Section of Experimental Medicine and Therapeutics

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DISCUSSION: GASTRIC BIOPSY AND THE INVESTIGATION OF THE MEGALOBLASTIC ANÆMIAS

Dr. R. K. Doig (Walter and Eliza Hall Institute and Royal Melbourne Hospital, Melbourne, Australia):

Gastric Biopsy and Gastritis

Gastric biopsy tube.—The principle of the gastric biopsy tube is that when the stomach is empty sudden suction applied to one end of a tube will draw mucosa into the other end lying in the stomach. If a knife be now pulled across this hole a fragment of mucosa will be cut off. The tube is made of Bowden wire covered with plastic and ending in a stainless steel cylinder with a lateral hole. A small cylindrical knife is moved up and down by a flexible wire past the lateral hole. The details of design and an initial report on the use of this instrument have been published elsewhere (Wood *et al.*, 1949). Instruments based on the same principle have been used by Palmer (1950), Tomenius (1950) and Rubin *et al.* (1953).

The biopsy.—This is carried out with the patient in the fasting state. After anæsthetizing the throat, the stomach is emptied and the tube passed to an appropriate depth. The biopsy is carried out by pushing the knife down, applying sudden suction and pulling the knife up. The procedure is repeated before the tube is removed. The specimens are fixed and sectioned perpendicular to the surface. The sections are stained with hæmatoxylin and eosin or mucicarmine, and also by a method which stains the pepsinogen granules (Motteram, 1951). The biopsy procedure is successful in about 85% of attempts and has been done in a wide variety of clinical conditions, including duodenal ulcer, chronic gastritis and pernicious anæmia (Wood and Joske, 1954). The technique may be described as a "blind" one and therefore has no place in the direct diagnosis of gastric cancer. Biopsy is not done on patients who have a tendency to bleed. Bleeding has been the sole complication and in a thousand attempts was clinically evident in 8 patients, 3 of whom needed transfusion.

The appearance of the normal gastric mucosa may be described as follows. The surface and gastric pits are lined by a regular tall columnar epithelium. The gastric glands occupy the greater part of the mucosa and lie perpendicular to the surface. The lamina propria is small, blood vessels are not prominent and interstitial cells are few. The abnormal mucosal appearances are conveniently divided into superficial gastritis, atrophic gastritis and gastric atrophy (Motteram, 1951). In superficial gastritis there is inflammatory change confined to the superficial part of the mucosa, the surface epithelium may be flattened and the pits may be deeper than normal. There is a variable degree of infiltration of the lamina propria with inflammatory cells. In atrophic gastritis there is more extensive change histologically which, on clinical grounds, is of longer duration. Arbitrarily, atrophic gastritis is diagnosed when more than half the gastric glands are absent. The surface epithelium is irregular and there may be islands of intestinal metaplasia, the pits are longer and pseudopyloric glands are common. The gastric glands are irregular in their disposition in the mucosa. Cellular infiltration is variable, but is often intense with definite aggregations of small round cells. Gastric atrophy is typically found in pernicious anæmia (Magnus and Ungley, 1938). There are few or no chief or parietal cells, the surface epithelium is regular and either gastric or intestinal in type. The glands may resemble intestinal crypts with striated border and goblet cells, or form pseudo-pyloric glands. Cellular infiltration is much less than in atrophic gastritis but there is still an increased amount of stroma. The cells are mainly lymphocytes and plasma cells.

A single sample has been accepted as sufficiently representative of the whole body mucosa in the absence of a local ulcerative lesion of the stomach for the following reasons. First, when two fragments are removed either at the same time or a short interval apart they usually look alike; and studies of satisfactory operation or autopsy material show that gastritis of the body of the stomach is usually a diffuse lesion (Hebbel, 1949; Williams, 1950). Second, the results of the histamine test meal and the biopsy appearance are related: the less the gland content of the section the lower the acidity and output of gastric juice (Funder and Weiden, 1952).

The clinical findings in 112 patients with superficial or atrophic gastritis but without peptic ulcer or gastric cancer have been reviewed (Doig and Wood, 1952). Half of this group had dyspepsia of a definite pattern. They complained of abdominal pain often in the epigastrium. The pain is of varying intensity, occurring shortly after meals and not usually relieved by alkaline powder. Of this group of 60 patients nausea was reported by 19 and occasional vomiting by 21. Atrophy of the tongue and slight epigastric tenderness were common. Anæmia due to overt hæmorrhage was found in 11 cases and due to various other causes in 16 cases. 6 patients were admitted with hæmatemesis and melæna and one with melæna alone; only one of these patients required transfusion. Alcoholism was the only significant ætiological factor and was found in 19 cases. Resolution occurred in 5 of these but was infrequent in other cases.

