Closer contact between geneticists and medical students is desirable if full advantage is to be taken of existing knowledge. Even more important is the assistance that properly directed researches in social and clinical medicine could give to the future development of human genetics. At the present time the most pressing need is the ascertainment of population frequencies of any diseases determined, to a significant degree, by genetical factors. Such ascertainment would enable the analysis of pedigree studies to be put upon a firm basis.

References

Doll, R., and Buch, J. (1950). *Ann. Eugen.*, **15**, 135.
Fölling, A. (1934). *Hoppe-Seyl. Z.*, **227**, 169.
Garrod, A. E. (1902). *Lancet*, **2**, 1616.
Goddard, H. H. (1914). *Feeble-mindedness: Its Causes and Consequences.* Macmillan, New York.
Harris, H. (1949). *Ann. Eugen.*, **14**, 293.
Hutchison, R., and Hunter, D. (1949). *Clinical Methods*, 12th ed. Cassell, London.
Kallmann, F. J. (1938). *The Genetics of Schizophrenia.* Augustin, New York.
Levine, P., Katzin, E. M., and Burnham, L. (1941). *J. Amer. med. Ass.*, **116**, 825.
Penrose, L. S. (1950). *Brit. J. Psychol.* (General Sect.), **40**, 128.
—— MacKenzie, Helen J., and Karn, Mary N. (1948). *Ann. Eugen.*, **14**, 234.
Pincus, G., and White, P. (1933). *Amer. J. med. Sci.*, **186**, 1.
Sedgwick, W. (1861). *Brit. Foreign med.-chir. Rev.*, **27**, 477; **28**, 198.

ABSORPTION OF VITAMIN B_{12} IN PERNICIOUS ANAEMIA

I. ORAL ADMINISTRATION WITHOUT A SOURCE OF INTRINSIC FACTOR

BY

C. C. UNGLEY, M.D., F.R.C.P.

Physician, Royal Victoria Infirmary, Newcastle-upon-Tyne

There are many who find difficulty in orientating recent work on vitamin B_{12} with teaching based on Castle's original hypothesis. Following experiments in which beef and gastric juice were fed separately and together to patients with pernicious anaemia, it was suggested that an intrinsic (gastric) factor interacted with an extrinsic (food) factor to produce the anti-pernicious-anaemia factor stored in liver. Efforts to obtain from mixtures of beef and gastric juice a product as heat-stable as the liver active principle were, however, unsuccessful. Moreover, the hypothesis failed to explain why the dosage required for orally administered liver extract so vastly exceeded the amount needed for injection. Even when it was shown that the simultaneous administration of a source of intrinsic factor allowed a greatly reduced oral dose of liver (Reimann and Fritsch, 1934) or liver extract (Fouts, Helmer, and Zerfas, 1935) most of us misinterpreted this to mean that liver contained extrinsic factor in addition to liver active principle.

The problem has been clarified by the behaviour of vitamin B_{12}. This heat-stable substance isolated from liver is effective both in pernicious anaemia (West, 1948) and in subacute combined degeneration of the cord (Berk, Denny-Brown, Finland, and Castle, 1948; Ungley, 1949b). It thus fulfils all the criteria of a true "liver active principle."

When administered orally, however, vitamin B_{12} behaves like Castle's extrinsic factor in that small amounts are effective only if accompanied by a source of intrinsic factor (Berk, Castle, Welch, Heinle, Anker, and Epstein, 1948). In this case at least "extrinsic factor" appears to be identical with "liver active principle," interaction with intrinsic factor being necessary only if the material is given by mouth.

The present work is an attempt (*a*) to determine how effectively vitamin B_{12} is absorbed when given alone or with gastric juice, and (*b*) to answer the question: Does Castle's intrinsic factor directly facilitate the absorption of B_{12} or merely protect it from destruction in the gastro-intestinal tract?

The results are described in consecutive papers, with the following subtitles: I. Oral administration without a source of intrinsic factor. II. Oral administration with normal gastric juice. III. Failure of fresh milk or whey concentrate to function as Castle's intrinsic factor or to potentiate the action of orally administered vitamin B_{12} (with Mr. G. A. Childs). IV. Administration into the buccal cavity, into a washed segment of intestine, or after partial sterilization of the bowel. No. V, on the influence of gastric and intestinal juice on the availability of vitamin B_{12} determined microbiologically (with Dr. W. F. J. Cuthbertson), will be published later.

