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

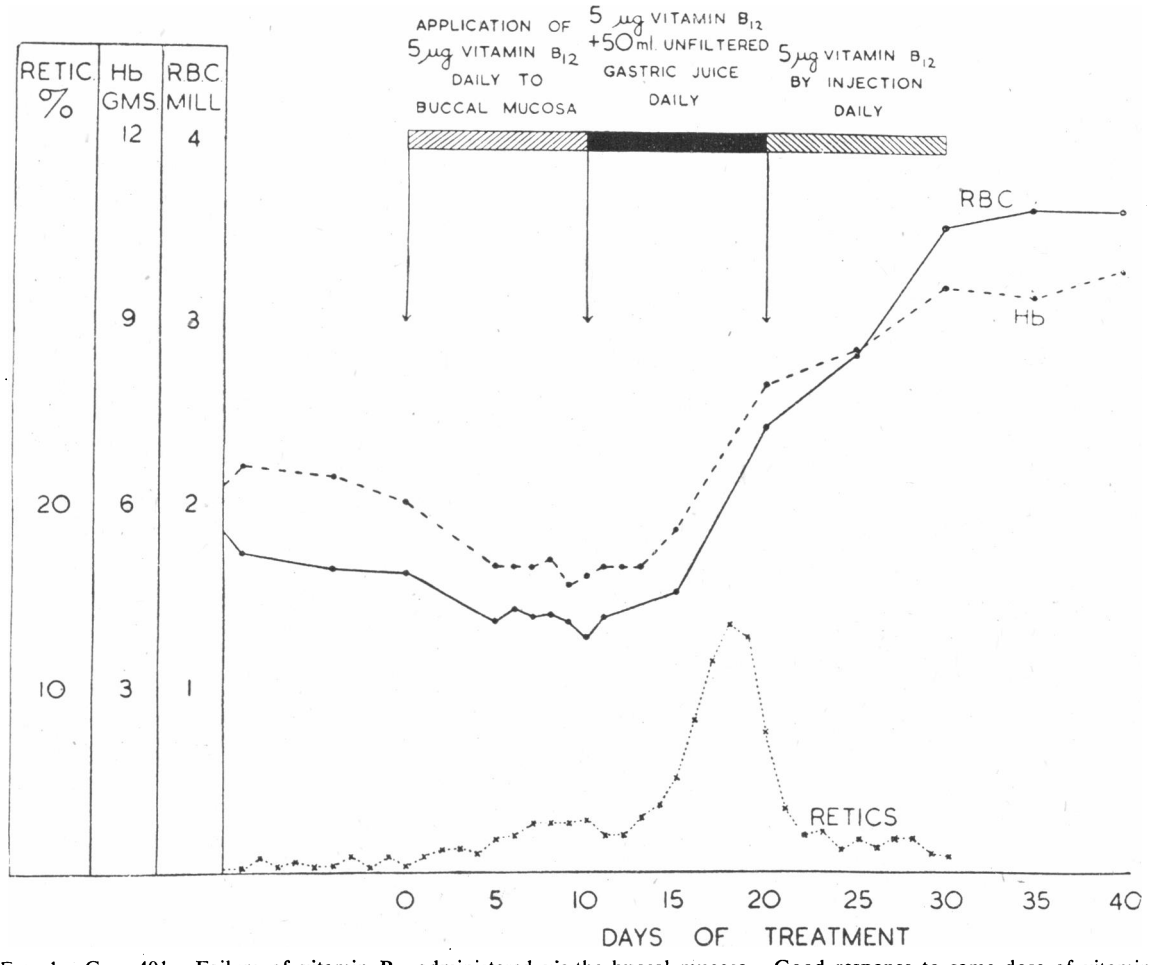

FIG. 1.—Case 401. Failure of vitamin B_{12} administered via the buccal mucosa. Good response to same dose of vitamin B_{12} given with gastric juice.

just before administration. The reticulocytes began to rise on the fourth day, reaching a peak of 13.4% on the eighth day. There was subjective improvement and the red blood cells increased.

