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Historical Review

A HISTORY OF PERNICIOUS ANAEMIA

This is a review of the ideas and observations that have led to our current understanding of pernicious anaemia (PA). PA is a megaloblastic anaemia (MA) due to atrophy of the mucosa of the body of the stomach which, in turn, is brought about by autoimmune factors. This is not a review of MA in general, nor of other forms of cobalamin (Cbl) deficiency that can also lead to a MA. Following the recommendation of a nomenclature commission, the name Cbl is used rather than vitamin B_{12}.

Progress comes with the acquisition of new facts either by new ideas, new methods, new measuring devices, availability of new science, etc. Once new tools appear several groups will apply them to their common problems and credit often has to be shared.

With apologies to James Combe of Edinburgh (1796–1883), Thomas Addison of London (1793–1860), Antoine Biemer of Zurich (1827–1892) and others, it is not possible to make a clinical diagnosis of PA among the case reports appearing in the first three-quarters of the nineteenth century. No mention of a smooth or painful tongue, not a touch of icterus, not a needle or pinprick sensation, let alone numbness, among the lot, make it difficult to accept a diagnosis of PA. Nevertheless, physicians were writing about a disease that often was PA and making admirable observations and perceptive suggestions about its causation. A case report by Osler & Gardner (1877) in Montreal could be that of PA. This anaemic patient had numbness of the fingers, hands and forearms; the red blood cells were large; at autopsy the gastric mucosa appeared atrophic and the marrow had large numbers of erythroblasts with finely granular nuclei. The increased marrow cellularity had also been noted by Cohnheim (1876).

MORPHOLOGICAL RECOGNITION

The key that was missing was a method of scrutinizing the blood. This arrived through the efforts of two cousins, Carl Weigert and Paul Ehrlich. Weigert developed staining of tissue sections with the new aniline dyes, while Ehrlich, still a medical student, applied these methods to staining heat-dried blood films placed on a copper plate, one end of which was warmed by a bunsen burner. Ehrlich (1854–1915) had been given a corner in the hospital basement by Wilhelm Waldeyer (1837–1921) and when he asked Ehrlich what he was doing was told 'Iche probe', which may be translated loosely as 'I am messing about'. Among other observations, Ehrlich (1880) (Fig 1) distinguished between cells he termed megaloblasts present in the blood in PA from normoblasts present in anaemia as a result of blood loss.

Not only were large red blood cells noted in PA, but irregular red cells, ? poikilocytes, were reported in wet blood preparations by Quincke (1877). Megaloblasts in the marrow during life were first noted by Zadek (1921). Hypersegmented neutrophils in peripheral blood in PA were described by Naegeli (1923) and came to be widely recognized after Cooke's study (Cooke, 1927). The giant metamyelocytes in the marrow were described by Tempka & Braun (1932).

FURTHER CLINICAL OBSERVATIONS

The association between PA and spinal cord lesions was described by Lichtheim (1887) and a full account was published by Russell *et al* (1900), who coined the term 'subacute combined degeneration of the spinal cord' (SCDC) although they were not convinced of its relation to PA. Arthur Hurst at Guy's Hospital, London, confirmed the association of the neuropathy with PA and added, too, the association of loss of hydrochloric acid in the gastric juice (Hurst & Bell, 1922). Cabot (1908) found that numbness and tingling of the extremities were present in almost all of his 1200 patients and 10% had ataxia. William Hunter (1901) noted the prevalence of a sore tongue in PA, which was present in 40% of Cabot's series.

THE SUCCESS OF ORAL LIVER

Many had wondered whether PA was the result of some inadequacy of diet; food supplements were tried with limited success, but some forays gave a flicker of hope which, alas, was not confirmed by others. George Hoyt Whipple (1878–1976) studied the rate of haemoglobin regeneration in dogs made anaemic by venesection; thereafter different foods were compared to test their efficacy in restoring the haemoglobin level. Liver was the most effective.

