported cases single doses of 25, 30, 50, 100, 400, and 1,000 μg. gave either negative or trivial results.

In one patient 75 μg. gave a "suboptimal response" and another showed a "maximal reticulocyte response" to 600 μg., but the authors do not give details.

A response to two doses of 800 μg. as concentrate was about equal to the response expected from 20 μg. by injection. The patient, however, was relatively unresponsive to injected vitamin B_{12}—25 μg. gave slightly less than the response expected from 5 μg. Thus the true oral-dose/injection-dose ratio may have been 16 : 1 rather than 80 : 1.

Ten daily doses of 450 μg. were followed by an increase of red blood cells equivalent to that expected from 10 μg. by injection.

An injection of 75 μg. in this patient is said to have given a "similar" response, which if literally true would suggest a response equivalent to 10 μg. Such a degree of unresponsiveness or "resistance" (7.5:1) would reduce the oral-dose/injection-dose ratio from an apparent 450:1 to a possible 60:1, but of course a valid correction cannot be made without fuller information.

In Case 101, given 80 μg. daily, the corrected oral-dose/parenteral-dose ratio was several hundreds to one.

Up to this point one had supposed that the occasional and usually rather poor responses were due to interaction between orally administered vitamin B_{12} and intrinsic factor, traces of which may persist in the gastric juice even in pernicious anaemia (Goldhamer, 1936).

The remarkable responses to a single dose of 3,000 μg. in five recent cases cannot easily be explained on this basis. The patients appear to have effectively absorbed amounts ranging from 80 to 160 μg. or possibly more. From results reported in Paper II it seems that at least 500 ml. of *normal* gastric juice is needed to ensure an adequate haemopoietic response from even 50 to 80 μg. of orally administered vitamin B_{12}. The equivalent volume in terms of pernicious anaemia gastric juice, even supposing traces of intrinsic factor to persist, would be enormous and far beyond the secretory capacity of the atrophic stomach. It would be surprising indeed if a patient with pernicious anaemia secreted enough intrinsic factor to combine with more than one or two micrograms, however large the dose.

These findings suggest that some vitamin B_{12} *can* be absorbed—not efficiently perhaps, but in quite considerable amounts if the dose is large enough—without first combining with the intrinsic factor.

Further speculation seems unjustified, since we do not yet know whether Castle's intrinsic factor combines stochiometrically with vitamin B_{12} or whether it acts as a catalyst. It will be interesting to determine whether a single large dose of vitamin B_{12} administered orally is more or less effective than the same amount of material given in divided doses.

Summary and Conclusions

The absorption of vitamin B_{12} was studied by comparing the effective oral dose with the parenteral dose expected to produce a similar increase of red blood cells in 15 days. The apparent oral-dose/parenteral-dose ratio was corrected if the observed response to injected material was below expectation, indicating a relative resistance to vitamin B_{12}.

In one case daily doses of 5 μg. gave no response. In another, even 80 μg. a day for 24 days produced an increase in red blood cells no greater than would have been expected in 15 days from the injection of 2.5 μg. The response to injected material was normal, so that the corrected oral-dose/parenteral-dose ratio was several hundreds to one. By contrast the oral-dose/parenteral-dose ratio in five cases given a single dose of 3,000 μg. was from 20 to 40 : 1, the increase of red blood cells in 15 days being so great as to suggest the absorption of 80 to 160 μg. or even more. Presumably very little of the vitamin absorbed can have interacted with Castle's intrinsic factor, considering the low quality and quantity of the secretion of the stomach in pernicious anaemia.

Further tests are necessary to determine whether in fact more vitamin B_{12} is absorbed after a single large dose than after the same quantity of material given in divided doses daily.

II. ORAL ADMINISTRATION WITH NORMAL GASTRIC JUICE

BY

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In the preceding paper it was shown that vitamin B_{12} administered orally without a source of intrinsic factor was not efficiently absorbed, although remarkable effects could be obtained with an enormous single dose. The present study attempts to answer two questions: How effectively is vitamin B_{12} absorbed when given with gastric juice? How much gastric juice is needed per microgram of vitamin B_{12}?

