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}

BY

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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.

For the first whey concentrate 7.5 litres of skimmed milk was treated at 37° C. with 455 ml. of diluted acetic acid to *p*H 4.5. The casein was filtered off to yield 6,800 ml. of crude whey. The whey was concentrated at *p*H 6.5 to 790 ml. and dialysed against distilled water overnight at 4° C. to eliminate salts and some of the lactose. During dialysis a precipitate composed mainly of lactose and lactoglobulin came down. This precipitate was combined with the concentrated supernatant fluid to make a total volume of approximately 650 ml., representing 7 litres of milk.

The second whey concentrate was prepared from 22 litres of milk. The casein was removed and the whey evaporated to small bulk at low temperature. This concentrated whey was then dialysed to remove most of the lactose.

Results

Two patients received fresh milk and three cases were given whey concentrates, in each case with added vitamin B_{12}.

Case 301

A woman aged 58 had pernicious anaemia with minor neurological involvement. The dietary history was normal. In a control period of 14 days, during which reticulocytes did not exceed 3.4%, the red blood cells fell from 1,610,000 to 1,070,000 per c.mm. After transfusion the count rose to 2,300,000 per c.mm. (Fig. 1). After a further control period of 13 days during which reticulocytes did not exceed 1.6%, the red blood cells numbered 1,480,000 per c.mm., haemoglobin 5.6 g. per 100 ml., reticulocytes 1%, M.C.V. 131.7 μ^3, M.C.H. 37.9 $\mu\mu$g., M.C.H.C. 28.2%, white blood cells 2,800 per c.mm. The marrow was megaloblastic.

In the first period she was given daily 500 ml. of preserved fresh milk to which 10 μg. of vitamin B_{12} was added before administration. There was a slight reticulocytosis, reaching 3% on the eighth and ninth days with little change in the anaemia.

In the second period she received daily 500 ml. of preserved fresh milk to which 100 μg. of folic acid was added before administration. There was no second reticulocyte response, but the anaemia improved.

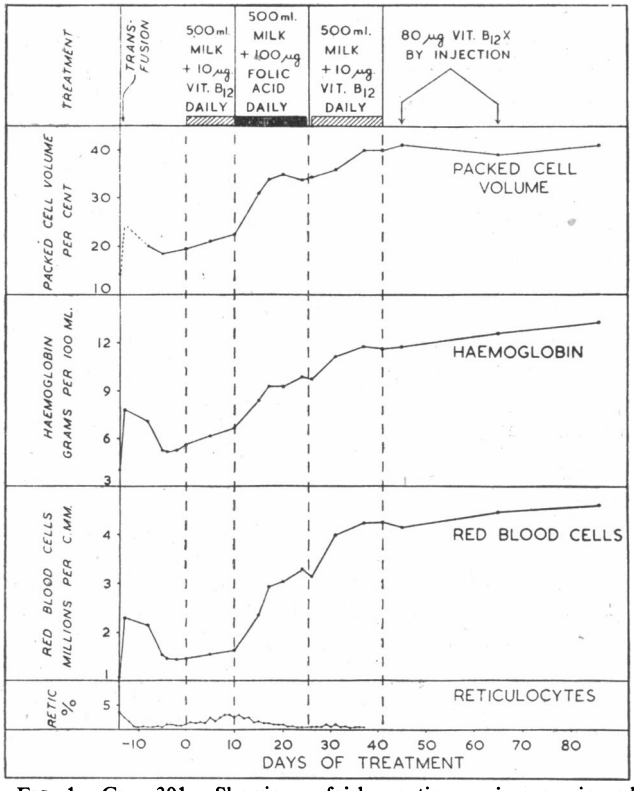

FIG. 1.—Case 301. Showing a fairly continuous increase in red blood cells during treatment, with only a slight reticulocytosis. This may have been a spontaneous remission.

	←First Period→		←Second Period→			
Days :	0	5	10	15	20	35
R.B.C. (mills./c.mm.)	1·48	1·54	1·62	2·39	3·03	3·23
Hb (g./100 ml.) ..	5·6	6·2	6·7	8·4	9·3	9·9
P.C.V. (%)	19·5	21·0	22·5	31·0	35·0	34·5

In the third period treatment reverted to the daily administration of 500 ml. of preserved fresh milk plus 10 μg. of vitamin B_{12}. The anaemia continued to improve.