George Richard Minot (1885–1950), like Whipple, became interested in the role of diet in relation to anaemia, particularly PA. He spent much time taking dietary histories and was impressed by the frequency with which patients limited themselves to a restricted diet that excluded meat. He advised patients to include meat and liver in the diet and, in some patients, improvement appeared to follow. Minot (Fig 2) had been shown a method of counting reticulocytes by James Homer Wright (1869–1938) that was useful for assessing blood responses and he persuaded a colleague, William Murphy, to join him. In 1923, they started a

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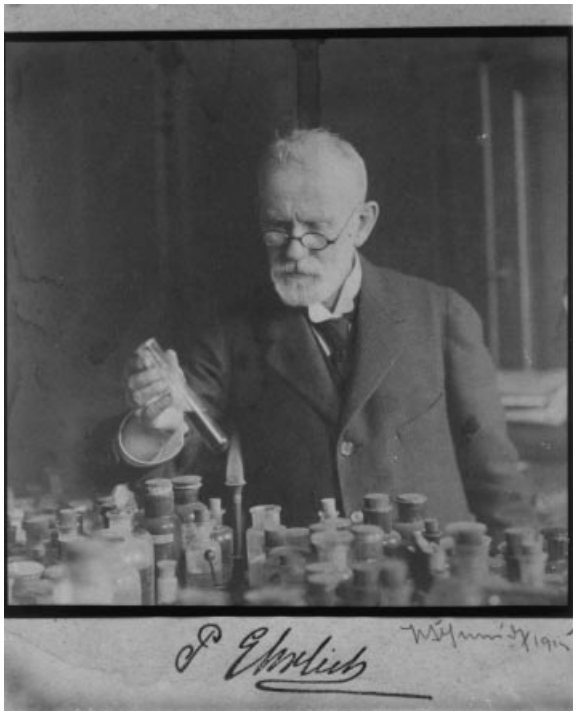


Fig 1. Paul Ehrlich (Wellcome Institute Library, London).

Fig 2. George Richard Minot (Wellcome Institute Library, London).

regimen that included 100–240 g of liver and 120 g of 'muscle meat', leafy vegetables, fruit, eggs and milk taken daily. Some patients noted clinical improvement and their reticulocytes increased within 4–5 d followed by rises in red cell count and haemoglobin levels. It took them 2 years to treat 45 patients who could tolerate the diet and all these responded (Minot & Murphy, 1926).

In 1934, the Nobel Prize in medicine and physiology was awarded to Whipple, Minot and Murphy. Was there ever an award more deserved? They saved the lives of their patients and pointed the way forward for further research.

What was there in liver that was lacking in patients with PA? The effect of liver in restoring the anaemia in Whipple's iron-deficient dogs was by supplying iron which is abundant in liver.

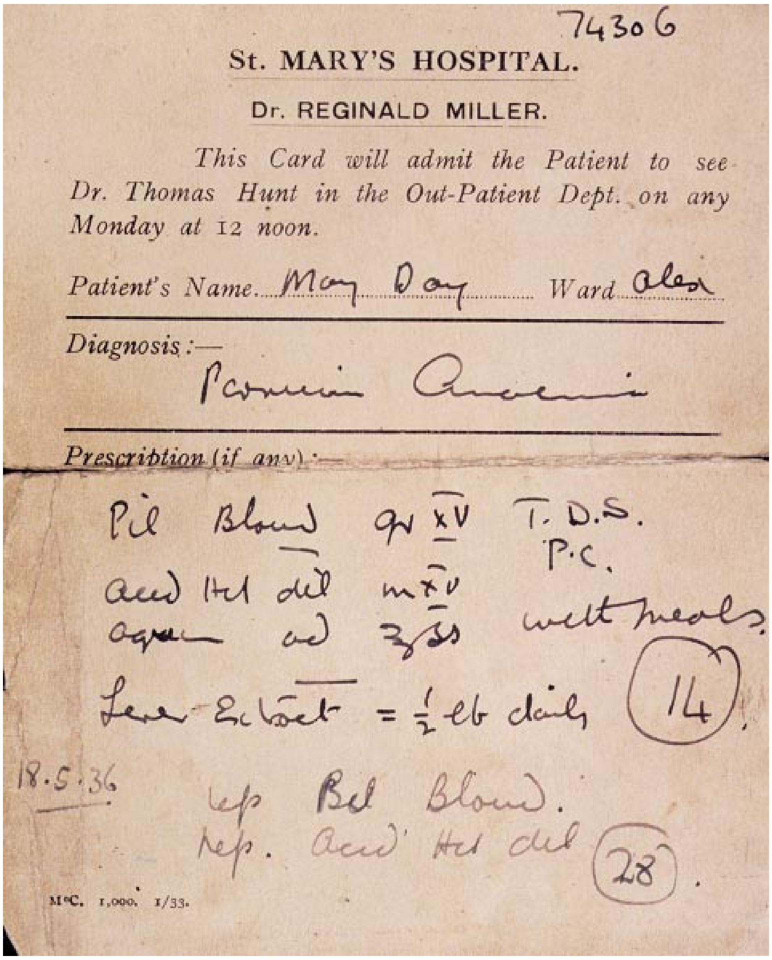
Liver given by mouth also provides Cbl and folic acid. But patients with PA cannot absorb Cbl, although some 1% of an oral dose can cross the intestinal mucosa by passive diffusion; this, presumably, is what happened when large amounts of liver were eaten. Beef liver contains about 110 μg of Cbl per 100 g and about 140 μg of folate per 100 g. Cbl is stable and generally resistant to heat; folate is labile unless preserved with reducing agents. The daily requirement of Cbl by man is l-2 μg. The liver diet, if consumed, had enough of these haematinics to provide a response in most MAs.