Gastric atrophy is the characteristic lesion of the mucosa of the body of the stomach in pernicious anæmia. In a study of 46 patients with pernicious anæmia or subacute combined degeneration of the cord, only 3 were atypical and had atrophic gastritis. Conversely in only 2 patients was there gastric atrophy without anæmia or cord signs (Doig and Wood, 1950). The histological appearance of the mucosa is the same whether the patient is untreated, or has had treatment for a long time or at a high intensity (Finckh and Wood, 1953).

In summary: gastric biopsy is a simple and safe technique by which an adequate and representative sample of the mucosa of the stomach can be obtained. The histological appearance may be normal, or may show a superficial or atrophic gastritis, or gastric atrophy. Half the patients with gastritis had a recognizable clinical story of indigestion. Gastric atrophy was almost always found in patients with pernicious anæmia or subacute combined degeneration of the cord and the gastric lesion was unaffected by treatment.

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Dr. Adam Turnbull (Nuffield Department of Clinical Medicine, The Radcliffe Infirmary, Oxford):

Experiences with Labelled Vitamin B_{12}

My object in this symposium is to give an account of our experiences at Oxford with radioactive vitamin B_{12}. When, just over one year ago, Dr. Lester Smith sent us our first supply of vitamin B_{12} labelled with the radioactive isotope of cobalt Co^{60}, the only published observations with this material in man were those of Heinle, Welch and their colleagues at Cleveland (Heinle *et al.*, 1952). They had given 0·5 µg. doses of the vitamin by mouth to 4 patients with pernicious anæmia and to 2 normal subjects, and had estimated the radioactivity which appeared in the fæces. They found that a much greater proportion of the radioactivity of this dose appeared in the fæces of the patients with pernicious anæmia than in those of the normal subjects, unless a source of intrinsic factor was given with the labelled vitamin B_{12}. In a personal communication Dr. Welch indicated the difficulties which they had encountered owing to the low specific activity of the available radioactive B_{12}, about 60 µc./mg., and he said that workers in another unit had not been able to confirm their findings.

We decided that we should first make sufficient observations in control subjects and patients with pernicious anæmia to confirm or disprove the findings of the Cleveland workers. We decided to use the same dose of 0·5 µg., but we hoped to gain greater accuracy from the much higher specific activity, about 420 µc./mg., of our radioactive vitamin B_{12}. We have given this dose with the patient fasting, and have estimated by means of a scintillation counter the radioactivity which subsequently

appears in the fæces. Full details of the technique and of our early results have been published elsewhere (Callender *et al.*, 1954*a*).

The procedure was first carried out in 10 control subjects who were chosen to be of comparable age to our patients with pernicious anæmia. They were in hospital for a variety of complaints, for example peptic ulcer and hypertension, but apart from one who had a moderate iron deficiency anæmia in addition to a fractured femur, none had any evidence of any blood disease. In 23 tests on these 10 control subjects between 20 and 40% of the radioactivity in the 0·5 µg. test dose usually appeared in the fæces. The mean of these observations was 31% and the observed limits 14 and 47%.

In contrast, in patients with pernicious anæmia between 80 and 95% of the radioactivity in this dose of labelled vitamin B_{12} usually appeared in the fæces, the mean of 39 observations in 22 patients being 88% and the observed limits 74 and 101%.

Nearly all of these patients with pernicious anæmia were in remission under treatment with vitamin B_{12} at the time of the tests. It might be said therefore that the difference between the results in the control patients and the patients with pernicious anæmia was due to the tissues of the latter being saturated with vitamin B_{12} given by injection. Labelled vitamin B_{12} was therefore given with intrinsic factor to 7 patients with pernicious anæmia. As the source of intrinsic factor pooled neutralized human gastric juice (100 ml.) was used in 3 patients, extracts of human gastric mucosa, prepared by Dr. W. H. Taylor of the Biochemistry Department of the Radcliffe Infirmary, in 2 patients, and in 2 others Fraction B, an intrinsic factor concentrate prepared by Prusoff and his colleagues (Prusoff *et al.*, 1950) and kindly supplied to us by Dr. Arnold Welch. In every case the recovery of radioactivity was much reduced whenever a source of intrinsic factor was also given, and with the more potent preparations the recoveries approached those found in the control patients. We concluded from these results that the difference between the findings in the control subjects and the patients with pernicious anæmia was due to the failure of the latter to secrete intrinsic factor.