	Third Period			
Days :	0	5	10	15
R.B.C. (mills./c.mm.)	3·16	4·00	4·24	4·26
Hb (g./100 ml.) ..	9·8	11·1	11·8	11·8
P.C.V. (%)	34·5	36·0	40·0	40·0

Comment.—The slight reticulocytosis in the first period might equally well have occurred from the daily oral administration of 10 μg. of vitamin B_{12} alone, without the milk. In the absence of any further reticulocyte response the improvement in the second and third periods cannot safely be attributed to treatment. Spontaneous remission is a distinct probability.

Case 302

A retired labourer aged 65 had pernicious anaemia without neurological involvement. The dietary history was normal.

After a control period of five days, during which the reticulocytes did not exceed 1%, the red blood cells numbered 2,010,000 per c.mm., haemoglobin 7.8 g. per 100 ml., reticulocytes 0.6%, M.C.V. 111.9 μ^3, M.C.H. 39.1 $\mu\mu$g., M.C.H.C. 34.7%, white blood cells 5,100 per c.mm. The marrow was megaloblastic.

In the first period he was given daily for 15 days 500 ml. of preserved fresh milk to which 10 μg. of vitamin B_{12} was added before administration. There was a slight reticulocytosis reaching 4.6% on the seventh day, but no increase of red blood cells (Fig. 2).

	First Period			
Days :	0	5	10	15
R.B.C. (mills./c.mm.)	2·01	1·86	1·78	1·93
Hb (g./100 ml.) ..	7·8	7·0	6·5	7·0
P.C.V. (%)	22·5	22·0	22·5	22·5

In the second period 1 μg. of vitamin B_{12} daily was given parenterally for 15 days. There was a second reticulocytosis of 4.6% on the eighth day, followed by subjective improvement and some increase in red blood cells.

	←Second Period→			←Third Period→			
Days :	0	5	10	15	20	25	30
R.B.C. (mills./c.mm.)	1·93	2·18	2·31	2·33	2·87	3·28	3·36
Hb (g./100 ml.) ..	7·0	8·0	8·9	9·3	10·7	11·3	11·7
P.C.V. (%)	22·5	24·0	28·0	29·0	33·0	34·0	36·0

In the third period 0.8 μg. of vitamin B_{12} plus 100 μg. of folic acid daily was given for 15 days. There was no reticulocytosis. The red blood cells continued to increase.

Comment.—In this case fresh milk was clearly not a satisfactory source of Castle's intrinsic factor. The reticulocyte response of 4.6% was no greater than might have been expected from the vitamin B_{12} alone, and there was no increase of red blood cells in 15 days.

The remaining cases were given whey concentrate instead of fresh milk.