Soon, liver concentrates were developed that could be given by mouth (Fig 3) and, subsequently, by injection, and rescued patients from a liver diet that, indeed, was horribly unpalatable. However, sensitivity to the animal protein present in the liver extract given by injection was the next problem and was only overcome when its use was replaced by the pure antianaemic factor.

The availability of liver extracts brought about interest in the nature of the haematological response. An optimal response required a peak rise of reticulocytes 5–7 d after the injection of liver extract and the height of the peak was greatest in those with severe anaemia; the flood of reticulocytes was as a result of a synchronous maturation of a vast number of megaloblasts into red cells. There is a steady rise in the red cell count to reach 3×10^{12}/l in the 3rd week (Minot & Castle, 1935). Many liver extracts did not have enough antianaemic factor to achieve this and some assayed by the author had only 1–2 μg of Cbl.

ISOLATION OF THE ANTIANAEMIC FACTOR

It took another 22 years for a pure antianaemic factor to be isolated, although, admittedly, the Second World War intervened; in 1948, an American group led by Karl Folkers and an English group led by E. Lester-Smith published, within weeks of each other, the isolation of a red crystalline substance termed vitamin B_{12} and subsequently renamed cobalamin (Lester-Smith, l948; Rickes *et al*, 1948). The slow progress was because the fractions that were isolated from liver had to be tested for activity in untreated PA patients, although this step was bypassed in the final stages using a microbiological assay with *Lactobacillus lactis* for the antianaemic factor by the American group (Shorb, 1947).


74306

St. MARY'S HOSPITAL.

Dr. REGINALD MILLER.

This Card will admit the Patient to see Dr. Thomas Hunt in the Out-Patient Dept. on any Monday at 12 noon.

Patient's Name May Day *Ward* Alex

Diagnosis:—

Pernicious Anaemia

Prescription (if any):—

Pil Blaud gr xv T.D.S. P.C.

acid Hcl dil m xv

aqua ad ℥ss with meals

Liver Extract = ½ lb daily (14)

18.5.36

rep Pil Blaud.

rep. acid Hcl dil (28).

McC. 1,000. 1/33.

Fig 3. A prescription which includes extract of half a pound of liver daily which was renewed for 2 years. This patient stopped treatment for pernicious anaemia in 1940 and was found to have severe MA again in 1961.

STRUCTURE OF COBALAMIN

The structure of this red crystalline compound was studied by the nature of its degradation products and by X-ray crystallography. It soon became apparent that there was a cobalt atom at the heart of the structure and this heavy atom was of great aid to the crystallographers, so much so that, with additional information from the chemists, they were the first to come up with the complete structure. To quote Dorothy Hodgkin: 'To be able to write down a chemical structure very largely from purely crystallographic evidence on the arrangement of atoms in space – and the chemical structure of a quite formidably large molecule at that – is for any crystallographer, something of a dream-like situation'. As Lester-Smith (1965) pointed out, it also required some 10 million calculations. In 1964, Dorothy Hodgkin was awarded the Nobel Prize for chemistry.