Methods

A general account of methods is given in Paper I. Normal human gastric juice was obtained almost entirely from students in response to two injections of 0.5 mg. of histamine at intervals of 30 minutes. Specimens without free HCl and those containing more than a trace of bile were discarded. After filtering through nainsook to remove mucus and debris, the juice was pooled and kept at approximately 5° C. The period of storage did not exceed three days, except in Case 404 (Paper IV), in which the juice had been stored 14 days.

The highly acid juice (*p*H 1 approx.) was neutralized to *p*H 7 with a predetermined quantity of N/1 NaOH. Vitamin B_{12} was then added. In Cases 201, 202, 401, and 402 the mixture was given within two hours, while in Cases 303, 304, 305, and 404 the interval was prolonged to six hours. The mixture was incubated for one hour in Case 304, but not in others. Food was withheld for four hours before and after the mixture. In Cases 303, 304, and 305 the intervals were prolonged to eight hours.

The gastric juice used for the second period of Case 201 was passed through a Seitz filter while still acid. As the Seitz-filtered juice was inactive this procedure was not used again.

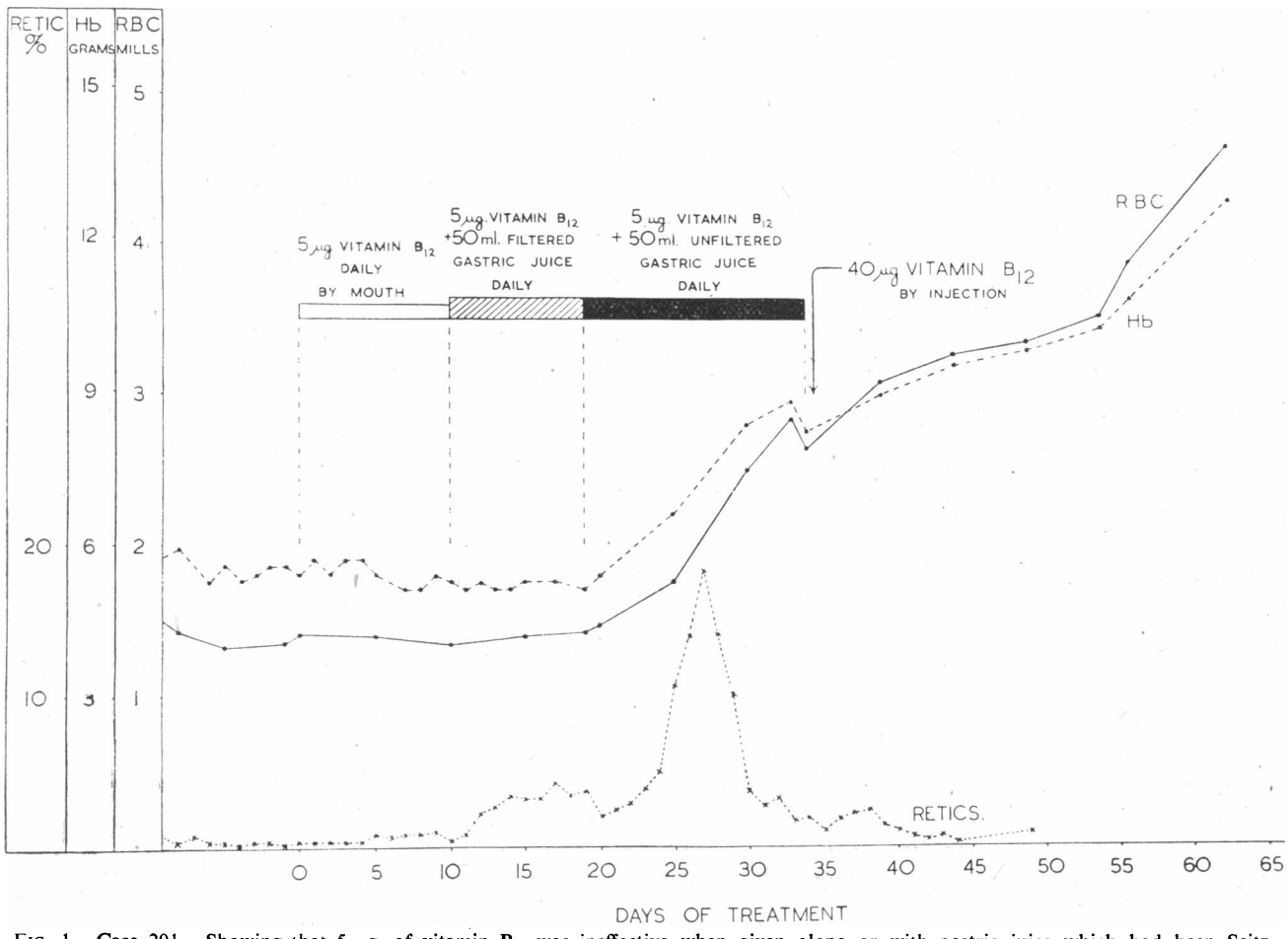

FIG. 1.—Case 201. Showing that 5 μg. of vitamin B_{12} was ineffective when given alone or with gastric juice which had been Seitz-filtered, but produced a good response when given with unfiltered gastric juice.

Results

Eight patients were treated with vitamin B_{12} and normal human gastric juice.

Case 201

A miner aged 58 had pernicious anaemia without neurological involvement. Having recently lost his teeth, he ate little meat, but otherwise his diet was satisfactory.

After 10 days' control period, during which reticulocytes remained less than 1%, the red blood cells numbered 1,400,000 per c.mm., with haemoglobin 5.3 g. per 100 ml., reticulocytes 0.4%, M.C.V. 130.6 μ^3, M.C.H. 38.2 $\mu\mu$g., M.C.H.C. 31.3%, white blood cells 1,700 per c.mm. The marrow was megaloblastic.

In the first period 5 μg. of vitamin B_{12} was given daily by mouth. There was neither reticulocytosis nor increase of red blood cells (Fig. 1).