	←Second Period→		←Third Period→		←Nil→		
Days:	0	5	10	15	20	25	30
R.B.C. (mills./c.mm.)	1·27	1·52	2·43	2·83	3·54	3·64	3·63
Hb (g./100 ml.) ..	4·7	5·5	7·8	8·4	9·5	9·3	9·8
P.C.V. (%)	14·0	—	25·0	29·1	31·0	32·5	35·0

In the third period he was given injections of 5 μg. of vitamin B_{12} daily for 10 days. There was no secondary reticulocytosis. The red blood cells continued to increase. Subsequent treatment with vitamin B_{12} brought the counts to normal.

Comment.—The absence of haemopoietic response in the first period suggested that little, if any, vitamin B_{12} was absorbed from the mouth. Disappearance of liquid from the chamber was not regarded as proof of absorption of vitamin B_{12}. The increase of red blood cells in 10 days after a total of 50 μg. of vitamin B_{12} given orally with gastric juice was equivalent to the response expected from a single injection of 20 μg. The adequacy of the response is further suggested by the absence of any secondary reticulocytosis in the third period when the same daily dose of vitamin B_{12} was given by injection. Incidentally the increase in red blood cells after a total of 50 μg. (plus any effect left over from the previous therapy) was equivalent only to the increase expected from 20 μg. Thus the true oral-dose/injection-dose ratio may have been close to unity; 50 μg. given orally with gastric juice and 50 μg. given by injection both produced a response equivalent to that normally expected from a single injection of 20 μg.

Instillation of Vitamin B_{12} into an Isolated Washed Segment of Intestine

This was performed twice in each of two patients.

Case 402

A man aged 54 had pernicious anaemia with minor neurological involvement. He disliked meat except bacon, but ate plenty of eggs, milk, cheese, fruit, and vegetables.

During the control period of nine days the reticulocytes were less than 1% and red blood cells fell from 2,650,000 to 2,090,000 per c.mm., with haemoglobin 7.4 g. per 100 ml., M.C.H. 35.5 μμg., M.C.V. 109 μ^3, M.C.H.C. 32.6%, and white blood cells 1,800 per c.mm.

After instillation of 40 μg. of vitamin B_{12} into a washed segment of intestine there was neither reticulocytosis nor increase of red cells (Fig. 2).

In the second period 40 μg. of vitamin B_{12} was instilled with 150 ml. of normal gastric juice. Unfortunately the gastric juice had been passed through a Seitz filter, which, as we learned subsequently, may have removed most of the intrinsic factor activity (Case 201). There was no reticulocytosis or increase of red blood cells.

In the third period the patient was given by mouth 150 ml. of unfiltered normal gastric juice neutralized just before administration and mixed with 40 μg. of vitamin B_{12}. The reticulocytes began to increase on the fourth day, reaching 7% on the sixth day. There was no increase of red blood cells, and clinical improvement was transient.