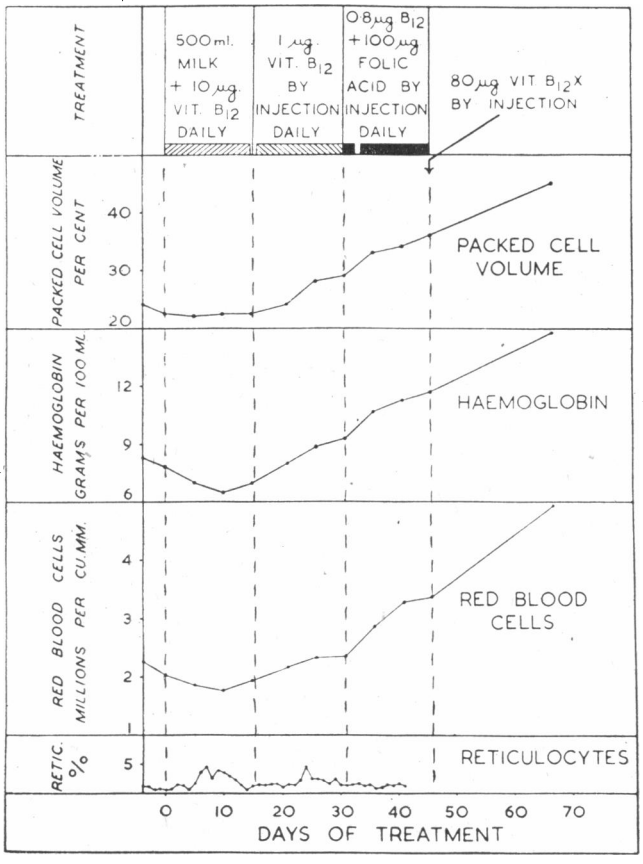

FIG. 2.—Case 302. The administration of milk and vitamin B_{12} produced only a small reticulocytosis without rise of red blood cells. Following the daily injection of 1 μg. vitamin B_{12} there was a secondary reticulocytosis, but the increase of red blood cells was below expectation. There was no further reticulocytosis when part of the vitamin B_{12} was replaced by folic acid.

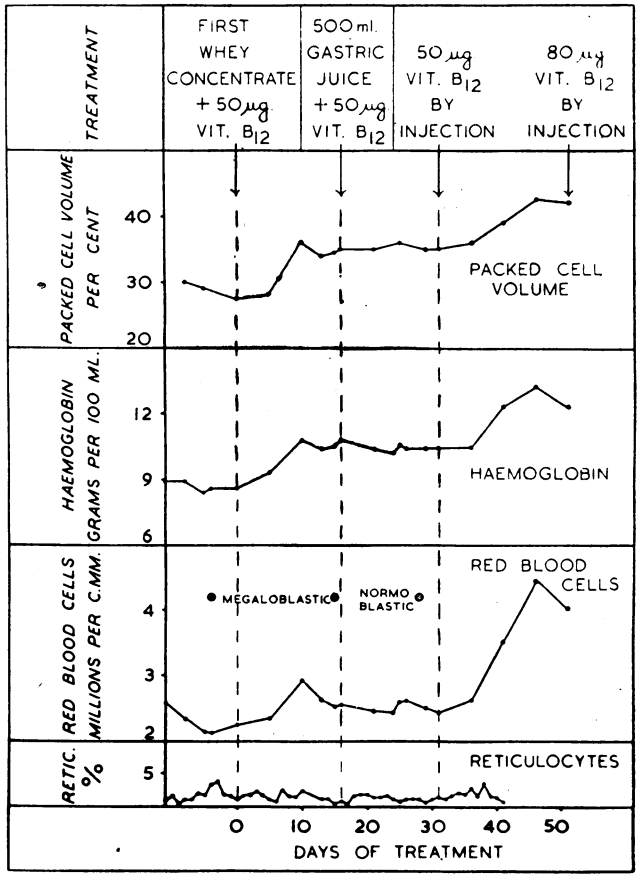

FIG. 3.—Case 303. In the control period there was a small spontaneous reticulocytosis without a rise of red blood cells. Following the administration of the whey concentrate with vitamin B_{12} there was a transient increase of red blood cells but no reticulocytosis, and the marrow remained megaloblastic. Vitamin B_{12} with gastric juice had no effect on reticulocytes or red blood cells, but the marrow became normoblastic. The response to an injection of 50 μg. of vitamin B_{12} was better than expectation.

Case 303

A housewife aged 73 had pernicious anaemia without neurological involvement. She ate little meat or cheese, and no eggs or tomatoes.

After a control period of 12 days, during which the reticulocytes did not exceed 3.8%, the red blood cells numbered 2,270,000 per c.mm., haemoglobin 8.6 g. per 100 ml., reticulocytes 1%, M.C.V., 121.1 μ^3, M.C.H. 37.9 μμg., M.C.H.C. 31.2%, white blood cells 5,100 per c.mm. The marrow was megaloblastic.

In the first period she received a whey concentrate derived from 7 litres of milk to which was added 50 μg. of vitamin B_{12}. The mixture stood at room temperature for seven hours and was then given by stomach tube approximately eight hours after the last meal and eight hours before breakfast. There was a slight variable reticulocytosis of 2.6% on the seventh day and some improvement in the anaemia (Fig. 3). The marrow remained megaloblastic.