	←—First Period—→		←—Second Period—→		
Days:	0	5	10	15	19
R.B.C. (mills./c.mm.)	1·40	1·38	1·33	1·39	1·41
Hb (g./100 ml.)	5·3	5·3	5·2	5·2	5·0
P.C.V. (%)	17·5	18·6	17·0	—	—

In the second period the same amount of vitamin B_{12} was mixed with 50 ml. of normal gastric juice neutralized just before administration. Before neutralization the gastric juice had been passed through a Seitz filter. Reticulocytes rose to only 4.2% on the seventh day and red cells did not increase.

In the third period the same amounts of vitamin B_{12} and gastric juice were administered, but this time the gastric juice was *not* Seitz-filtered. Reticulocytes rose on the fourth day and reached 18.2% on the eighth day. There was subjective improvement and red cells increased.

	←——Third Period——→			←——Fourth Period——→		
Days:	0	6	11	15	20	25	30
R.B.C. (mills./c.mm.)	1·41	1·75	2·48	2·63	3·09	3·28	3·36
Hb (g./100 ml.) ..	5·0	6·5	8·3	8·1	8·9	9·5	9·8
P.C.V. (%)	—	21·0	27·0	28·0	33·0	33·0	35·0

In the fourth period an injection of 40 μg. of vitamin B_{12} led to a negligible reticulocytosis of 2.4% on the fourth day. Red blood cells continued to increase.

Comment.—The daily oral administration of 5 μg. of vitamin B_{12} alone or with Seitz-filtered gastric juice was ineffective. The same quantity with unfiltered gastric juice was effective. The increase of erythrocytes in 15 days after giving a total of 75 μg. with unfiltered gastric juice was equivalent to the response expected from an injection of 10 μg. In fact, however, the patient was relatively "resistant" to injected material in that the increase of red blood cells after 40 μg. was slightly less than that expected from 5 μg. Even then some of the increase may have been due to previous therapy. Correction for this resistance reduces the oral-dose/parenteral-dose ratio from an apparent 7.5 : 1 to the region of unity.

Case 202.—Administration of 50 μg. Vitamin B_{12} with 500 ml. of Gastric Juice by Stomach Tube

A woman aged 63 had pernicious anaemia with sore smooth tongue and minor neurological involvement. The dietary history was normal.