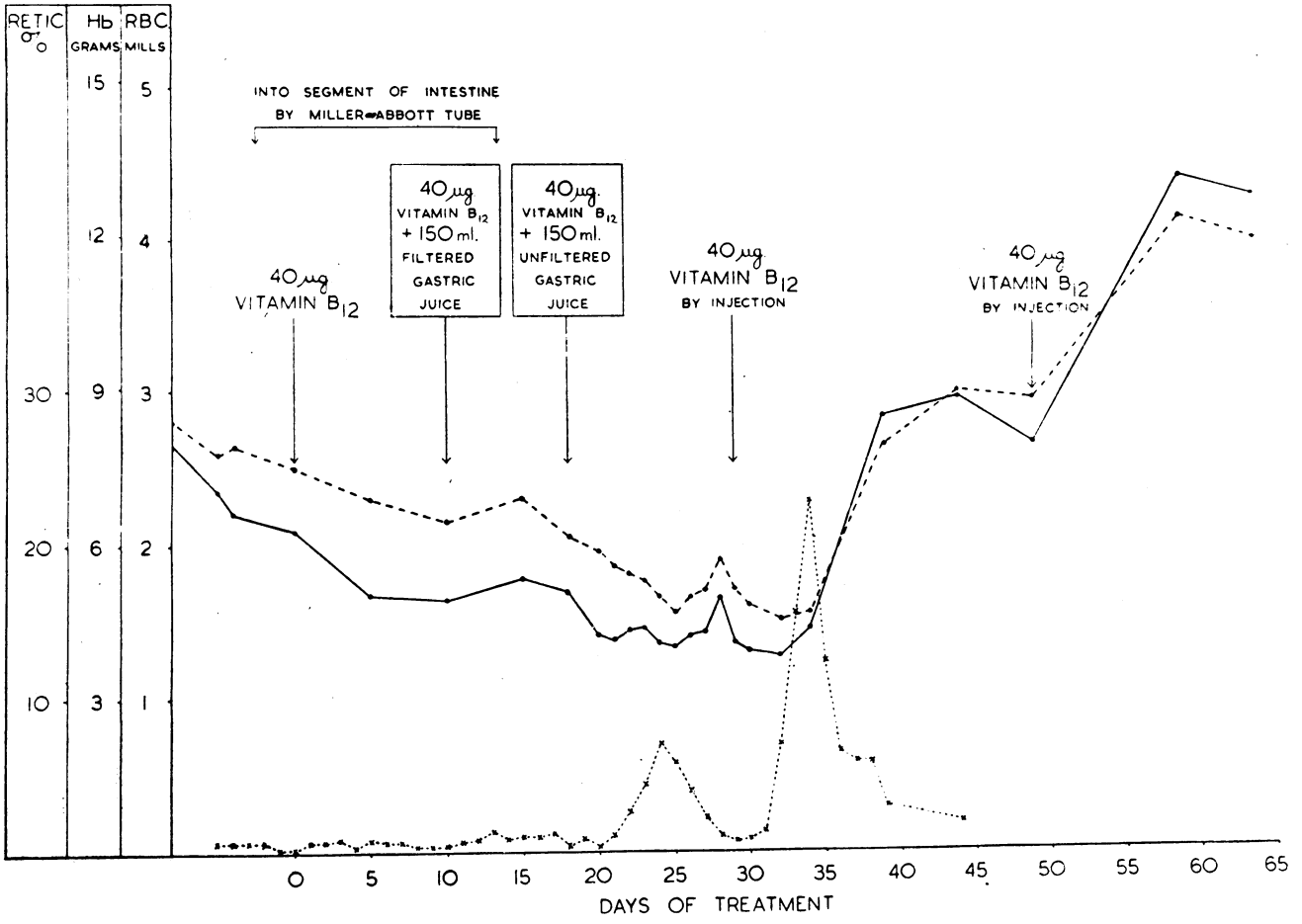

FIG. 2.—Case 402. Failure of vitamin B_{12} administered into an isolated washed segment of small intestine—either alone or with Seitz-filtered (and thus probably inactivated) gastric juice. Poor response to same dose of vitamin B_{12} given orally with 150 ml. of unfiltered gastric juice. (150 ml. is probably not enough for the absorption of 40 μg.) Normal response to injection of 40 μg.

	←Third Period→			←Fourth Period→		
Days:	0	5	11	16	21	26
R.B.C. (mills./c.mm.)	1·68	1·45	1·35	1·44	2·83	2·96
Hb (g./100 ml.) ..	6·1	5·2	5·0	4·6	7·8	8·9
P.C.V. (%)	19·0	16·5	—	18·0	30·0	31·0

In the fourth period a single injection of 40 μg. of vitamin B_{12} led to a reticulocytosis of 22.8% on the fifth day and the red blood cells increased rapidly. Further injections of vitamin B_{12} brought the counts to normal.