	First Period				Second Period			
Days :	0	5	10	15	16	21	26	31
R.B.C. (mills./c.mm.)	2·27	2·36	2·92	2·51	2·55	2·45	2·61	2·44
Hb (g./100 ml.)	8·6	9·3	10·8	10·5	10·8	10·4	10·4	10·4
P.C.V. (%)	27·5	28·0	36·0	34·5	35·0	35·0	36·0	35·0

In the second period she received 500 ml. of normal gastric juice with 50 μg. of vitamin B_{12}; the mixture was left at room temperature for seven and a half hours and given by stomach tube in the same manner as before. There was no reticulocytosis and no rise of red blood cells in 15 days; after 12 days the marrow picture was predominantly normoblastic and macronormoblastic.

In the third period 50 μg. of vitamin B_{12} was given parenterally. A slight reticulocytosis of 3.4% on the seventh day was followed by a good increase in red blood cells.

	Third Period			
Days :	0	5	10	15
R.B.C. (mills./c.mm.)	2·44	2·61	3·50	4·41
Hb (g./100 ml.)	10·4	10·4	12·3	13·2
P.C.V. (%)	35·0	36·0	39·0	42·5

Comment.—The result in the first period, when whey concentrate was given as a possible source of Castle's intrinsic factor, was probably negative, the response being no greater than might be expected from the vitamin B_{12} alone, but for some reason the patient did not respond to B_{12} and gastric juice. She was not "resistant" to vitamin B_{12}, for the increase in red blood cells after an injection of 50 μg. was greater than would be expected from an injection of 80 μg.

Case 304

A housewife aged 67 had pernicious anaemia with minor neurological involvement. She ate very little meat or vegetables.

After a control period of 11 days, during which the reticulocytes did not exceed 1.2%, the red blood cells numbered 2,460,000 per c.mm., haemoglobin 8·9 g. per 100 ml., reticulocytes 1%, M.C.V. 118 μ^3, M.C.H. 36.1 μμg., M.C.H.C. 30.6%,

white blood cells 3,700 per c.mm. Sternal marrow showed megaloblastic erythropoiesis.

In the first period she received the second whey concentrate derived from 3 litres of milk which was incubated for one hour with 50 μg. of vitamin B_{12} and given by stomach tube approximately eight hours after the last meal and eight hours before breakfast. In 10 days there was neither reticulocytosis nor rise of red blood cells (Fig. 4).

	First Period		
Days :	0	5	10
R.B.C. (mills./c.mm.)	2·46	2·34	2·21
Hb (g./100 ml.)	8·9	9·3	8·9
P.C.V. (%)	29·0	28·0	27·0

In the second period she was given 500 ml. of normal gastric juice which was neutralized, incubated for one hour with 50 μg. of vitamin B_{12}, kept at 5° C. for seven and a half hours, and given by stomach tube in the same manner as before. Reticulocytes rose to 7.8% on the sixth day and the red blood cells increased.

	←Second Period→			←Third Period→			
Days :	0	5	10	15	20	25	30
R.B.C. (mills./c.mm.)	2·25	2·34	2·73	2·84	3·05	3·79	3·47
Hb (g./100 ml.)	8·7	8·9	10·4	9·9	10·8	12·1	11·1
P.C.V. (%)	27·0	27·0	33·0	29·0	32·0	36·0	36·0

