Occasional Review

Vitamin B_{12}: an area of darkness

D M MATTHEWS, J C LINNELL

British Medical Journal, 1979, **2**, 533-535

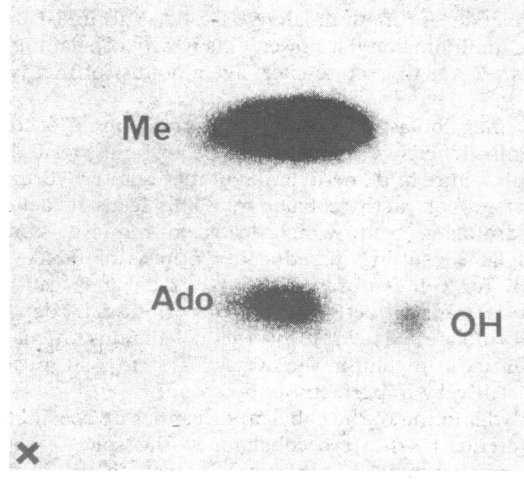
If a doctor prescribes "vitamin B_{12}," as many still do, his patient may well receive cyanocobalamin, a compound admittedly effective in the commoner forms of "B_{12}-deficiency," but no more effective than hydroxocobalamin and possibly actually harmful in certain conditions. This is because strictly vitamin B_{12} and cyanocobalamin remain synonymous. Ignorance and confusion about the several forms of vitamin B_{12} and their functions are widespread, while most of the inborn errors of vitamin B_{12} metabolism that have now been described are unknown to many doctors, even to paediatricians. In short, the whole subject of vitamin B_{12} has become an area of darkness in the medical mind.

The researches of the past ten years, described and summarised at the Third European Symposium on Vitamin B_{12} and Intrinsic Factor[1] held in Zurich in March, have shown that this continuing confusion is quite unnecessary, and we have written this summary of present views in an attempt to reduce it. If the reader finds our account of interest and remembers at the end of it two facts (1) that hydroxocobalamin, *not* cyanocobalamin, is the form of vitamin B_{12} most suitable for therapeutic use and (2) that a "normal serum (or plasma) B_{12}" value from a reliable laboratory does *not* guarantee that the patient is not B_{12}-deficient, or indeed that his vitamin B_{12} metabolism is not grossly disordered, we shall be well satisfied.

Historical background

The present state of confusion has arisen for historical reasons. The long quest for the "antipernicious anaemia factor" in liver seemed to have ended in 1948 when pure cyanocobalamin was isolated in the form of red crystals. Cyanocobalamin was assigned the next free number on the list of B vitamins and so became synonymous with vitamin B_{12}. It was very active therapeutically when given intramuscularly in pernicious anaemia and the other common forms of B_{12}-deficiency known then, and was non-toxic in extremely high doses. There followed a sort of golden age in which innumerable papers were published on methods of estimating serum B_{12} microbiologically, normal values were established, and pathological values were reported for almost every condition under the sun. Meanwhile, the mechanism of absorption of cyanocobalamin was intensively studied, and tests of its absorption suitable for clinical use were devised. Few of us in those days would have doubted that cyanocobalamin (possibly mixed with a little of its photolytic breakdown product, hydroxocobalamin) was the form of vitamin B_{12} present in the body, or that this compound was what we were estimating in serum. Yet in both these assumptions we were totally wrong.

The picture began to change in the early 1960s, when two coenzyme forms of cobalamin were found in the animal body, adenosylcobalamin in the liver by Barker and his colleagues and methylcobalamin in plasma and liver by Lindstrand and Ståhlberg. Since then it has been shown[2-4] that the bodies of man and animals contain three main cobalamins: (1) hydroxocobalamin (or very closely related compounds, or both), (2) adenosylcobalamin, and (3) methylcobalamin. Cyanocobalamin is present only in traces and is often undetectable. Its biochemical significance, if any, is uncertain. The reason why cyanocobalamin was the form in which vitamin B_{12} was first isolated was merely that the techniques used for isolation tended either to eliminate other cobalamins, or to convert them to cyanocobalamin, which is relatively stable. The proportions of the different cobalamins vary considerably among organs and in different species of animal. In man, however, blood plasma



Individual cobalamins in a typical sample of normal human blood plasma. Sample contains a large proportion of methylcobalamin, a smaller proportion of adenosylcobalamin, and a small proportion of hydroxocobalamin. No cyanocobalamin was detectable. The cobalamins were separated by thin-layer chromatography and located by bioautography using a cobalamin-dependent strain of *Escherichia coli*.[1] They can be quantitated by photometric scanning. X marks origin of chromatogram.