Observations were then made on patients who had undergone total gastrectomy. Our results are being reported in full elsewhere (Callender *et al.*, 1954*b*). Apart from one patient, the amounts of radioactivity which appeared in the fæces when labelled B_{12} was given alone were similar to those observed in the patients with pernicious anæmia. When a source of intrinsic factor was also given the recovery was always reduced. It seems that there is the same reduction or absence of the secretion of intrinsic factor after total gastrectomy as occurs in pernicious anæmia, and this supports the view that in man the stomach is the only gastrointestinal source of intrinsic factor. These patients are presumably living on their stores of vitamin B_{12} and they would not be expected to develop macrocytic anæmia until the stores are exhausted, a matter of about three years. In one patient the amount of radioactivity recovered was intermediate between the levels found in the controls and the patients with pernicious anæmia. It seemed that he secreted some intrinsic factor. Perhaps the gastrectomy was not strictly total, or possibly he had an island of ectopic gastric mucosa in the œsophagus which was secreting intrinsic factor. Such findings would account for the failure to develop macrocytic anæmia in some patients even for many years after "total gastrectomy".

A few observations have been made in patients with steatorrhœa and megaloblastic anæmia of pregnancy. The patients with steatorrhœa appear to fall into three groups. Normal recoveries were found in 3 in whom it appears that both the secretion of intrinsic factor and the absorption of vitamin B_{12} were normal. In 2 patients the recoveries were greater than we have come to expect in the normal, but were not reduced by the addition of intrinsic factor. It seems probable that there was some defect in absorption in these patients which was not due to lack of intrinsic factor. In one patient with severe steatorrhœa the recovery when labelled vitamin B_{12} was given alone was similar to that found in patients with pernicious anæmia, and it was greatly reduced when intrinsic factor was also given. Unfortunately only one such observation could be made but it appeared that there was a lack of intrinsic factor in this patient.

In 2 patients with megaloblastic anæmia of pregnancy the recoveries of radioactivity were within normal limits. Both were receiving folic acid at the time. We have found that in a patient with Addisonian pernicious anæmia giving folic acid does not affect the recovery of radioactivity after a dose of radioactive vitamin B_{12}. It is possible, however, that therapy with folic acid had caused the resumption of the secretion of intrinsic factor in these patients or its return to normal levels.

There are a number of points arising from these results. Most interesting, perhaps, are that the control subjects do not appear to absorb the whole of this small dose of vitamin B_{12}, and that the patients with pernicious anæmia appear to absorb a small part of it, about 10%. It must be emphasized, however, that it is the radioactivity of the Co^{60} atom which is being estimated and that this may not still be in a molecule of vitamin B_{12}. If breakdown of vitamin B_{12} does occur in the gastrointestinal tract degradation products containing cobalt might be absorbed. Finally it must be remembered that the test depends on the absorptive powers of the gastrointestinal tract at the

time at which the dose is given. These provisos notwithstanding, it appears that the technique provides a useful indirect measure of the secretion of intrinsic factor which it is hoped will assist in the further investigation of the role of vitamin B_{12} and of intrinsic factor in the megaloblastic anæmias.

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Dr. J. Badenoch (Nuffield Department of Clinical Medicine, Oxford): *The Use of Labelled Vitamin B_{12} and Gastric Biopsy in the Investigation of Anæmia*

In Oxford Dr. W. C. D. Richards and I have been using the flexible gastric biopsy tube to study the pathology of the mucosa of the stomach for about two years, and we are indebted to Dr. I. J. Wood and Dr. R. K. Doig and their colleagues for their help in the course of our work. We have been interested mainly in the changes which occur in the mucosa of the stomach in anæmia and in the correlation between our histological findings and the results of the assay of intrinsic factor as measured by the absorption of radioactive vitamin B_{12}.

Our method of carrying out the biopsy is similar to that already described by Dr. Doig. Our experience is not nearly so extensive as that of the Melbourne workers but we have attempted the procedure in over 160 cases and have obtained a specimen of gastric mucosa in 130 of these. In only one instance has the biopsy been followed by bleeding which was severe enough to be recognized clinically.

The specimens of mucosa from the body of the stomach obtained with our biopsy tube are about 2 mm. in diameter and they usually extend from the superficial epithelium to the muscularis mucosæ. In normal subjects histological examination reveals closely packed body glands containing numerous chief and parietal cells and very few cells can be seen in the interstitial tissue. In contrast in pernicious anæmia we find gross atrophy of the specific glandular tissue and no normal chief and parietal cells can be seen. In some there is confluent intestinal metaplasia. The degree of cellular infiltration in the interstitial tissue is variable but it can be quite marked. A similar picture is seen in patients with subacute combined degeneration of the cord even in the absence of a macrocytic anæmia.