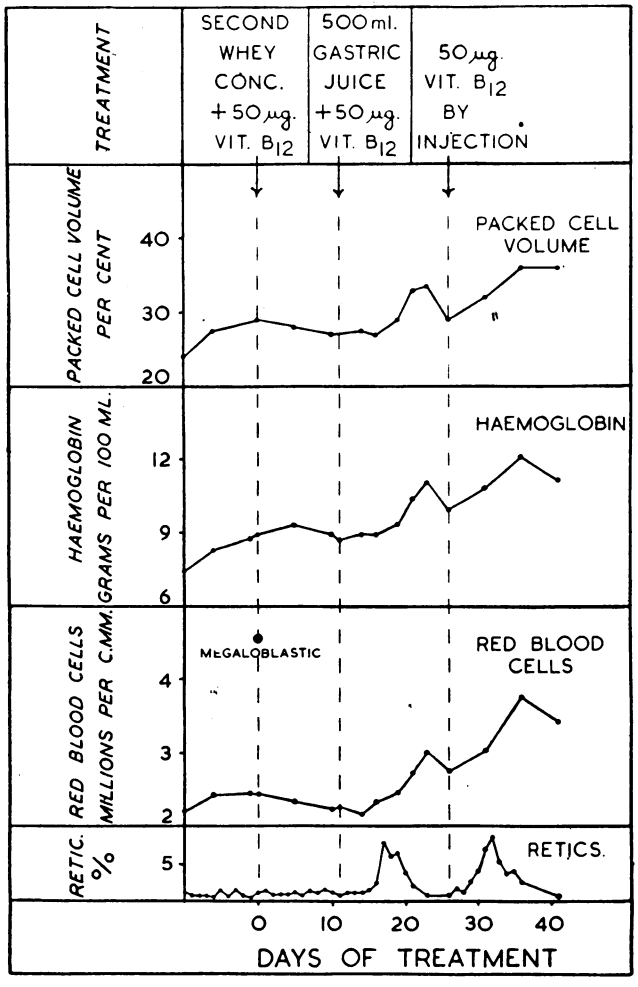

FIG. 4.—Case 304. There was no response to the administration of a whey concentrate and vitamin B_{12}. The response to 50 μg. of vitamin B_{12} with gastric juice was less than that which would be expected from an injection of 5 μg. The response to 50 μg. of vitamin B_{12} by injection was below expectation.

In the third period 50 μg. of vitamin B_{12} was administered parenterally. There was a second reticulocytosis of 8.6% on the sixth day and the red blood cells increased.

Comment.—The result in the first period when whey concentrate was given was completely negative. The rise of red blood cells after giving 50 μg. of vitamin B_{12} and 500 ml. of gastric juice was less than that expected from an injection of 5 μg. The response from the injected dose of 50 μg. was also slightly less than that expected from an injection of 5 μg. Thus the oral-dose/parenteral-dose ratio was probably 1 or 2 : 1.

Case 305

A woman aged 60 had pernicious anaemia with minor neurological involvement. She ate little meat, eggs, fish, cheese, or fruit, but plenty of milk, cereals, and vegetables.

After a control period of nine days, during which the reticulocytes did not exceed 1.6%, the red blood cells numbered 1,810,000 per c.mm., haemoglobin 6.5 g. per 100 ml., reticulocytes 0.6%, M.C.V. 116 μ^3, M.C.H. 32.8 $\mu\mu$g., M.C.H.C. 31%, white blood cells 4,000 per c.mm. The marrow was megaloblastic.

In the first period she was given on two successive nights 575 ml. of the second whey concentrate derived from 3 litres of milk which was incubated for one hour with 25 μg. of vitamin B_{12} and given by stomach tube approximately eight hours after the last meal and eight hours before breakfast. There was a slight reticulocytosis of 4.6% on the sixth day (Fig. 5). The red blood cells had increased at the tenth day,