Comment.—Vitamin B_{12} in a dose of 40 μg. administered into a washed segment of intestine twice failed to produce a haemopoietic effect. This suggests that little or no vitamin B_{12} was absorbed. The high concentration of vitamin B_{12} in the aspirate even after one hour (see Methods) is perhaps further evidence that absorption was absent or incomplete, but one cannot be certain how efficiently the tube had been washed clear of vitamin B_{12} before the final aspiration. The poor response in the third period suggests that 150 ml. of the gastric juice used was enough for the absorption of only a small fraction of the dose of 40 μg. of vitamin B_{12}. A similar reticulocytosis without rise of red blood cells has been produced by doses as low as 1.25 or 2.5 μg. by injection. In the fourth period the increase of red blood cells in 15 days after 40 μg. by injection (plus any effect left over from previous therapy) was close to the expected increase. This suggests that there was no "resistance" to vitamin B_{12}.

Case 403

A crane-driver aged 56 had pernicious anaemia without neurological involvement. The dietary history was normal.

On admission red blood cells numbered 1,670,000 per c.mm., haemoglobin 5.9 g. per 100 ml., reticulocytes 0.4 %, P.C.V. 18%, M.C.V. 108 μ^3, M.C.H. 35.5 μμg., M.C.H.C. 33%, white blood cells 5,300 per c.mm. During a control period of 11 days the reticulocytes did not exceed 1.6% and the red cells did not increase. The marrow was megaloblastic.

After the instillation of 80 μg. of vitamin B_{12} into a washed segment of intestine reticulocytes rose slightly to 4.2% on the fifth day, but there was no increase of red cells (Fig. 3).

	←First Period→			←Second Period→		
Days:	0	5	10	14	19	25
R.B.C. (mills./c.mm.)	1·58	1·56	1·53	1·45	1·33	1·38
Hb (g./100 ml.) ..	6·2	6·4	5·9	5·6	5·3	5·9
P.C.V. (%)	19·0	19·0	18·0	18·0	17·0	17·0

In the second period the distance between the balloons was doubled and 80 μg. of vitamin B_{12} with 500 ml. of normal gastric juice was instilled in two lots for an hour each. Reticulocytes rose to a peak of 5.4% on the sixth day and after 11 days the red cell count was essentially unchanged. A further marrow puncture showed that, although there was some maturation compared with the control period, erythropoiesis was still predominantly megaloblastic.

In the third period the patient was given an intramuscular injection of 80 μg. of vitamin B_{12}. The reticulocytes rose on the third day and reached a peak of 27.8% on the sixth day. The red blood cells increased rapidly.