We have had the opportunity to study 4 such patients in whom a provisional diagnosis of subacute combined degeneration of the cord has been made. In the first patient the diagnosis lay between subacute combined degeneration of the cord and disseminated sclerosis and in the second between subacute combined degeneration of the cord and hysteria. Neither had a megaloblastic anæmia. In both, the changes in the stomach resembled those found in pernicious anæmia and in both the absorption of radioactive vitamin B_{12} was impaired. This led us to make a firm diagnosis of subacute combined degeneration of the cord which was confirmed by the good response to treatment with vitamin B_{12}. In the third patient the stomach showed the changes of an atrophic gastritis but normal parietal cells were present in some numbers and the absorption of radioactive vitamin B_{12} was within our normal range. We felt that the diagnosis of subacute combined degeneration of the cord could be excluded with confidence.

The fourth patient, a boy of 15, was interesting because he was a strict vegetarian who developed a dietary deficiency of vitamin B_{12}. He suffered from classical subacute combined degeneration of the cord which responded dramatically to treatment with vitamin B_{12} alone. As might be expected from his history, both the results of gastric biopsy and the absorption of radioactive vitamin B_{12} were normal.

We believe that in pernicious anæmia there is a virtual absence of normal parietal cells in the mucosa of the stomach and if these cells can be found in any numbers the diagnosis should be carefully reviewed, and a search for other causes of megaloblastic anæmia such as intestinal strictures or occult steatorrhœa should be carried out. In fact in the patients in whom the biopsy findings have been anomalous the excretion of uropepsin and the vitamin B_{12} absorption studies have also indicated that the patient is not suffering from Addisonian anæmia.

However, in some patients without Addisonian anæmia but with achlorhydria, gastric biopsy revealed an atrophy of the specific glandular tissue which approached that seen in pernicious anæmia.

This stimulated us to look for other similar cases and we have been investigating all the patients in our wards who have histamine-fast achlorhydria. We were particularly interested to study patients with hypochromic anæmia and achlorhydria since this type of anæmia has been reported to be common in the close relatives of patients with pernicious anæmia.

We have carried out a gastric biopsy on 24 patients with hypochromic anæmia and achlorhydria and although the histological picture of the stomach appears to be very variable we have found 6 with a gross atrophy of the body mucosa, and 1 in whom the changes were indistinguishable from those found in pernicious anæmia. This last patient was very interesting. Two specimens of mucosa were obtained from her stomach and both showed the same changes, while further evidence that there was a generalized atrophy of the gastric mucosa was provided by the fact that the output of uropepsin in twenty-four hours was 32 units, which is well within the range we have observed in pernicious anæmia. She was a woman of 51 years of age who had had a severe hypochromic anæmia for seven years. She had lived on a bread-and-tea type of diet, she suffered from menorrhagia, and she had a smooth tongue, angular stomatitis and dysphagia. At the time of her admission to hospital her hæmoglobin was 55% with a colour index of 0·6. Her anæmia responded fully to iron by mouth and she has remained well for the past twelve months with no other form of treatment.

We have studied the absorption of radioactive vitamin B_{12} in the patients with hypochromic anæmia and achlorhydria who have gross atrophy of the gastric mucosa.

3 of the 6 patients in whom the degree of atrophy of the body mucosa of the stomach approached that seen in pernicious anæmia absorbed less of the radioactive vitamin than any of the control subjects we have examined so far, but in the 2 of these in whom the test was repeated with the addition of a source of intrinsic factor there was no significant difference in the result.

However, in the patient in whom the changes in the gastric mucosa were indistinguishable from those found in pernicious anæmia the absorption of radioactive vitamin B_{12} was greatly reduced and it could be restored to normal levels by the addition of intrinsic factor. There is every reason to suppose that this patient would develop pernicious anæmia in the future.

We have also used the gastric biopsy and the study of the absorption of radioactive B_{12} in the investigation of obscure cases of megaloblastic anæmia. Recently 2 young women with megaloblastic anæmia were referred to Professor L. J. Witts by the kindness of Dr. J. Lowe of Swindon and Dr. E. K. Blackburn of Sheffield. The clinical pictures they presented were remarkably similar.