However, this is not the whole story. Unknown to the scientists who had worked on the isolation of Cbl, the use of cyanide to produce cyanoCbl, as well as exposure to light, had knocked off an important structure. Barker *et al* (1958) published an account of the metabolism of glutamate by a *Clostridium*. The glutamate underwent an isomerization and an orange-coloured co-enzyme was involved that turned out to be Cbl with a deoxyadenosyl group attached to the cobalt. This Cbl co-enzyme, deoxyadenosylCbl, is the major form of Cbl in tissues; it is also extremely sensitive to light, being changed rapidly to hydroxoCbl.

DeoxyadenosylCbl is concerned with the metabolism of methylmalonic acid in man (Flavin & Ochoa, 1957). The other functional form of Cbl is methylCbl involved in conversion of homocysteine to methionine (Sakami & Welch, 1950). Both these pathways are impaired in PA in relapse.

Cbl consists of a ring of four pyrrole units very similar to that present in haem. These, however, have the cobalt atom in the centre instead of iron and the ring is called the corrin nucleus. The cobalamins have a further structure, a base, termed benzimidazole, set at right angles to the corrin nucleus and this may have a link to the cobalt atom (base-on position).

ISOTOPICALLY LABELLED COBALAMIN

By the time Cbl had been isolated from liver it was already known that it was also present in fermentation flasks growing bacteria such as streptomyces species. Other organisms gave higher yields so that kilogram quantities of pure Cbl were obtained; these sources have replaced liver

in the production of Cbl. By adding a radioactive form of cobalt to the fermentation flasks instead of ordinary cobalt, labelled Cbl became available (Chaiet *et al*, 1950). The importance of labelled Cbl is that it made it possible to carry out Cbl absorption tests in patients, to design isotope-dilution assays for serum Cbl, to design ways of assaying intrinsic factor (IF), to detect antibodies to IF and even to measure glomerular filtratration rate, as free Cbl is excreted by the glomerulus without any reabsorption by the renal tubules. It also forms the basis for many research studies related to Cbl. The most useful isotope is Cbl labelled with ^{57}Co which provides the least amount of radiation exposure, but has a scintillation spectrum with a single energy peak that provides a high degree of counting efficiency.

THE STOMACH

By the nineteenth century, it was known that there was acid present in the gastric juice and that it was not uncommon for this to be absent in PA patients (Castle, 1980). Hurst (1924) showed absence of acid in the gastric juice in PA and that it preceded the development of anaemia by years. Levine & Ladd (1921) found no hydrochloric acid in the gastric juice from 99% of 150 PA patients. Further, the volume of gastric juice in PA is much reduced and not increased by a stimulant to secretion. The association of achlohydria and PA was confirmed by better methods for stimulating gastric secretion and better ways of making a positive diagnosis of PA. The presence of acid in the gastric juice excludes a diagnosis of PA.

Atrophy of the gastric mucosa in PA in post-mortem specimens examined microscopically was reported by Samuel Fenwick at the London Hospital (Fenwick, 1870). He also found impaired digestion of egg white by an extract of the gastric mucosa in PA, in contrast with an extract of a normal stomach.

Faber & Bloch (1900) injected formalin into the abdomen or stomach immediately after death and obtained the first reliable microscopic picture of gastric atrophy involving the proximal two-thirds of the stomach, but sparing the antrum.

The modern era in the study of gastric and intestinal morphology was ushered in by the use of a flexible biopsy tube by Wood *et al* (1949) in Australia. In PA, the normal mucosa is replaced by mucus-secreting cells and there is lymphocytic and plasma cell infiltration. It should be emphasized that the stomach changes are not unique to PA and identical changes are present in persons who are otherwise healthy and do not develop PA (Chanarin, 1979). Such persons also have achlorhydria.

Gastric atrophy is accompanied by high serum levels of the hormone, gastrin (McGuigan & Trudeau, 1970), which is secreted by G-cells in the gastric antrum and duodenum. It is the major stimulant of gastric secretion. The release of acid into the gastric antrum in turn is the stimulus for inhibition of gastrin release. The achlorhydria present in PA, as well as in severe gastric atrophy without PA, results in loss of the switch-off mechanism and, hence, very high serum gastrin levels in 80% of PAs.