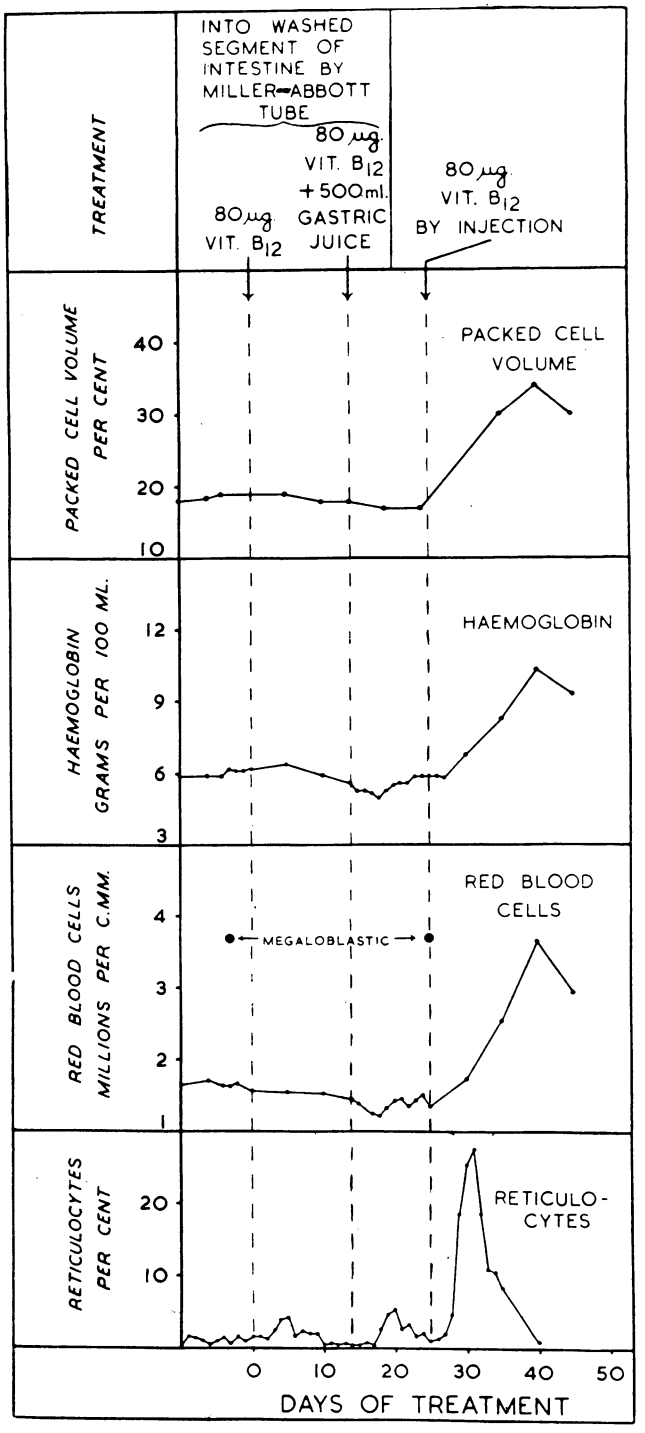

FIG. 3.—Case 403. Showing that the instillation of 80 µg. of vitamin B_{12} with or without gastric juice into a washed segment of intestine failed to produce anything more than a slight reticulocytosis. There was afterwards a good response to 80 µg. of vitamin B_{12} by injection.

	Third Period				
Days:	0	5	10	15	20
R.B.C. (mills./c.mm.)	1·38	1·76	2·58	3·69	2·95
Hb (g./100 ml.)	5·9	6·8	8·3	10·4	9·3
P.C.V. (%)	17·0	19·0	30·0	34·0	30·0

The patient ate well and had no soreness of the tongue during any of the experimental periods, but notable improvement in strength and colour was observed in the third period.

Comment.—Vitamin B_{12} instilled into a washed segment of intestine, either with or without gastric juice, was not effectively absorbed. The small reticulocytosis observed in the first and second periods was no greater than might have been observed from the oral administration of 80 µg. of vitamin B_{12}. The response to injected vitamin B_{12} was better than expectation.

Not much reliance can be put on the negative results of these intubation experiments. The segment of intestine may have been too small or sited in an area unsuitable for absorption of vitamin B_{12}. Moreover, the protection against contact with intestinal contents was anything but complete.

Oral Administration of Vitamin B_{12} after Partial Sterilization of the Intestine

The object of this experiment was to determine whether vitamin B_{12} would be effectively absorbed if intestinal bacteria which might take up the vitamin were destroyed.

Case 404

A coal-screener aged 56 had pernicious anaemia without neurological involvement. His diet had been satisfactory. During a control period of 12 days the reticulocytes did not exceed 1% and the red cells fell from 1,700,000 to 1,260,000 per c.mm. He was transfused with blood of a different but compatible group so that his own cells could be enumerated. The next day the total count was 1,730,000.

In the first period intestinal antiseptics were given as follows:

1st day: Phthalylsulphathiazole 4 g., six times.
2nd day: Phthalylsulphathiazole 2 g., six times; also "aureomycin" 0.75 g., four times.
3rd day: Same as second, with dihydrostreptomycin 1 g., four times.
4th, 5th, and 6th days: All three drugs as on third day.