Both were young women who suffered from epilepsy and both had received various anticonvulsants including Epanutin and phenobarbitone for the control of their fits. In both the presenting symptom of the anæmia was soreness and ulceration of the mouth and one was known to have a leucopenia at the time the symptoms developed. Both had been faddy about their food and neither liked meat, but neither had been on a strict vegetarian regime prior to the onset of the illness. The results of the investigations carried out in these 2 patients are summarized in Table I.

TABLE I.—SUMMARY OF INVESTIGATIONS CARRIED OUT ON 2 PATIENTS WITH EPILEPSY AND MEGALOBLASTIC ANÆMIA

(Dr. D. L. Mollin kindly estimated the levels of vitamin B_{12} in the sera)

Patient	Free HCl in gastric juice	% dietary fat adsorbed	X-rays gastro-intestinal tract	Gastric biopsy	% Co^{60} B_{12} in fæces	Serum B_{12} level
M. K., female aged 22	0	Normal 98%	Normal	Normal mucosa	Normal 17%	Low normal 120 μμg./ml.
B. C., female aged 17	+	Normal 95%	Normal	Normal mucosa	Normal 12%	Normal 215 μμg./ml.

The anæmia in 1 of these patients responded to vitamin B_{12} alone, while in the other folic acid was tried and a good response was obtained.

These 2 young women appear to us to be suffering from a hitherto undescribed form of megaloblastic anæmia. As judged by the result of the gastric biopsy and the absorption of radioactive B_{12}, neither is suffering from true Addisonian anæmia and we have done our best to exclude the recognized causes of intestinal megaloblastic anæmia. It is tempting to ascribe the anæmia to a dietary deficiency of vitamin B_{12} but the normal serum levels of vitamin B_{12} are hardly compatible with this hypothesis. In any event they present a different picture from that usually seen in a dietary deficiency of the vitamin in this country, for when such a deficiency occurs signs of subacute combined degeneration of the cord usually precede the onset of a megaloblastic anæmia.

It is remarkable that both of these patients were epileptic and we have been wondering if the anticonvulsant drugs they had received could have played a part in producing the anæmia. It is true that the barbiturates will inhibit maturation of red cells in marrow cultures *in vitro*, but these drugs are in common use to-day and it is hard to believe that such a toxic effect would have escaped clinical recognition.

We have no adequate explanation for the development of a megaloblastic anæmia in either of these patients but we hope that a combination of the study of the histology of the stomach with the absorption of radioactive vitamin B_{12}, and the estimation of vitamin B_{12} in the serum when applied to other puzzling cases, may help to solve some of the problems which still exist in the pathogenesis of the megaloblastic anæmias.

Dr. D. L. Mollin, and Dr. G. I. M. Ross (Department of Pathology, Postgraduate Medical School of London):

Vitamin B_{12} Deficiency in the Megaloblastic Anæmias

The previous speakers have shown that presumptive evidence of vitamin B_{12} deficiency may be obtained in certain patients with megaloblastic anæmia by means of gastric biopsy and by studies with radioactive B_{12}. If such patients are anæmic or suffering from neurological symptoms it is possible to confirm the presence of vitamin B_{12} deficiency by following their hæmatological or clinical response to treatment with the vitamin.

Vitamin B_{12} deficiency in patients with megaloblastic anæmia may also be recognized by demonstrating an abnormally low concentration of B_{12} in their serum. For the last three years we have been trying to determine the incidence of vitamin B_{12} deficiency in the various types of megaloblastic anæmia both by measuring the pre-treatment serum B_{12} concentration in these patients and, whenever possible, by following their responses to treatment with vitamin B_{12}, folic acid or folinic acid.

In this paper we summarize the results we have obtained. The sera from more than 280 patients with various types of megaloblastic anæmia have been studied. About 100 of these were patients at Hammersmith Hospital. Sera from the remainder were kindly sent by colleagues at other hospitals.

Methods

The serum B_{12} concentrations were measured by microbiological assay using the green alga *Euglena gracilis* var. *bacillaris* as test organism (Ross, 1952).

Details of the hæmatological methods have been given elsewhere (Mollin and Ross, 1952).

Results

Normal subjects.—The serum B_{12} concentration of 126 normal subjects ranged from 100 to 900 μμg./ml. The mean concentration was 360 μμg./ml. Similar concentrations in a smaller group of normal subjects were previously reported (Mollin and Ross, 1952).

Patients with pernicious anæmia.—The serum B_{12} concentrations of patients with untreated pernicious anæmia were much lower. The mean serum B_{12} concentration in each of 190 patients was less than 100 μμg./ml. In the majority of patients the concentrations were less than 50 μμg./ml.