To facilitate cross-reference, the cases are numbered from 101 in Paper I, 201 in Paper II, and so on.

Methods

Haematological methods, dietary control, and selection of cases have been described (Ungley, 1949a). All patients had pernicious anaemia with megaloblastic marrow and complete achlorhydria even after histamine and alcohol. All showed at least one characteristic reticulocyte response to potent material and ultimately attained complete remission, usually after further treatment with vitamin B_{12} or liver extract. Only relevant parts of the case histories are included.

Dietary histories are summarized because of the possibility—still being explored—that even in pernicious anaemia the chance of a patient responding to orally administered vitamin B_{12} may be greater if low intake as well as deficient absorption had contributed to the development of deficiency; and because deficiency of other dietary factors such as folic acid could interfere with the response to vitamin B_{12} even if the latter were injected parenterally.

Pure vitamin B_{12} was used except in the first period of Case 101, in which vitamin B_{12c} was given, and in five cases receiving 3,000 μg. as concentrate, to be reported fully later.

The time of administration was considered in relation to the phases of digestion and the nature of foods ingested within six hours which might affect the availability of vitamin B_{12} to bacteria or to the host. The large doses of vitamin B_{12} concentrate ($\equiv$3,000 μg.) were given fasting, and no food was ingested until six hours afterwards.

Responses were assessed on the increase of red blood cells in 15 days rather than on the reticulocytosis, although the latter sometimes afforded valuable confirmation. Haemoglobin and packed-cell volume were recorded as a further check. The double reticulocyte response method of Minot and Castle (1935) was used in some cases.

To facilitate comparison and to avoid the need for ambiguous terms such as "good," "maximal," or "optimal" response, the increase of red blood cells in 15 days was expressed as equivalent to the reponse expected from a single injection of so many micrograms of vitamin B_{12} (Ungley, 1949a, Tables II and V). Oral preparations were sometimes given daily, whereas standards for parenteral therapy are based on the response to a single dose. Nevertheless the total amount could be compared with the parenteral dose which would be expected to give the same response (see Table). When subsequent tests showed that the patient's actual responsiveness to injected material was below expectation the oral-dose/injection-dose ratio was corrected accordingly.

Even the "corrected" ratios are very approximate. The expected rise of red blood cells in 15 days from a given dose (between 5 and 80 μg.) of injected material has a standard

deviation of 300,000 per c.mm. The sources of deviation are partly technical and partly individual variations in response. Similar deviations in the observed response to oral therapy—and they might be greater—could produce wide and not necessarily linear variations in the parenteral dose which would be expected to produce an equivalent response. This means that the results of any single experiment can give only a rough indication of the oral-dose/parenteral-dose ratio.

Results

Daily doses of 5 μg. were ineffective in Case 201 (see Paper II).

Daily doses of 80 μg.* had a slight effect in the following case.

Case 101

A woman aged 59 had pernicious anaemia without neurological involvement. The dietary history was satisfactory except that she took no cheese and little milk. After a satisfactory control period of 13 days red blood cells numbered 1,700,000 per c.mm., haemoglobin 6.2 g. per 100 ml., reticulocytes 1%, M.C.V. 128 μ^3, M.C.H. 36.7 μμg., M.C.H.C. 30.3%, white blood cells 2,400 per c.mm. The marrow was megaloblastic.

First and Second Periods.—The daily oral administration of 80 μg. of vitamin B_{12a} (Lester Smith, 1950; Ungley, 1950) led to a reticulocytosis, beginning on the third day and reaching 8.8% on the ninth day, and to a small rise of red blood cells. Substituting pure vitamin B_{12} for vitamin B_{12a} led to a secondary reticulocytosis of 7.2% on the seventh day but no further increase of red blood cells.

	First Period			Second Period			Third Period	
Days:	0	5	10	14	19	24	29	34
R.B.C. (mills./c.mm.)	1·70	1·80	1·92	2·21	1·98	2·14	2·45	3·07
Hb (g./100 ml.) ..	6·2	6·8	7·1	8·0	7·6	8·3	8·9	10·5
P.C.V. %	21·0	—	22·0	23·0	22·5	25·0	28·0	—

In the *third period* 1 μg. of vitamin B_{12} was injected daily. Reticulocytes did not exceed 3%, but red blood cells increased rapidly.