WILLIAM CASTLE AND GASTRIC INTRINSIC FACTOR

William Castle at the Thorndike Memorial Laboratory, Boston City Hospital, devised experiments to explore the relationship between gastric juice, the antianaemic factor that Castle assumed, correctly, was also present in beef, and the response in PA. The question Castle asked was 'Was it possible that the stomach of the normal person could derive something from ordinary food that for him was equivalent to eating liver?'.

The experiment in untreated patients with PA consisted of two consecutive periods of 10 d or more during which daily reticulocyte counts were made. During the first period of 10 d, the PA patient received 200 g of lean beef muscle (steak) each day. There was no reticulocyte response. During the second period, the contents of the stomach of a healthy man were recovered 1 h after the ingestion of 300 g of steak; about 100 g could not be recovered. The gastric contents were incubated for a few hours until liquefied and then given to the PA patient through a tube. This was done daily. On day 6 there was a rise in reticulocytes reaching a peak on day 10, followed by a rise in the red cell count. The response was similar to that obtained with large amounts of oral liver.

Control studies showed that 150 ml of normal gastric juice given alone to untreated PA patients each day did not produce any reticulocyte response. Heated gastric juice was not effective, suggesting that the factor in gastric juice was heat labile (Castle *et al*, 1930). Thus, Castle concluded that a reaction was taking place between an unknown intrinsic factor (IF) in the gastric juice and an unknown extrinsic factor in beef muscle. Whereas Minot & Murphy (1926) found that 200–300 g of liver daily was needed to get a response in PA, 10 g liver was adequate when incubated with 10–20 ml normal gastric juice (Reiman & Fritsch, 1934). Castle's extrinsic factor is the same as the antianaemic factor that is Cbl, and IF is needed for its absorption. Presumably the gastric juice in PA lacks IF.

Klein & Wilkinson (1934), as well as Abels & Schilling (1964), showed that IF is more resistant to both heat and enzyme digestion when it is combined with Cbl and this has been abundantly confirmed since (reviewed by Chanarin, 1969). The elegant studies of Hoedemaeker *et al* (1964) in Holland using autoradiography of frozen sections of human stomach incubated with [^{57}Co]-Cbl showed that IF was produced in the gastric parietal cell. The binding of Cbl to the parietal cell was abolished by first incubating the section with a serum containing antibodies to IF. The parietal cell in man is thus the source of both hydrochloric acid and IF. The parietal cell is the only source of IF in man as a total gastrectomy is invariably followed by a MA due to Cbl deficiency. IF is a glycoprotein with a molecular weight of 45 000.

An assay for human IF was described by Ardeman & Chanarin (1963) and Abels *et al* (1963). Gastric juice has two binding proteins that can link to Cbl. The one is IF and the other is the R-binder, present in all body fluids and which appears to have no known physiological role. The IF

content of gastric juice can be measured by the amount of labelled Cbl it binds, but binding to R-binder has to be subtracted; indeed R-protein is the main Cbl binder in gastric juice in PA. Thus, total binding of Cbl by gastric juice is measured. Serum containing IF antibody is added to a further aliquot of gastric juice; the antibody now occupies the Cbl-binding site on IF. Cbl added now can bind only to the R-protein. For example, 1 ml of gastric juice binds 100 ng of Cbl. After the addition of an IF antibody it only binds 20 ng of Cbl, which is a measure of R-binder uptake; the residual binding of 80 ng of Cbl is as a result of binding to IF. A unit of IF was proposed as the amount that took up 1 ng of Cbl. Thus, the hypothetical sample had an IF content of 80 units of IF per ml gastric juice. In these studies, an excess of Cbl was added and unbound Cbl was removed, the simplest method being adsorption to activated charcoal, as used by Ardeman & Chanarin (1963).