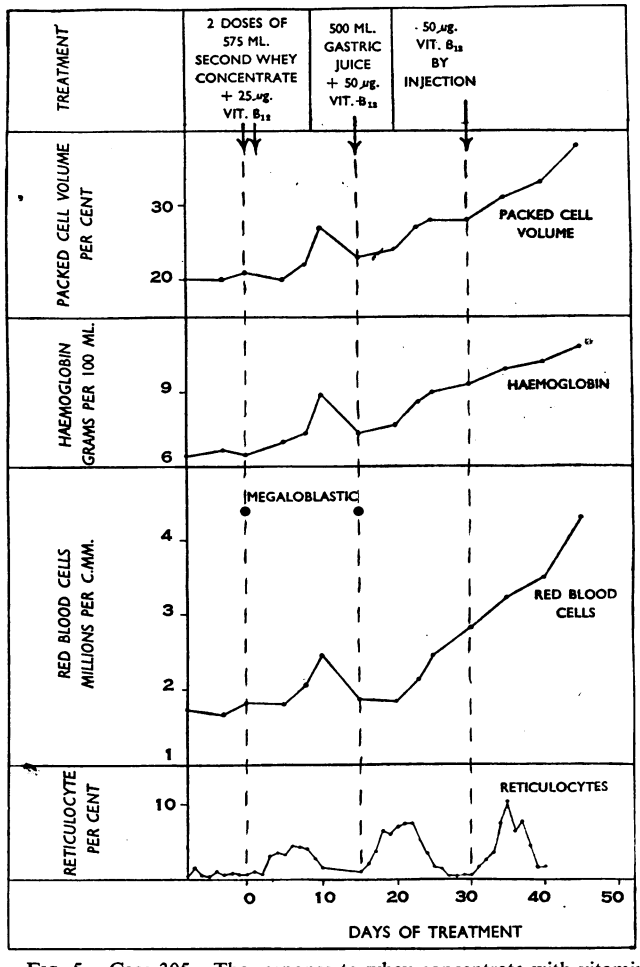

FIG. 5.—Case 305. The response to whey concentrate with vitamin B_{12} was poor and no greater than might have been obtained by giving the vitamin B_{12} by mouth alone. The response to 50 μg. of vitamin B_{12} given with gastric juice was equivalent to that which would have been expected from an injection of 5 μg. The response to an injection of 50 μg. of vitamin B_{12} was up to expectation.

but by the fifteenth were back to their former level. The marrow was still megaloblastic.

	First Period			
Days :	0	5	10	15
R.B.C. (mills./c.mm.)	1·81	1·80	2·46	1·88
Hb (g./100 ml.)	6·5	7·0	8·9	7·4
P.C.V. (%)	21·0	20·0	27·0	23·0

In the second period she received a single dose of 500 ml. of normal gastric juice which was neutralized, incubated for one hour with 50 μg. of vitamin B_{12}, kept at 5° C. for seven hours, and given by stomach tube eight hours after the last meal and eight hours before breakfast. Reticulocytes rose to a second peak of 7.4% on the sixth and seventh days, and this time the improvement in the anaemia was sustained.

	←Second Period→			←Third Period→			
Days :	0	5	10	15	20	25	30
R.B.C. (mills./c.mm.)	1·88	1·86	2·46	2·83	3·21	3·50	4·32
Hb (g./100 ml.)	7·4	7·7	9·0	9·3	9·9	10·2	10·8
P.C.V. (%)	23·0	24·0	28·0	28·0	31·0	33·0	38·0

In the third period 50 μg. of vitamin B_{12} was given parenterally. Reticulocytes rose to 10.2% on the fifth day. Improvement in the anaemia was accelerated.

Comment.—The result with the whey concentrate plus vitamin B_{12} was regarded as negative as there was only a slight reticulocytosis of 4.6% with no rise of red blood cells in 15 days—that is, a very slight response which might well have occurred from the oral administration of similar doses of vitamin B_{12} without the whey concentrate. In the second period the rise in red blood cells after giving 50 μg. of vitamin B_{12} plus 500 ml. of gastric juice was equivalent to that expected from an injection of 5 μg. In the third period the response to the injected dose of 50 μg. was greater than expected. The patient therefore was not "resistant," and the oral-dose/parenteral-dose ratio was probably about 10 : 1.

Conclusions

Neither fresh milk nor a whey concentrate in the doses given was an adequate substitute for normal human gastric juice as a source of Castle's intrinsic factor.

IV. ADMINISTRATION INTO BUCCAL CAVITY, INTO WASHED SEGMENT OF INTESTINE, OR AFTER PARTIAL STERILIZATION OF BOWEL

BY

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The object of these experiments was to determine whether vitamin B_{12} even without intrinsic factor would be efficiently absorbed if destruction by intestinal contents could be avoided or reduced. This was attempted in three ways: (1) by the application of vitamin B_{12} to the buccal mucosa; (2) by instillation into a washed segment of the intestine; and (3) by the oral administration of vitamin B_{12} after preliminary partial sterilization of the gut.

Methods

A general account of methods is given in Paper I.