In some of the less anæmic patients (R.B.C. 2·0 million per c.mm., or more) the serum B_{12} concentrations were between 50 and 100 μμg./ml. but the concentrations were still subnormal even in patients with normal or almost normal blood counts (Table I). For reasons given elsewhere we think that the serum B_{12} concentration only falls definitely below the normal range when the reserves of B_{12} in the tissues are almost exhausted (Mollin and Ross, 1953). We consider therefore that these patients are suffering from quite severe B_{12} deficiency in spite of their normal or almost normal blood counts. Such patients almost invariably complain of symptoms similar to those of treated patients with pernicious anæmia who are not receiving quite enough vitamin B_{12} to keep them in complete remission. When they are treated with vitamin B_{12} the slight abnormalities that may be present in their blood disappear, and even more definite change occurs in their clinical condition; there is an increase in appetite, weight and general well-being.

TABLE I.—THE BLOOD COUNT AND SERUM B_{12} CONCENTRATION OF PATIENTS WITH VERY MILD UNTREATED PERNICIOUS ANÆMIA

Number of patients	R.B.C. M. per c.mm.		Hb grammes %		Serum B_{12} concentration μμg./ml.	
	Range	Mean	Range	Mean	Range	Mean
9	3·9–4·3	4·1	13·1–14·4	13·9	20–95	68
3*	3·9–4·5	4·1	13·5–13·7	13·6	<20–20	<20

*These patients had subacute combined degeneration of the cord.

In 3 patients with little or no anæmia but with subacute combined degeneration of the cord (S.C.D.) the serum B_{12} concentrations were very low indeed (Table I). We have always found very low serum B_{12} concentrations in patients with this condition irrespective of the degree of their anæmia. Presumably in the patients with S.C.D. and little or no anæmia the blood count is maintained by adequate amounts of folic acid.

The changes in the serum B_{12} concentrations, blood count, and bone marrow of patients with pernicious anæmia treated with B_{12}

We have described elsewhere the way in which we have tried to correlate the changes in the B_{12} concentrations of serum and the hæmatological changes that follow treatment with B_{12} (Mollin and Ross, 1953).

After therapeutically effective intramuscular injections of B_{12} the serum B_{12} concentrations of patients with pernicious anæmia in relapse rise to within the normal range and remain elevated for a variable time depending on the size of the injection, the amount excreted, and on the individual

requirements of the patient for B_{12}. While the serum B_{12} concentrations are within the normal range the marrow remains normoblastic. When the serum B_{12} concentrations fall below about 90 to 120 μμg./ml. megaloblastic hæmopoiesis reappears in the marrow.

Similar changes follow the administration of large oral doses of B_{12} (Ross *et al.*, 1954) or smaller oral doses given with a source of intrinsic factor. Fig. 1 shows the changes in the blood count and

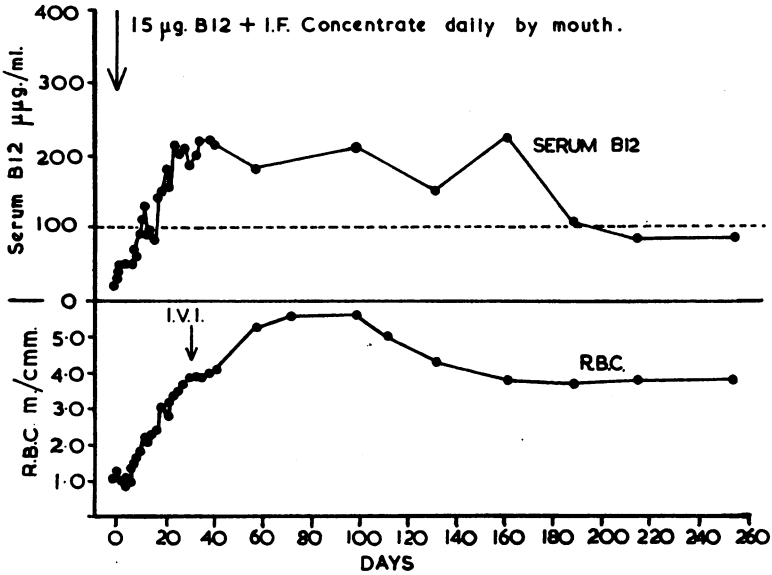

FIG. 1.—The changes in the red cell count and the serum B_{12} concentration of a patient with pernicious anæmia treated with vitamin B_{12} and intrinsic factor given daily by mouth. The broken line in the upper half of the chart represents the lower limit of the range of serum B_{12} concentrations in normal subjects.