Comment.—The reticulocyte responses in the first and second periods point to some therapeutic effect from orally administered material, although equally definite responses have been observed after single injections of only 2.5 μg. Moreover, the increase in red blood cells in 24 days after a total quantity of 1,920 μg. (Fig. 1) was not significantly greater than would have been expected in 15 days from a single injection of 2.5 μg. (Ungley, 1949a, Table V). In other words, the apparent oral-dose/injection-dose ratio was several hundreds to one. The increase of red blood

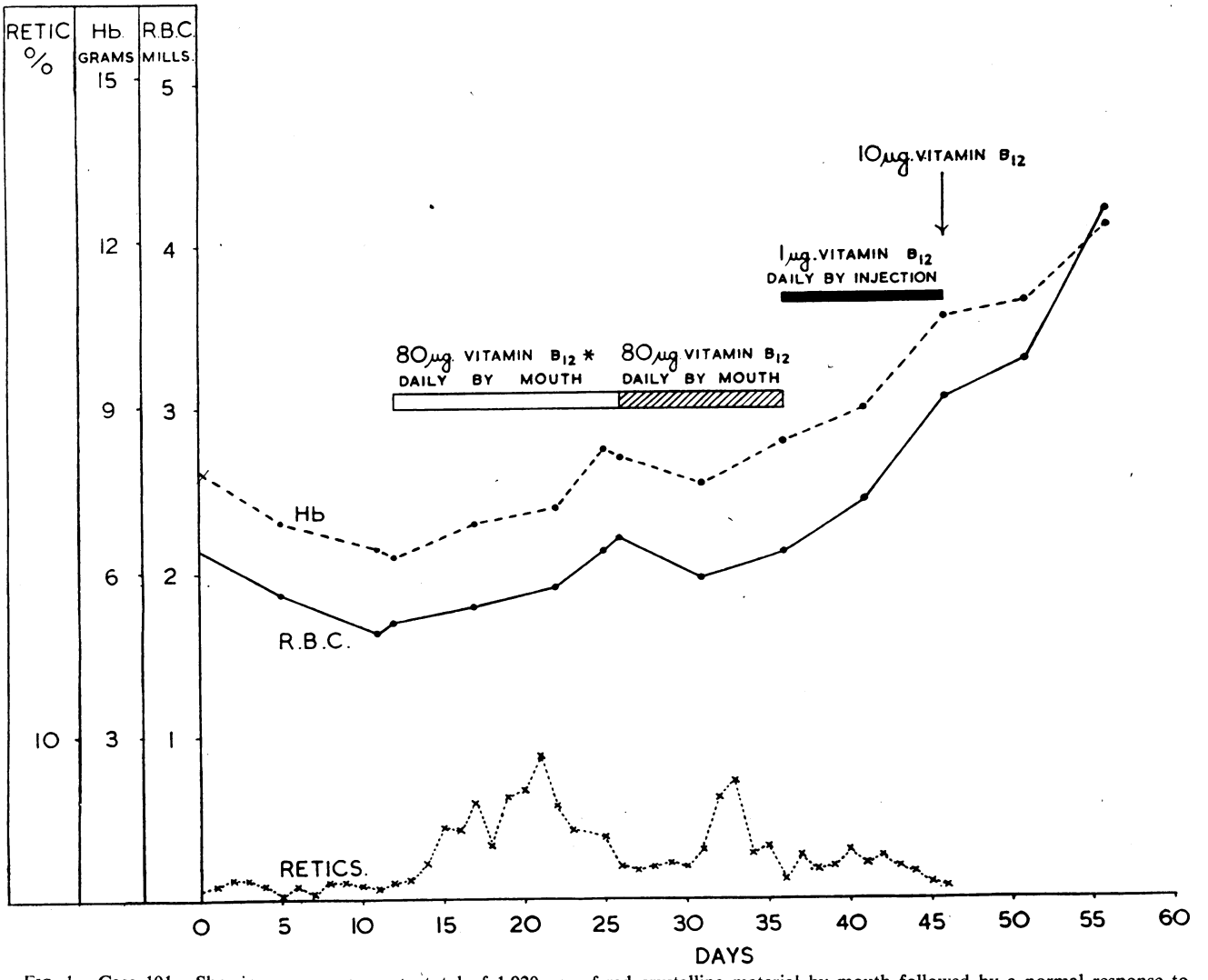

FIG. 1.—Case 101. Showing poor response to total of 1,920 μg. of red crystalline material by mouth followed by a normal response to daily injections of 1 μg.