Bacteriological examination (Dr. C. A. Green) showed that *Bact. coli* had disappeared from the stools by the third day and cocci by the fifth day. Yeasts persisted even up to the last examination on the seventh day. On the evening of the fifth day the patient received orally 80 µg. of vitamin B_{12}. There was neither reticulocytosis nor increase of red blood cells (Fig. 4).

In the second period 80 µg. of vitamin B_{12} with 500 ml. of gastric juice was given at 7.30 p.m. No food was given for four hours before or after the mixture. The reticulocytes rose to 15.8% on the sixth day. Changes in blood counts were as follows:

	Second Period			
Day:	0	5	10	15
R.B.C. (mills./c.mm.)	1·34	1·51	2·13	2·16
Hb (g./100 ml.)	5·3	5·9	7·4	7·7
P.C.V. (%)	16·0	20·0	24·0	23·0

In the third period 80 µg. of vitamin B_{12} was injected. There was another reticulocytosis, reaching 17.2% on the fifth day. Changes in counts were as follows:

	Third Period			
Day:	0	5	10	15
R.B.C. (mills./c.mm.)	2·16	2·24	3·21	3·70
Hb (g./100 ml.)	7·7	8·0	9·3	10·8
P.C.V. (%)	23·0	24·0	33·0	37·0

Comment.—Preliminary partial sterilization of the intestine did not permit absorption of vitamin B_{12} given orally. The rise of red blood cells in 15 days after giving 80 µg. with 500 ml. of gastric juice was rather less than that expected from the injection of 5 µg. The response to the injected dose of 80 µg. was almost equal to that expected from 40 µg. Thus the oral-dose/injection-dose ratio was probably more than 8 : 1.

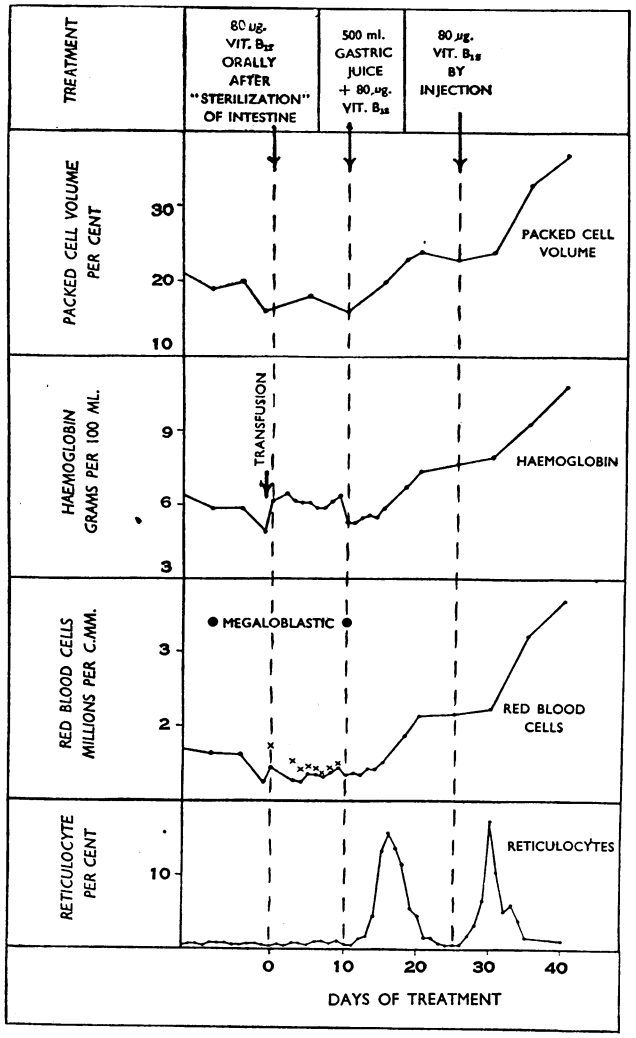

FIG. 4.—Case 404. Showing that 80 μg. of vitamin B_{12} administered orally after partial sterilization of the intestine did not lead to any reticulocytosis or increase of red blood cells. Thereafter the same dose of vitamin B_{12} given with gastric juice produced a response rather less than that expected from an injection of 5 μg. The injection of 80 μg. of vitamin B_{12} was followed by a response almost equal to that expected from 40 μg. (In the section for red blood cells the crosses represent the total count including transfused cells, whereas the continuous line represents the patient's own cells, the donor's cells have been separated by differential agglutination.)