Department of Experimental Chemical Pathology, Vincent Square Laboratories of Westminster Hospital, London SW1V 2RH
D M MATTHEWS, MD, PHD, professor of experimental chemical pathology
J C LINNELL, PHD, MRIC, lecturer in experimental chemical pathology

contains mainly methylcobalamin (mean about 70%), the remainder being adenosylcobalamin and hydroxocobalamin (see figure 1). Human liver contains mainly adenosylcobalamin and hydroxocobalamin, with a small percentage of methylcobalamin. In other organs adenosylcobalamin and hydroxocobalamin predominate, though methylcobalamin is never absent, and in some organs, such as the spleen, the proportion of methylcobalamin is as high as 35-40%.

Structure of cobalamins

The structure of the various cobalamins, and their general functions, are not hard to describe. All cobalamins contain a corrin ring and a nucleotide, as depicted in relevant textbooks. The individuality of each cobalamin is determined by the nature of the chemical group attached to the central cobalt atom of the corrin ring on the opposite side to the nucleotide. In cyanocobalamin this is a cyano group, CN, in hydroxocobalamin, a hydroxyl group, OH, and in methylcobalamin, a methyl group, CH_3. Finally, in adenosylcobalamin it is a large and complex adenosyl group. Functionally, hydroxocobalamin may be regarded as a precursor of the two coenzyme forms, or an intermediary between them. In vitro, it may also be produced by photolysis of the coenzyme forms, which are very sensitive to light. Methylcobalamin is concerned in transmethylation reactions, accepting a CH_3 group from one compound and transferring it to another; for example, in the presence of the enzyme methionine synthetase it accepts methyl groups from methylfolate and transfers them to the amino-acid homocysteine to give methionine. Adenosylcobalamin is concerned in isomerisation reactions, which are intramolecular rearrangements; for example, it is required for the conversion of methylmalonyl-CoA to succinyl-CoA by the enzyme methylmalonyl mutase. This is why cobalamin-deficient patients are apt to excrete excessive amounts of methylmalonic acid.

The megaloblastic failure of haemopoiesis occurring in cobalamin deficiency in man appears to be associated in large part with failure of demethylation of methyltetrahydrofolate due to deficiency of methylcobalamin.[5] This leads to deficiency of tetrahydrofolate itself, which leads to reduced synthesis of thymidylate, resulting in reduced synthesis of DNA, which is essential for cell maturation. (Folate deficiency causes megaloblastosis more directly, naturally resulting in deficiency of tetrahydrofolate). The role of the cobalamins in maintaining the integrity of myelin in the nervous system is, it must be confessed, still very imperfectly understood.

Bodily handling of the cobalamins cannot be considered without reference to the transcobalamins, the specific proteins to which they are bound in plasma. In man there are two main transcobalamins, TC I and TC II. Cobalamins attached to TC II, though only a very small proportion of total plasma cobalamins, have a much more rapid turnover than cobalamins attached to TC I. TC II may be essential for cellular uptake of cobalamins, and TC II deficiency induces megaloblastic anaemia, whereas TC I deficiency appears to have no serious effect.

Fortunately, the realisation that very little of the cobalamin in food is in the form of cyanocobalamin has not invalidated the work on intestinal absorption carried out with this compound, since probably all cobalamins are absorbed in a basically similar way: there are special mechanisms by which they are combined with intrinsic factor, the cobalamin/IF complex taken up by the ileal mucosa, and the cobalamin transferred to the blood.

Possibility of inborn errors

The above outline should make it easier to see that many inborn errors in cobalamin metabolism are possible, such as the following.

Failure of intestinal absorption of cobalamins may result from defective secretion of intrinsic factor ("juvenile pernicious anaemia") or from a defect in the ileal mechanism for absorption of the cobalamins. These conditions are associated with low total plasma cobalamin (B_{12}) concentrations and megaloblastic anaemia.

Defective production of one of the transcobalamins—In the case of defective production of TC I the total plasma cobalamin is low but there are no signs of cobalamin deficiency. In the case of TC II deficiency the total plasma cobalamin is within normal limits, but the patient is liable to megaloblastic anaemia.

Failure of tissue uptake of cobalamins, or defective synthesis of one or both of the cobalamin coenzymes—Failure of tissue uptake of cobalamins as distinct from TC II deficiency has not been described. Defective synthesis of adenosylcobalamin leads to methylmalonicaciduria, which may be reduced by treatment with hydroxocobalamin. Total plasma cobalamin is within normal limits, but analysis of the plasma or red cells for individual cobalamins shows that adenosylcobalamin is abnormally low.