*The variety of vitamin B_{12} used in the first period was vitamin B_{12a}.

Results Obtained by Various Workers

Author	Case	Haemopoietic Response				Oral Therapy B_{12} (μg.)	Apparent Ratio		Correction Based on Responsiveness to Injected B_{12}		Corrected Ratio		Presumed Absorption	
		E_0	Retic. Peak	I_{10}	I_{15}		Total 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	Per Dose (μg.)	%
Berk *et al.*	101	1·56	5·7	0·35	—	5×10	50	: Less than 2·5	No record				Less than 0·25*/5	Less than 5*
Ungley ..	201	1·40	Nil	Nil	—	5×10	50	: Nil	40‡	: 5			Nil/5	Nil
Hall *et al.*	Fig. 3	1·50‖	5·9	Nil	Nil	5×7	35	: Possibly 1·25	13×5 =65‡	: 80	Possibly 30	: 1	Less than 0·2/5	?3 or 4
,,	Fig. 1	1·75‖	Nil	Nil	—	25	25	: Nil	25	: 5	—		Nil/25	Nil
Spies *et al.*	2	2·24	Nil	Nil	—	30	30	: Nil	25	: 5	—		Nil/30	Nil
,,	2	1·99	Nil	Nil	—	50	50	: Nil					Nil/50	Nil
,,	3	2·70	2·6	Negligible	—	100	100	: ?Nil	45	: Less than 5	—		Nil/100	Nil
,,	3	2·70	2·0		—	400	400	: Nil					Nil/400	Nil
Campbell *et al.*	—	No record	"Submaximal response"			75	75	: ?	No record		—		Some*/75	Some*
,,	—	,,	"Failed to respond"			1,000	1,000	: Nil	,,	,,	—		Nil*/1,000	Nil*
West ..	—	,,	"Maximal reticulocyte response"			600	600	: ?	,,	,,			Some*/600	Some*
Spies *et al.*	2	1·28	21·0	—	1·40†	800×2 =1,600	1,600	: 20	25	: 5	16	: 1	50/800	6
,,	1	2·27	35·7	—	1·04‖	450×10 =4,500	4,500	: 10	Said to have had similar response to 75 μg.		Doubtful		1*/450	0·2*
Ungley ..	101	1·70	8·8	—	0·44 in 24 days	80×24 =1,920	1,920	: More than 2·5	10×1 =10	: 10	Several hundred	: 1	0·1 or 0·2/80	Less than 0·3
,, ..	102	1·52	32·8	—	2·30	3,000	3,000	: Much over 160§	R.B.C. level too high		—		Much over 160*/3,000	5 or 10*
,, ..	103	1·67	26·8		2·00	3,000	3,000	: 160§	,,	,, ,, ,,	—		160*/3,000	5*
,, ..	104	1·80	24·6		1·72	3,000	3,000	: 80	,,	,, ,, ,,	—		80*/3,000	2 or 3*
,, ..	105	2·56	26·0		1·75	3,000	3,000	: Nearly 160§	,,	,, ,, ,,	—		Nearly 160*/3,000	5*
,, ..	106	1·91	29·6		1·72	3,000	3,000	: 80	,,	,, ,, ,,	—		80*/3,000	2 or 3*

E_0 = Initial erythrocyte count. I_{10} = Increase of erythrocytes in 10 days. I_{15} = Increase of erythrocytes in 15 days.