The average output of IF in gastric juice in man is about 3000 units/h in the resting state and increases to an average of 10 000 units or more/h after a stimulus of gastric juice secretion such as histamine, gastrin, etc. The output over 24 h is about 70 000 IF units or more. About 500 units are required for normal absorption of a 1 μg dose of Cbl in PA. The output in women is about 70% of that in men. Following a stimulus to gastric secretion, the output of IF reaches a peak in the first 15 min as compared with hydrochloric acid which peaks at 45 min. The early IF peak is because of a release of preformed IF. With continual infusion of a stimulus to secretion, the output of IF remains above baseline level. These studies were reviewed by Chanarin (1969,1979).

In PA there is a decline in both concentration and amount of IF. The concentration of IF in PA averaged 3 units per ml of gastric juice compared with 72 units in controls and the total output in PA over an hour is usually zero to a maximum of 200 units, too little to allow of normal absorption of 1 μg of Cbl (Ardeman & Chanarin, 1965a).

As loss of IF from the gastric juice appears to be the cause of PA, it seemed reasonable to treat patients by giving them oral IF. Preparations containing IF were prepared from hog stomach and Wilkinson (1949) claimed to have maintained all but 15 out of 441 PA patients on an oral pig-stomach preparation for 6 years. Purer preparations of IF were prepared and eventually capsules of about 100 mg of IF with 10 μg of Cbl were marketed under various names. However, both Mollin & Ross (1954) in London and Glass et al (1954) in the United States noted that the level of serum Cbl fell in these patients after about a year, followed by return of a MA, including neuropathy. In time it was shown that the relapse was as a result of the appearance of antibodies against the foreign IF.

THE SERUM COBALAMIN ASSAY

Euglena gracilis is a unicellular, chlorophyl-containing protozoon found in pond water and it has an absolute requirement for Cbl. It was used by Hutner *et al* (1949) for the assay of the antianaemic factor and the assay was modified by Ross (1950) for the assay of Cbl in human serum. Subsequently, a range of assay organisms have been used with success and, latterly, isotope-dilution methods have replaced many of the microbiological procedures. Ross found that the diluted serum had to be heated in boiling water to release Cbl from various binding proteins in serum before it could be taken up by *Euglena.* At that time, Ross was a registrar in microbiology at Hammersmith Hospital, London, and he joined forces with David Mollin in the Haematology Department.

Mollin & Ross (1952) showed that all untreated patients with PA had an abnormally low serum Cbl level. This has proved one of the major procedures for sorting the sheep from the goats. With rare exceptions of patients with abnormally high serum levels of Cbl-binding proteins, all patients with Cbl deficiency have a low serum Cbl.

However, life is never simple; low serum Cbl levels occur in the absence of any changes in the blood or elsewhere. A 'low' serum Cbl level, although always present in untreated PA, does not diagnose PA; there are other reasons for a low serum Cbl level than PA (Chanarin, 1979).

THE COBALAMIN-BINDING PROTEINS

Assay of protein fractions of serum after electrophoresis showed that endogenous Cbl is in the position of α-1 globulin. Chromatography of serum after addition of [^{57}Co]-Cbl on Sephadex G-200 showed that Cbl was attached to two proteins, one eluting before the albumin termed transcobalamin I (TCI) and the other after the albumin termed transcobalamin II (TCII). Charles Hall showed that, when labelled Cbl given by mouth is absorbed, it first appears in the position of TCII and later in the position of TCI as well (Hall and Finkler, l965). They concluded that TCII is the prime Cbl transport protein carrying Cbl from the gut into the blood and then to the liver from where it is redistributed by both new TCII as well as TCI. Congenital absence of a functional TCII causes a severe MA in the first few months of life owing to an inability to transport Cbl. Most of the Cbl in serum is on TCI because it has a relatively long half-life of 9–10 d, whereas the half-life of TCII is about 1·5 h. Thus, in assaying the serum Cbl level, it is mainly TCI-Cbl that is being assayed.

In untreated PA, the very low Cbl level on TCI enables the serum to bind more added Cbl than normal sera where TCI is relatively saturated with Cbl. TCI too, is one of the R-Cbl binders.