Defective synthesis of both adenosylcobalamin and methylcobalamin, probably due to a defect in the metabolism of a common precursor, occurs in mild to severe forms. In the mildest form, the patient may show nothing apart from possible slight mental retardation. In the most severe form the condition is potentially lethal at an early age. There is megaloblastic anaemia, crippling neurological damage with dementia, methylmalonicaciduria, and homocystinuria. What is so deceptive about the dual defect of coenzyme synthesis is that just as in defective synthesis of adenosylcobalamin alone and TC II deficiency, total plasma cobalamin remains within normal limits, despite the fact that the tissues are grossly cobalamin-depleted, and analysis of the plasma or red cells for individual cobalamins shows an abnormal pattern with reduction of coenzyme forms and excess hydroxocobalamin. Treatment with massive doses of hydroxocobalamin may produce improvement. An isolated defect of synthesis of methylcobalamin has not yet been described.

We have made no attempt to describe the many investigations, some very specialised, that may be required to elucidate the details of an inborn error of cobalamin metabolism; international collaboration is often necessary. Nor have we space to describe all the conditions in which the pattern of plasma and tissue cobalamins is altered. Before concluding, however, we should add that there are several conditions in which plasma cyanocobalamin, normally either undetectable or present as less than 8% of total plasma cobalamins, is raised, sometimes to values as high as 35%. These include tobacco amblyopia, Leber's optic atrophy, and dominantly inherited optic atrophy. In tobacco amblyopia there is an increase in cyanide intake due to the cyanide content of tobacco smoke, though normal smokers do not have a significant increase in plasma cyanocobalamin. Hydroxocobalamin is concerned in the detoxication of cyanide by conversion to cyanocobalamin, and possibly in all these conditions there is a derangement of cyanide metabolism. Some of the optic neuropathies appear to respond to massive doses of hydroxocobalamin, and have been claimed to be adversely affected by administration of cyanocobalamin. Common sense would suggest the inadvisability of raising by a very large factor a plasma cyanocobalamin concentration that is already abnormally high. After therapeutic administration of cyanocobalamin, increased concentrations of this compound persist in the plasma for some days or weeks, so that if a patient's plasma cyanocobalamin is high, the possibility that the condition is iatrogenic must be considered.

Finally, we should say that cases of serious discrepancies between the results of microbiological and radioisotopic assays for total cobalamins are now giving rise to controversy and concern.[6] We think that every laboratory should specify on its reports the assay method used to obtain its values for total cobalamins, and that the term "vitamin B_{12}" should be abandoned and replaced by "the cobalamins."

Our work on distribution and metabolism of cobalamins has been

generously supported by grants from the Wellcome Trust. The title of this article was suggested by the title of a book by V S Naipaul.

References

[1] *Proceedings of the Third European Symposium on Vitamin B_{12} and Intrinsic Factor*, ed B Zagalak and W Friedrich. Berlin, W de Gruyter, 1979. In press.
[2] Matthews, D M, and Linnell, J C, *The Cobalamins*, ed H R V Arnstein and R J Wrighton, p 23. Edinburgh, Churchill Livingstone, 1971.
[3] Linnell, J C, *Cobalamin: Biochemistry and Pathophysiology*, ed B M Babior, p 287. New York, John Wiley, 1975.
[4] Matthews, D M, *Proceedings of the Third European Symposium on Vitamin B_{12} and Intrinsic Factor*, ed B Zagalak and W Friedrich. Berlin, W de Gruyter, 1979. In press.
[5] Das, K C, and Herbert, V, *Clinics in Hematology*, 1976, **5**, 697.
[6] England, J M, and Linnell, J C, *Proceedings of The Third European on Vitamin B_{12} and Intrinsic Factor*, ed B Zagalak and W Friedrich. Berlin, W de Gruyter, 1979. In press.

(*Accepted 4 July 1979*)

MATERIA NON MEDICA

Lice on the ocean wave

Fifty years ago an 8000-ton liner steamed through the Red Sea, homeward bound from India, carrying a full complement of passengers. Many were teaplanters and their families having a spell of home leave. A large swarm of locusts flew overhead going from east to west bent on devastation in north-east Africa. A number landed on board and were captured as pets for the children. Peel an orange and the locusts will do the rest. Several mothers were frightened of what their children might catch in spite of my assurance that locusts produced only famine, of which there was no danger on board. The locusts died fairly quickly or else flew away.

Shortly afterwards, when we were in the Mediterranean, an anxious mother told me that some young insects, very small, had hatched out on some cushions in the second saloon. I was thrilled and bewildered as any amateur entomologist might be. Inspection revealed the new generation to be not infantile locusts but head lice. This presented one of those teasers that ships' surgeons have to get used to. My problems were: (1) To find the carriers without offence. (2) To treat them also without offence (those were the days of sassafras). (3) To clean and disinfect the second saloon. (4) To stop adverse gossip.