For *buccal administration*, vitamin B_{12} was applied daily to the floor of the mouth in the sulcus between the alveolar margin and the cheek. Over a period of 30 minutes a solution containing 5.5 μg. was added gradually by syringe to a pledget of cotton-wool lying on the mucous membrane which formed the base of an artificial cavity created by a dental appliance, designed to prevent leakage from the sulcus. For the next 30 minutes water alone was added. Afterwards the cotton-wool pledgets were stored for microbiological assay by Dr. W. F. J. Cuthbertson, 10 pledgets yielding 8 μg. of vitamin B_{12}.

There were four experiments in which B_{12} with or without gastric juice was *instilled into a washed segment of intestine.* In Case 402 a Miller–Abbott tube with two balloons was passed until *x*-ray examination confirmed that the end was in the small intestine. The balloons were inflated, and the intervening section of intestine was washed out with normal saline until the washings were clear. Then a solution of 40 μg. of vitamin B_{12} in 10 ml. of water was instilled, followed by water to wash through the tube. After one hour the remaining material was aspirated and the segment washed out again. A sample of the aspirate contained 0.5 μg. of vitamin B_{12} per ml.

In the second period the Miller–Abbott tube was again passed into the small intestine and the balloons were inflated as before. After washing out, the segment between the balloons was filled with 150 ml. of Seitz-filtered normal gastric juice mixed with 40 μg. of vitamin B_{12}.

In Case 403 in the first period a Miller–Abbott tube with two balloons attached 15 cm. apart was passed as in Case 402 until the end was well down the small intestine. The balloons were inflated and the intervening section of intestine was washed out with 200 ml. of normal saline until the washings were almost clear. Then 80 μg. of vitamin B_{12} in 10 ml. of normal saline was instilled, followed by 30 ml. of saline to wash through the tube (approximately 10 ml. would remain in the tube). After two hours the material remaining in the segment of intestine was aspirated, and the segment was washed again. The aspirate contained 0.45 μg. of vitamin B_{12} per ml.

In the second period a Miller–Abbott tube was passed as before but with the balloons 30 cm. apart. Intestinal juice collected on the way down contained 0.01 μg. of vitamin B_{12} per ml. The balloons were inflated and the segment was washed out with 500 ml. of distilled water, of which 300 ml. was recovered and found to contain 0.018 μg. of vitamin B_{12} per ml. Then 40 μg. of vitamin B_{12} in 250 ml. of normal gastric juice adjusted to *p*H 7 was instilled very slowly into the segment and left in place for one hour. Aspiration after one hour yielded 14 ml. of clear yellow fluid containing 0.05 μg. of vitamin B_{12} per ml. Washings yielded 35 ml. of clear pale yellow fluid containing no vitamin B_{12}. A further 40 μg. of vitamin B_{12} with 250 ml. of normal gastric juice was instilled. This caused colic, and the patient vomited 150 ml. of dark-brown fluid containing approximately 0.001 μg. or less of vitamin B_{12} per ml. Aspiration after one hour yielded 20 ml. of dark-brown fluid containing 0.025 μg. of vitamin B_{12} per ml. The segment was again washed out with 500 ml. of water, 118 ml. of which, containing 0.02 μg. of vitamin B_{12} per ml., was recovered.

An attempt to *sterilize the small intestine* is described in Case 404.

Application of Vitamin B_{12} to Buccal Mucosa

Case 401

A farm labourer aged 50 had pernicious anaemia with sore tongue but no neurological involvement. The dietary history was normal.

After a satisfactory control period of 11 days the red blood cells numbered 1,620,000 per c.mm., haemoglobin 5.9 g. per 100 ml., M.C.H. 36 $\mu\mu$g., M.C.V., 111 μ^3, M.C.H.C. 32.9%, reticulocytes 0.4%, and white blood cells 1,800 per c.mm. The marrow was megaloblastic.

After the daily instillation of approximately 5 μg. of vitamin B_{12} into the buccal cavity there was a negligible reticulocytosis of 2.8% on the tenth day and the red blood cells fell to 1,270,000 per c.mm. (Fig. 1).

In the second period the patient received 5 μg. of vitamin B_{12} daily mixed with 50 ml. of unfiltered gastric juice, neutralized