THE INTESTINAL ABSORPTION OF COBALAMIN

With the availability of labelled Cbl, Cbl absorption tests began to be widely used in the 1950s. The commonest method was the urinary excretion test described by Schilling (1953). Here, an oral dose of radioactive Cbl is followed by an injection of 1000 μg of cyano-Cbl. The free cyano-Cbl is largely excreted into the urine over the next 24 h and carries with it about one third of the absorbed labelled Cbl. With a 1·0 μg dose of labelled Cbl, 10% or more of the oral dose is excreted in the urine by normal subjects. But there are other even more satisfactory ways of assessing the

result, including whole body counting (when the 1000 μg injection of Cbl is omitted) and the plasma radioactivity in a sample taken about 10 h after the oral dose; these methods do not rely on the patient achieving a complete urine collection.

In PA, there is impaired absorption of Cbl and it is corrected when the test is repeated with labelled Cbl given with oral IF (Schilling, 1953). The latter part of the test indicates IF deficiency as the cause of the Cbl malabsorption. The improvement of Cbl absorption in PA when the test is done with added IF generally does not reach the level found in healthy subjects and sometimes may be so poor as to raise the question of intestinal malabsorption as the prime cause of the Cbl deficiency. This is related to the potency of IF antibodies at various sites, but it should be remembered that incomplete urine collection can also result in a misleadingly low result.

Rose & Chanarin (1971) noted that the mean result of a urinary excretion test with a 1·0 μg dose of Cbl in 32 control subjects was 19·2% (range 11·2–32·0%). In 29 patients with PA, the mean urinary excretion result with added IF was 12·8% (range 3·8–23%).

Further analysis of these results indicated that, in the six PAs where IF antibody was absent from both serum and gastric juice, the results were similar to controls with a mean urinary excretion of 19·3%. Among another six PA patients, where antibody was present only in serum, the mean urinary excretion was 14·4%. In 10 PA patients with antibody present only in gastric juice, the mean urinary excretion with IF was 11·1%, and, finally, in seven PA patients where IF antibody was found in both serum and gastric juice, the urinary excretion test result with IF was 8·4%. Thus, the more abundant the IF antibodies the poorer is the response to added IF.

There is a further reason why the absorption of Cbl with added IF in PA may be poor. The site of Cbl absorption in man is the distal ileum (McIntyre *et al*, 1956; Booth & Mollin, 1957). In untreated PA, it is many years since the ileal receptors for the IF–Cbl complex have seen as much as a molecule of IF and it can take several months for poor absorption of the IF–Cbl complex to be restored. This state of affairs has been termed 'transient' Cbl malabsortion and occurs in a small proportion of patients with long-standing Cbl deficiency, not only in PA but also in nutritional Cbl deficiency in life-long vegetarians, etc. The ileum is also the source of the TCII that carries Cbl into portal blood (Chanarin *et al*, 1978).

PERNICIOUS ANAEMIA AS AN AUTOIMMUNE DISEASE

Association with other autoimmune diseases

There is a high frequency of PA among those disorders that have antibodies against the target organ. Thus, among 286 patients with myxoedema, 9·0% also had PA (Chanarin, 1979), as compared with a frequency of PA of about 1 per 1000 (0·01%) in the general population. Of 102 consecutive patients with vitiligo, eight also had PA.

The association is strongest among the rarer disorders. Thus, 12 out of 31 patients (39%) with acquired hypogammaglobulinaemia also had PA. Six cases with both PA and pure red cell aplasia have been reported. In the polyendocrinopathy syndrome starting in the first or second decade of life, PA, hypoparathyroidism, mucocutaneous candidiasis and Addison's disease manifest themselves and all show appropriate antibodies.

Parietal cell and thyroid antibodies

Parietal cell antibodies (Taylor *et al*, 1962) are present in serum in 76–93% of different series of PAs and in the serum of 36% of the relatives of PA patients. The antibody is present in sera from 32% of patients with myxoedema, 28% of patients with Graves' disease, 20% of relatives of thyroid patients and 23% of patients with Addison's disease. Parietal cell antibodies are found in between 2–16% of controls, the high 16% figure being in elderly women. There is a higher frequency of PA in women, the female to male ratio being 1·7 to 1·0. The parietal cell antibody is probably important in the production of gastric atrophy.

Thyroid antibodies are present in sera from 55% of PAs, in sera from 50% of PA relatives, in 87% of sera from myxoedema patients, in 53% of sera in Graves' disease and in 46% of relatives of patients with thyroid disease.