serum B_{12} concentration of a patient who was treated with a daily dose of 15 μg. of vitamin B_{12} with a concentrate of hog's stomach containing intrinsic factor (Bifacton 2 tablets daily). The serum B_{12} concentration gradually rose and was consistently above the normal range after about twenty days of treatment. The blood count rose steadily until about the thirtieth day when signs of iron deficiency began to appear in the patient's blood. She was treated with intravenous iron and her blood count then rose to 5·0 million per c.mm. Both the serum B_{12} concentration and the blood count subsequently fell. We think this was because the patient did not take the tablets regularly, but the patient consistently denied this.

Patients with other megaloblastic anæmias.—The results of the pre-treatment serum B_{12} assays in these patients are summarized in Table II.

TABLE II.—THE PRE-TREATMENT SERUM B_{12} CONCENTRATIONS OF PATIENTS WITH VARIOUS MEGALOBLASTIC ANÆMIAS

Condition	Number of patients	Pre-treatment serum B_{12} concentration μμg./ml. Normal >100	Low <100
Pernicious anæmia	190	0	190
Total gastrectomy	4	0	4
Partial gastrectomy	9	2	7
Intestinal anastomosis; strictures	7	2	5
Idiopathic steatorrhœa	33	20	13
Non-tropical nutritional megaloblastic anæmia*	6	3	3
Megaloblastic anæmia of pregnancy	31	29	2

*The 3 patients with non-tropical nutritional megaloblastic anæmia who had low serum B_{12} concentrations were vegetarians. 2 of the other 3 patients with this condition were suffering from scurvy.

The great majority of *patients with normal pre-treatment serum B_{12} concentrations* did not respond to treatment with B_{12} but responded to treatment with folic or folinic acid. The effect of treatment

with B_{12} and folinic acid on such a patient is illustrated in Fig. 2. The patient was suffering from megaloblastic anæmia of pregnancy. She was treated first with B_{12} and then with folinic acid. There was little or no response to a single injection of 40 μg. of B_{12} or to the single very small injection of 60 μg. of folinic acid. There was a satisfactory response to treatment with larger doses of folinic acid. Presumably such patients are suffering from a pure and complete, or almost complete, deficiency of folic acid.

There are, however, a few patients with *severe* megaloblastic anæmia whose pre-treatment serum B_{12} levels are within the normal range but who yet show some response to treatment with large doses of B_{12}. The concentrations in these patients though normal are a low normal, being between 100 and 150 μμg./ml. The hæmatological responses of these patients are usually suboptimal and incomplete even when large doses of B_{12} are given and are very definitely suboptimal to doses of 20 to 40 μg. of B_{12}—doses which are capable of producing dramatic responses in patients with pure B_{12} deficiency. Dr. Badenoch has referred to one such patient. The fact that the serum B_{12} concentrations of these patients are within the normal range indicates that they are not suffering from severe B_{12} deficiency, though minor degrees of deficiency may be present. It is unlikely that the severe anæmia in these patients is due solely to B_{12} deficiency; deficiency of folic acid or failure to

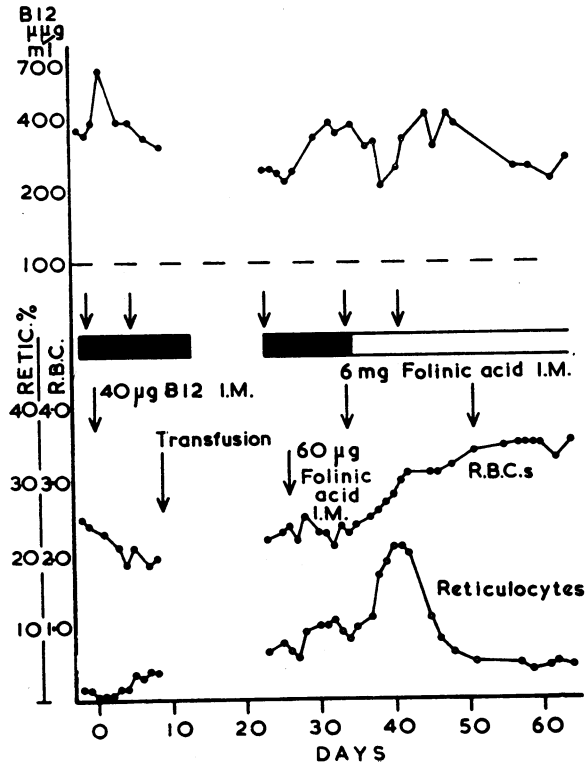

FIG. 2.—The changes in the blood count, bone marrow and serum B_{12} concentration of a patient with megaloblastic anæmia of pregnancy treated first with an injection of vitamin B_{12} and then with injections of folinic acid. The serum B_{12} concentrations are shown in the upper part of the figure. The broken line represents the lower limit of the range in normal subjects. The marrow changes are indicated diagrammatically above the blood count. The black shading indicates a megaloblastic marrow; the absence of shading a normoblastic marrow. The arrows indicate marrow punctures.