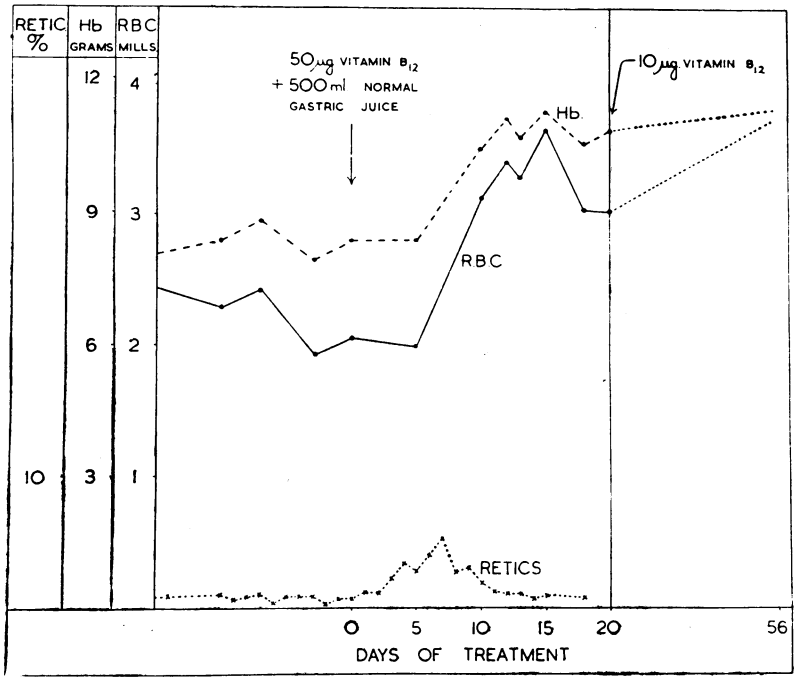

FIG. 2.—Case 202. Response to single dose of 50 μg. of vitamin B_{12} with 500 ml. of normal gastric juice given by stomach tube.

After a satisfactory control period of several weeks the red blood cells numbered 2,050,000 per c.mm., with haemoglobin 8.3 g. per 100 ml., M.C.H., 40.4 μμg., M.C.V. 127 μ^3, M.C.H.C. 31.9%, reticulocytes 0.6%, and white blood cells 4,400 per c.mm.

A single dose of 500 ml. of pooled normal gastric juice was neutralized and mixed with 50 μg. of vitamin B_{12} just before administration by stomach tube. Reticulocytes began to rise on the fourth day, reaching 5.2% on the seventh day (Fig. 2).

Red blood cells increased rapidly, sore tongue was relieved, appetite and well-being were restored. These effects lasted fully 15 days, but by the twentieth day the tongue was again sore and the counts were beginning to fall. Injections of vitamin B_{12} subsequently brought about complete remission.

Days:	0	5	10	15	20
R.B.C. (mills./c.mm.)	2·05	1·99	3·13	3·66	3·02
Hb (g./100 ml.)	8·3	8·3	10·4	11·3	10·8
P.C.V. (%)	26·0	27·0	35·0	37·0	31·5

Comment.—The increase of red blood cells in 15 days slightly exceeded the response expected from an injection of 40 μg. Because of this excellent result there can be no doubt about the patient's responsiveness to vitamin B_{12}, even though the actual effect of injection was not measured. Thus the apparent oral-dose/parenteral-dose ratio of near unity needs no correction for " resistance " to vitamin B_{12}.

Remaining Six Cases

These six cases are described in Papers III and IV. Case 401 was given 5 μg. daily with 50 ml. of gastric juice. Case 402 received 40 μg. with 150 ml. of gastric juice. Case 404 received 80 μg. with 500 ml. Cases 303, 304, and 305 received 50 μg. and 500 ml. of gastric juice after a preliminary trial of milk or whey as a source of intrinsic factor.

The findings in the eight patients are summarized in the accompanying Table along with other published cases.

Discussion

Differences in dietary control and in the source, filtration, storage, and time of administration of the gastric juice may account for some of the variation in the results observed (see Hall, Morgan, and Campbell, 1949).

In the first period of treatment in three of the cases described by Berk, Castle, Welch, Heinle, Anker, and Epstein (1948), vitamin B_{12} and gastric juice were given twelve hours apart and there was no haemopoietic effect.

Vitamin B_{12} given two hours before the gastric juice was effective in one case (Hall, Morgan, and Campbell, 1949). In all other cases cited in the Table the gastric juice and vitamin B_{12} were given simultaneously.

In the cases of Berk *et al.* and in mine, the diet was restricted and no food was given four hours before or after the administration of gastric juice mixtures. In the cases of Hall *et al.* the mixtures were given only one hour before the morning meal and the patients were allowed meat and eggs later in the day. Thus the effects attributed to gastric juice and vitamin B_{12} may have been partly due to the interaction of gastric juice with other extrinsic factor(s) in food.