Intrinsic factor antibodies

Studies on why patients had become refractory to oral treatment with hog IF preparations showed that their sera, when given by mouth with hog IF to healthy volunteers, prevented Cbl absorption in a urinary excretion test. The activity was in the globulin fraction of the serum (Schwartz, 1958). Had an IF antibody developed?

Further, oral administration to normal subjects of radioactive Cbl with 10 ml of serum from PA patients who had not been exposed to hog IF, also inhibited the absorption of Cbl by these volunteers. This was the case with sera from 18 out of 52 patients with PA (Taylor, l959; Schwartz, l960). The IF antibody in the PA serum had neutralized the human IF. These *in vivo* observations were translated into an *in vitro* test by Ardeman & Chanarin (1963). They showed that the binding of labelled Cbl to IF in gastric juice was inhibited by PA sera that had antibody to IF. This was the case with 325 sera out of a total of 605 PA sera tested (54%) in 10 published series (Chanarin, 1969).

Intrinsic factor antibody in gastric juice

Fisher *et al* (1966) found free IF antibody in five out of 14 gastric juice samples from PA patients. Rose and Chanarin (1969) dissociated IF antibody from IF–antibody complexes in gastric juice and identified IF antibody in 16 out of 28 gastric juice samples from PA patients. Eight patients who did not have a serum antibody had an antibody in the gastric juice. All three samples in gastric juice that were tested were IgA immunoglobulins and Goldberg *et al* (1968) showed that such an antibody had the secretory end piece characteristic of an intestinal antibody. Serum antibodies are almost invariably IgG immunoglobulins. All this points to the stomach as the source of the intestinal antibodies.

Camilleri *et al* (1973) showed that there was an excess of immunoglobulin-staining cells in the gastric mucosa in PA.

Cell-mediated immunity to intrinsic factor

Patients with acquired hypogammaglobulinaemia are unable to make humoral antibodies; nevertheless, one-third have PA as well. This cannot be as a result of action of IF antibodies and must be because of specific cell-mediated immunity. Tai & McGuigan (1969) demonstrated lymphocyte transformation in the presence of IF in six out of 16 PA patients and Chanarin & James (1974) found 10 out of 51 tests were positive.

Greater success was obtained by Chanarin & James (1974) with a test for migration inhibition factor where migration of white blood cells from the end of a glass capillary tube is measured in the presence of antigen. This test was positive in 27 out of 39 PA patients tested. All controls were negative. In all, 86% of patients with PA had a positive result for cell-mediated immunity against IF.

All four patients with hypogammaglobulinaemia and PA tested had cell-mediated immunity to IF.

Twenty-five patients with PA were tested for the presence of humoral IF antibody in serum and gastric juice and for cell-mediated immunity against IF. All but one gave positive results in one or more tests. It was concluded that these findings establish the autoimmune nature of PA and that the immunity is not merely an interesting byproduct. These tests are often capricious in performance and the failure to get a positive in one out of 25 PA patients is probably technical (Chanarin and James, 1974).

THE EFFECT OF TREATING PERNICIOUS ANAEMIA WITH STEROIDS

Patients with PA treated with steroids show a reversal of the abnormal findings characterizing the disease. If they are still megaloblastic, the anaemia will respond in the first instance (Doig *et al*, 1957), but in the longer term Cbl neuropathy may be precipitated. The absorption of Cbl improves and may become 'normal' (Frost & Goldwein, 1958). There is a return of IF in the gastric juice (Kristensen and Friis, 1960) and a decline in the amount of IF antibody in serum (Taylor, 1959). In some patients there is return of acid in the gastric juice. Gastric biopsy shows a return of parietal and chief cells (Ardeman & Chanarin, 1965b; Jeffries, 1965). All this is as a result of suppression of cell-mediated immunity against the parietal cell and against IF. Withdrawal of steroids leads to a slow return to the status quo.

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The author has dipped freely into the two volumes by the late M. M. Wintrobe. These are: Wintrobe, M.M. (1985) *Hematology, the Blossoming of a Science*. Lea & Febinger, Philadelphia, and Wintrobe, M.M. (ed.) (1980) *Blood, Pure and Eloquent*. McGraw-Hill, New York.

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