‖ From chart. † Calculated from I_{10}. ‡ Some of the response may have been due to previous therapy. § These are the calculated equivalents, but the dose-response curve for injected B_{12} has not yet been carried beyond 160 μg. and must flatten out sooner or later. It follows that the amounts of B_{12} actually absorbed may have been greater than those indicated in the table. * Ratios and absorption figures thus marked are uncorrected because the actual response to parenteral therapy was not observed. "Nil" = Response not measurable, but some vitamin B_{12} may have been absorbed.

cells after 10 daily injections of 1 μg. was equivalent to the rise expected from a single dose of 10 μg. Some of this increase may have been due to therapy in the preceding period, but at least there is no suggestion of "resistance" to vitamin B_{12}, and the question of correcting the ratio does not arise.

Five Other Cases

Five patients (Cases 102–106) have recently been given orally a single dose of 3,000 μg. as a concentrate. The cases are the first of a new series, and a full report will be published later. The reticulocyte response was maximal in all, and the increase of red blood cells in 15 days was equivalent to that which would have been expected from single injections of 80 μg., 80 μg., nearly 160 μg., 160 μg., and much over 160 (? 320) μg. The apparent oral-dose/parenteral-dose ratio was between 10 and 40 : 1. The red cell levels reached were so high that comparative tests with parenterally administered vitamin B_{12} were impracticable, but these large responses are perhaps enough to indicate that the patients were not resistant to vitamin B_{12} (see Table).

Indeed, these responses are as great as any observed with injected vitamin B_{12}, which has not been tested in doses over 160 μg. Up to this level there was still a straight-line relationship between mean response and logarithm of dose. Sooner or later the dose-response curve presumably flattens out—i.e., there comes a point beyond which any further increase in dose makes no difference to the rise of red blood cells in 15 days. If 160 μg. is this upper

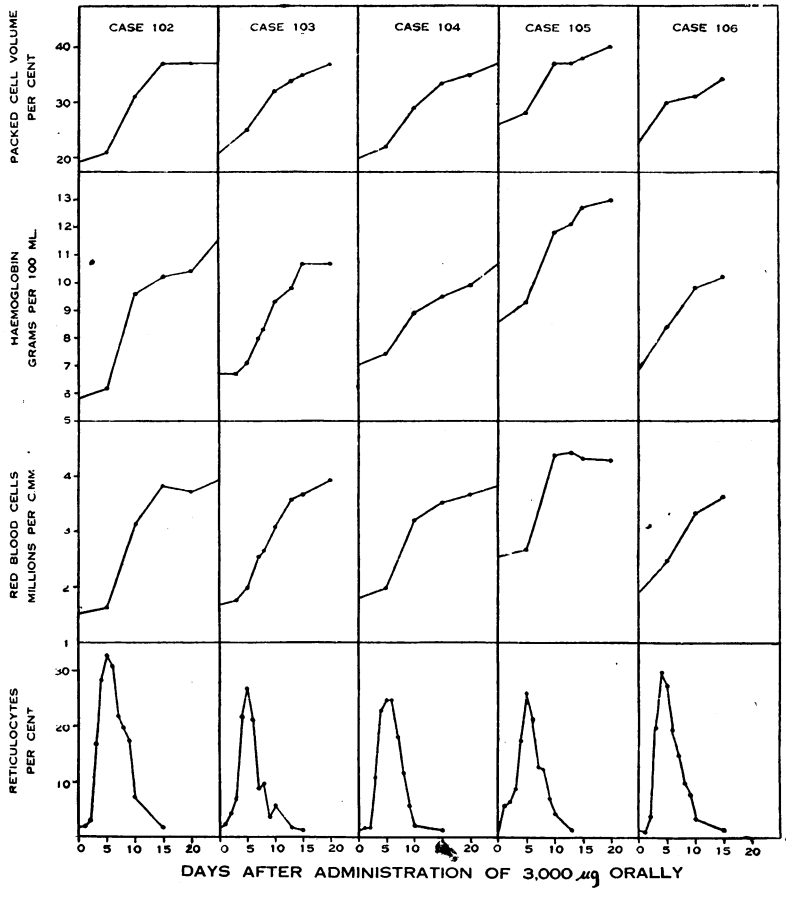

FIG. 2.—Effect in five cases of a single dose of 3,000 μg. of vitamin B_{12} as a concentrate.

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

C. C. UNGLEY, M.D., F.R.C.P.

Physician, Royal Victoria Infirmary, Newcastle-upon-Tyne

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.