I consulted, in order, Vavaseur Elder's excellent *Ships' Surgeons Handbook*, which gave no help; the purser, who was highly amused; and the captain, who was furious that anything so degrading should occur in his ship.

I put up a notice saying that some insects had emerged in the saloon that might get into people's hair and that I wished to carry out an inspection of heads. Most mothers came along straight away with their progeny. My eye was on three teenagers and their mother who did not seem very co-operative—all their heads were crawling with *Pediculus capitis*. I soaked their heads in sassafras and banished them to the stern for the rest of the day, where the west wind we were sailing into could diseminate the smell and relieve their embarrasment. I closed the saloon, had it cleaned out, and then—all doors and windows hopefully sealed—I lit my first and last sulphur candle, just escaping before being overwhelmed by SO_2 fumes. The next day life resumed its normal sway as it always does on board ship and the matter was forgotten. I do not think that many of the passengers swallowed the idea that the infestation had come from locusts, but so far as I know the ship maintained its high reputation for cleanliness. I hope it did, she was a happy ship. That was in 1929. I last saw her as I was leaving Colombo Harbour in 1945. She looked well though a trifle older.—J C HAWKSLEY (retired physician, East Kennett, Wiltshire).

A 1979 Irish victory

"After all, we beat the Welsh last year—battered them into submission—holders of the Triple Crown. They took it very badly. And we did go on to thrash the All Blacks in the third test in New Zealand—Queensland has beaten all-comers in the last two years and they are the backbone of the Australian team." Thus spake not Zarathustra, but the Rugby Union experts before the arrival of the Irish Fifteen due for their first visit to Australia for many years.

Feelings towards the Irish in Australia are coloured by so many variants: many Irishmen arrived as migrants or convicts in the early times in the new colony; Northern Ireland, so far away with its much publicised persistent violence; Irish bar-room jokes with their taunting allusions to Irish simpletons and simplicities. So, when the pride of Ireland ran on to the biased turf of Ballymore in Brisbane, white legs matching their white shorts, yet complementing the startling green jerseys, spectators, used to sunkissed, bronzed Australian brawn, wondered how they could possibly survive.

Eighty minutes later, an at first grudgingly admiring crowd had seen their gladiators soundly beaten by those same fragile-looking invaders, with the help of the boot of a five-eighth by the name of Campbell—surely of Scottish background. This victory was to be repeated just two weeks later in Sydney—again that same Campbell boot kicked a loud death knoll.

An Australian Rugby Union correspondent asked Tony Shaw, the Australian Captain, what had happened to the tough, fast, furious, all-hating, all-conquering pack that had subdued the astonished Welsh the previous year. "It's very difficult to work up a hate towards such a nice bunch of guys."

No ugly forward brawls: no referee baiting: no broken jaws: no aftermatch accusations. Surely this is what International Rugby Union is all about.—J P COLQUHOUN (Cleveland, Queensland, Australia).

Aikido

For a long time I have taken up an entirely new interest every five years or so. In the past these have included swimming, gastronomy, motoring in Europe, colour photography, Scottish country dancing, and, most recently, Victorian oil paintings. This time I selected Aikido.

When a new sports centre opened last year near my home I wrote to the director to say that I should like to have some instruction in self-defence, mentioning that I was no longer in my first youth. In his reply he said that aikido might meet my requirements. He described aikido as a Japanese martial art which is non-competitive and non-aggressive. It is based on the principle of harmony, and strength does not play a significant part. It is a relatively recent Japanese martial art and was introduced by Morihei Uyeshiba about 60 years ago.

Following a talk with the Aikido coach, I attended a practice session as a spectator. The group numbered 20, the average age being about 22 years. I was encouraged, however, by a notice on the gymnasium door which announced that all ages between 9 and 90 were welcome. This has since been changed to 12 to 90. My main impression of this practice was sheer amazement that major joint and bone injuries did not occur frequently. I was assured, however, that such accidents were extremely rare and were mainly due to faults in technique, particularly failure to relax. By this time it was apparent that Aikido was extremely complex. I asked several people how long it took to achieve a reasonable level of performance and was given widely differing views, the longest being 30 years.

I decided, in spite of all this, to attend a few practice sessions as a participant. These were then held weekly and later this was increased to twice weekly. I found all the members of the group, and particularly the coach and his deputy, extremely helpful and pleasant. To begin with, I often left the class limping and conscious of pains in various joints and muscles of which I had not been aware for many years. Gradually, however, things improved and there were no ill effects except when I tried something outside my very limited repertoire. After more than a year, I feel fitter and more mobile than I did when I started and I look forward with enthusiasm to each practice session. 90 seems a long way off.—ARTHUR SUTHERLAND (gynaecologist, Glasgow).