Discussion

Results in Case 401 suggest that there was little, if any, absorption of vitamin B_{12} from the buccal mucosa. Negative results are also reported by Erf and Wimer (1949).

These negative findings and our failure on four occasions to procure effective absorption from a washed isolated segment of intestine lend no support to the idea that vitamin B_{12} given via the alimentary tract might prove effective if contact with intestinal juice were prevented.

The intubation experiments were, however, not altogether satisfactory (see Cases 402 and 403). In another type of experiment an attempt was made to destroy intestinal bacteria which might otherwise take up orally administered vitamin B_{12} and render it unavailable to the host. Using phthalylsulphathiazole, aureomycin, and dihydrostreptomycin, we succeeded in sterilizing the stools except for yeasts. The small intestine was presumably equally sterile. Even so, the administration of 80 μg. of vitamin B_{12} orally was without effect.

Moreover, in microbiological tests we showed that "pernicious anaemia" intestinal juice did not destroy added vitamin B_{12}, in the presence either of "pernicious anaemia" or normal gastric juice (Ungley and Cuthbertson, 1950—Paper V of this series, to be published).

The results reported here are negative but not conclusive. They do not support, but neither do they entirely rule out, the hypothesis that Castle's intrinsic factor acts by protecting vitamin B_{12} from destruction in the alimentary tract. Further work is in progress.

Summary and Conclusions

Efforts were made to determine whether vitamin B_{12} would be effectively absorbed if the possibly destructive action of intestinal juice could be reduced or avoided.

Application of 5 μg. daily to the buccal mucosa gave a negative response in a patient who subsequently responded to the same quantity of vitamin B_{12} given with gastric juice.

In four experiments in two patients the instillation of vitamin B_{12} with or without gastric juice into a washed segment of intestine isolated between the two balloons of a Miller–Abbott tube led to negative or trivial responses. In each case the subsequent administration of vitamin B_{12} by injection or orally with gastric juice was effective.

Even after preliminary partial sterilization of the intestine with aureomycin and other drugs the oral administration of 80 μg. of vitamin B_{12} was ineffective. The patient subsequently responded to the same dose of vitamin B_{12} given with gastric juice.

These negative findings are against, but do not entirely exclude, the possibility that Castle's intrinsic factor acts by protecting vitamin B_{12} from destruction in the gastro-intestinal tract.

ADDENDUM.—Since these papers were submitted additional evidence on the effect of orally administered vitamin B_{12} and the role of Castle's intrinsic factor has been reviewed by Dr. Byron E. Hall (*British Medical Journal*, September 9, 1950, p. 585).

I am grateful to the medical, nursing, and lay staff of the hospital and medical school and to many general practitioners for their co-operation. I have to thank Dr. L. W. Carstairs for intubating the small intestine and Dr. R. B. Thompson for marrow biopsies. Special thanks are due to students and others who donated gastric juice. The dental appliance used in Case 401 was constructed with the kind co-operation of Professor John Boyes and Mr. H. G. Hanley, L.D.S., of the Dental School, King's College, University of Durham. Dr. E. Lester Smith, of the Research Division of Glaxo Laboratories, Ltd., Greenford, supplied the crystalline vitamins B_{12} and B_{12c} and also the vitamin B_{12} concentrate used in these investigations. Dr. W. F. J. Cuthbertson, also of Glaxo Laboratories, was responsible for all the microbiological assays.

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