Author	Case	Haemopoietic Response				Oral Therapy		Apparent Ratio	Correction Based on Responsiveness to Injected B_{12}	Corrected Ratio	Presumed Absorption (approx.)	
		B_0	Retic. Peak	I_{10}	I_{15}	Gastric Juice Given (ml.)	B_{12} (μg.)	Total Oral Dose Given (μg.) : Parenteral Dose Expected to Give Similar Response (μg.)	Dose Injected (μg.) : Dose Expected to Give Similar Response (μg.)	Oral Dose : Parenteral Dose (approximate)	Per Dose (μg.)	%
Berk *et al.*	101	1·82	9·3	0·90	—	150×10	5×10	50 : 10	No record	—	1†/5	20†
,,	102	1·65	16·7	0·80	—	150×10	5×10	50 : 5	,, ,,	—	0·5†/5	10†
,,	103	1·90	3·8	0·64	—	150×10	5×10	50 : 5	,, ,,	—	0·5†/5	10†
,,	104	2·14	3·8	0·04	—	125×10	5×10	50 : Possibly 1·25	5×10 : Less than 5 =50	Doubtful	Doubtful	Doubtful
Hall *et al.*	Fig. 2	1·50	31·7	—	0·86*	150×15	5×15	75 : 5	No record	—	0·3†/5	6†
,,	Fig. 4	1·63	16·5	—	1·37*	150×15	5×15	75 : 20	,,	—	1·2†/5	24†
Ungley	401	1·27	13·4	1·16	—	50×10	5×10	50 : 20	5×10 : 20 =50	1 : 1	5/5	100
,,	201	1·41	18·2	—	1·22	50×15	5×15	75 : 10	40 : 5	About 1 : 1	5/5	100
,,	202	2·05	5·2	—	1·61	500	50	50 : More than 40	R.B.C. level too high	—	Over 40†/50	Over 80†
,,	303	2·55	1·8	—	−0·11	500	50	50 : Nil	50 : More than 80	Oral therapy failed	0/50	Nil
,,	304	2·25	7·8	—	0·59	500	50	50 : Less than 5	50 : Less than 5	About 1 or 2 : 1	About 25 or 50/50	50 to 100
,,	305	1·88	7·4	—	0·95	500	50	50 : 5	50 : More than 40	10 : 1	5/50	10
,,	404	1·34	15·8	—	0·82	500	80	80 : Less than 5	80 : 40	More than 8 : 1	Less than 10/80	Less than 15
,,	402	1·68	7·0	—	−0·03	150	40	40 : Possibly 1·25	40 : 40	Possibly 30 : 1 (Insufficient gastric juice)	Possibly 1·25/40	Possibly 3

* From graph. † Ratios and absorption figures marked † are uncorrected, because the actual response to parenteral therapy was not observed.

Except in one patient (Case 404) who received juice stored for 14 days, the juice was either fresh or not more than three days old. Other workers have used pooled gastric juice which may have been stored. Campbell, Hall, and Morgan (1949) state that the intrinsic factor in gastric juice is not destroyed by storage at 5° C. for three months, but did not indicate whether there is a lessening of activity.

Hall *et al.* (1949) found that Berkefeld-filtered gastric juice was active in a proportion of 150 ml. per 5 μg. of vitamin B_{12}, but comparisons with unfiltered gastric juice are not mentioned. In our Case 201 Seitz filtration of gastric juice apparently led to loss of all or most of its intrinsic factor activity.

How Efficiently is Vitamin B_{12} Absorbed When Given Orally With Gastric Juice?

It is obvious from the Table that the results are very variable. In four out of eight of my patients the effect of vitamin B_{12} given orally with gastric juice was about as effective as if the same dose of vitamin had been injected into these patients. In two others the corrected oral-dose/parenteral-dose ratio was about 10 : 1. In one case oral therapy failed completely, and in another the amount of gastric juice was too small.

Calculations similar to those used for my cases can be applied to the first three cases reported by Berk *et al.* (1948). These patients received 5 μg. of vitamin B_{12} with 150 ml. of normal gastric juice daily for 10 days. The increase in red blood cells in 10 days was approximately equivalent to that which would have been expected from single injections of 10, 5, and 5 μg. In other words, the amounts of vitamin B_{12} given orally with gastric juice were approximately 5, 10, and 10 times as great as would probably have been needed to produce a similar response with injected material. This is assuming that the patients of Berk *et al.* would have been normally responsive to injected vitamin B_{12}. Their fourth case is excluded from discussion because of the poor response not only to orally but to parenterally administered material.