utilize folic acid must play a considerable part in the production of the anæmia. Presumably these patients cannot be completely deficient in folic acid or they would not respond to treatment with large doses of B_{12}.

The great majority of *patients with low pre-treatment serum B_{12} concentrations* respond to treatment with B_{12} as readily and completely as do patients with uncomplicated pernicious anæmia. In these patients the megaloblastic anæmia appears to be entirely due to deficiency of vitamin B_{12}.

However, there are a few patients with severe megaloblastic anæmia and pre-treatment serum concentrations below the normal range who respond inadequately to treatment with vitamin B_{12} alone. The concentrations may be as low as those found in pernicious anæmia of comparable severity or they may be rather higher lying between 60 and 100 μμg./ml. (an unusual finding in a

patient with a severe uncomplicated pernicious anæmia). Such patients may show an "optimal" reticulocyte response and "optimal" or almost "optimal" red cell response to single injections of 100 to 200 µg. of vitamin B_{12}. But megaloblastic change reappears in the marrows of these patients while their serum B_{12} concentrations are still within the normal range. If these patients are then treated with folic or folinic acid they will respond hæmatologically to this treatment but the serum B_{12} concentrations will gradually fall once more to low levels. This is in contrast to the results of treatment in patients with pernicious anæmia in whom the marrow does not again become megaloblastic until the serum B_{12} level has fallen below the normal range.

Conclusions

We have briefly discussed some of the ways in which the assay of the B_{12} concentrations of serum may be of value in the investigation of the megaloblastic anæmias.

The method has proved particularly valuable for demonstrating B_{12} deficiency in patients who have little or no hæmatological evidence of such deficiency. In such patients serum B_{12} concentrations of about 100 $\mu\mu$g./ml. or less indicate B_{12} deficiency. The method is useful for confirming the diagnosis of subacute combined degeneration of the cord in patients without anæmia; in these patients the serum B_{12} concentrations are always very low.

Because the assay of the concentration of B_{12} in the serum is more sensitive for demonstrating B_{12} deficiency than ordinary hæmatological techniques it is valuable for determining the exact incidence of B_{12} deficiency in various special populations, e.g. in patients following gastrectomy, in vegetarians or in the relatives of patients known to be suffering from pernicious anæmia. By studying such groups it should be possible to discover how long an interval may elapse before demonstrable anæmia develops after the serum B_{12} level has fallen below the normal range. In some patients with pernicious anæmia we have found the serum B_{12} level to be low but the blood picture normal or almost normal for periods of at least one to two years.

It is also a most convenient method of determining the specific deficiency in patients with florid megaloblastic anæmia and used in conjunction with the hæmatological response to treatment it can help to prove a double deficiency of B_{12} and folic acid.

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PRESIDENT'S ADDRESS

By G. A. H. BUTTLE, O.B.E., M.A., M.R.C.S., L.R.C.P.

IT has become commonplace to refer to the enormous developments in Therapeutics. As judged by the rather crude standard of the number of new drugs introduced, there have been as many new ones since 1900 as in the whole of the previous history of the subject. Whereas fifty years ago improvements in treatment were dependent only on clinical observation of patients, recently the great majority, but not all, of the advances have come in the first instance from the results of animal experiment. The initiative has rested to a great extent with the pharmacologist and the chemist whose responsibility it had become to suggest substances for clinical trial. Of course it only too often happens that many substances which are suggested do not fulfil the hopes originally placed in them and the trial and final assessment must always be the result of years of clinical work.

These great developments are by no means all due to animal experiment and the new knowledge has so awakened interest in therapeutics that a great number of discoveries are now being made during the treatment of patients themselves. Thus, the effect of cortisone and ACTH in rheumatoid arthritis would never have been predicted but for the work on patients. This field of clinical experiment is developing fast and may lead to as rich a harvest in therapeutic discovery in the future as animal experiment has done in the immediate past.