In one of Hall, Morgan, and Campbell's cases the increase of red blood cells in 15 days after a total of 75 μg. with gastric juice was less than would be expected from 5 μg. by injection. In another case the response to a total of 75 μg. with gastric juice was slightly less than would have been expected from 20 μg. by injection. The apparent oral-dose/injection-dose ratios were approximately 15 and 3.75, but the actual responsiveness of these patients to injected material was not determined.

Some of the factors which may have contributed to the difference in the results obtained by the three observers have already been discussed.

How Much Juice is Needed?

This can roughly be estimated from (*a*) the total amount of gastric juice given, and (*b*) the amount of vitamin B_{12} absorbed (judging this by the haemopoietic response and correcting for "resistance," if any).

In the cases of Berk *et al.* (1948) and Hall *et al.* (1949) a correction for responsiveness to injected vitamin B_{12} was possible in only one instance. So far as one can judge from the uncorrected oral-dose/parenteral-dose ratio each 100 ml. of gastric juice seems to have been enough for the absorption of only 1 μg. or less. In all their patients and in the first two of mine the material was given daily and not as a single dose.

In Case 401 a total of 50 μg. was adequately absorbed when administered with a total of 500 ml. of gastric juice. In other words, each 100 ml. was enough for the absorption of 10 μg. Similar calculations for all my cases in the Table suggest that 100 ml. of gastric juice was enough for the absorption of the following amounts of vitamin B_{12} (μg.): 10, 10, 10, *nil*, 5 or 10, 1, less than 2, less than 1.

It is interesting to compare these figures with those of Ternberg and Eakin (1949) for the vitamin B_{12}-binding power of samples of normal gastric juice, which were: 2, 6 (pooled sample from at least three subjects), 6, and 1.5 μg. per 100 ml. of gastric juice.

It will be even more interesting, in the future, to measure microbiologically in this way the activity of samples of gastric juice which are to be clinically tested for their power to promote absorption of vitamin B_{12} in pernicious anaemia.

Summary and Conclusions

The effect of vitamin B_{12} given with normal gastric juice was observed in eight patients with pernicious anaemia. The total amounts were usually 50 μg. + 500 ml. given as a single dose or in divided doses.

One patient failed to respond to oral therapy. In one who received 40 μg. with only 150 ml. of gastric juice the response was very poor. In two the oral-dose/parenteral-dose ratio was about 10:1, suggesting that about 10% of the vitamin had been absorbed. In the remaining four the response was about as good as would have been expected if the same dose of vitamin B_{12} had been injected. Thus small doses of vitamin B_{12} given with normal gastric juice are *sometimes* efficiently absorbed.

In these eight experiments each 100 ml. of gastric juice seemed to be enough for the absorption of 0, less than 1, 1, less than 2, 5 or 10, 10, 10, and 10 μg. of vitamin B_{12}. Thus the amount of gastric juice necessary to promote the absorption of a given amount of B_{12} varied considerably. These variations are perhaps only partly due to differences in the amount of intrinsic factor in the samples of gastric juice; differences in the recipients may have been equally important.

III. FAILURE OF FRESH MILK OR CONCENTRATED WHEY TO FUNCTION AS CASTLE'S INTRINSIC FACTOR OR TO POTENTIATE THE ACTION OF ORALLY ADMINISTERED VITAMIN B_{12}

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In another paper we have confirmed the findings of Ternberg and Eakin (1949) that normal gastric juice combines with vitamin B_{12} and renders it unavailable to certain bacteria (Ungley and Cuthbertson, 1950: Paper V of this series, to be published).

The possibility that milk might have similar properties has been investigated. For this purpose microbiological tests proved useless, milk being an unsatisfactory material for the microbiological assay of vitamin B_{12}. Nevertheless, exploratory clinical tests have been made to determine whether milk would be effective as a source of Castle's intrinsic factor.

Methods

A general account of methods is given in Paper I.

For the first two tests fresh cow's milk was cooled quickly immediately after milking and kept in a vacuum flask for not more than four hours before administration.