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The Laboratory Diagnosis of Megaloblastic Anemias

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The diagnostic approach to megaloblastic anemia involves four usually sequential steps. The first step, recognition of megaloblastosis, requires attention to altered blood cell size and morphology. These changes may sometimes be subtle or masked. The cornerstone of the second step, identification of the specific vitamin deficiency, is assay of serum vitamin B_{12} and folic acid levels, although they may occasionally be misleading. The third step, identification of the specific disease entity responsible for the vitamin deficiency, generally revolves around tests of absorption and gastric function. The fourth step, reevaluation after replacement therapy, is often not thought of as a diagnostic step but carries important diagnostic implications and is sometimes the only way in which coexisting abnormalities can be unmasked and identified.

THE READY AVAILABILITY of vitamin assays has greatly simplified the diagnosis of megaloblastic anemias. However, the identification and therapy of vitamin deficiency both now appear so easy that the approach to megaloblastic anemia has come to be routine and occasionally even careless. Therefore, for example, a review of patients with vitamin B_{12} deficiency who were admitted to a Midwestern, university-affiliated, voluntary hospital over a four-year span showed incomplete investigation or incorrect diagnosis in approximately a third of cases in which the vitamin deficiency was identified by assay (unpublished data). A common error was to automatically label all cases of vitamin B_{12} deficiency as pernicious anemia, usually without resorting to a Schilling test but in several instances even when Schilling test results were incompatible with such a diagnosis. In this setting, significant and specifically treatable causes such as ileitis, intestinal bacterial overgrowth and sprue were sometimes not diagnosed. While management of folic acid deficiency in the same population was not similarly analyzed, folate-deficient patients rarely received further studies by their physicians for malabsorption or other diseases. They were simply given folic acid supplementation.

Perhaps more serious is the recent documentation that managing physicians often disregard

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evidence of possible vitamin deficiency uncovered by assays that they had themselves requested.[1] I have encountered this phenomenon frequently also, particularly if the assay result was not extremely low. Unfortunately, in a few cases the deficiency was thereby allowed to progress, sometimes to significant neurologic disability in the case of vitamin B_{12} deficiency.

The purpose of this review is to outline the armamentarium available for the optimal laboratory diagnosis of megaloblastic anemia, with emphasis on problems in interpreting results and on causes of misleading results. Well-established, reasonably accessible laboratory tests will be presented, as well as several other tests deserving wider attention. Since laboratory errors are inherent in any procedure, only common errors will be mentioned.

The diagnostic approach to megaloblastic anemia can be divided into four usually sequential steps.

1. Recognition of Megaloblastosis

Blood count and morphology usually establish the diagnosis of megaloblastic anemia. Occasionally, however, the picture may be modified or mild, and recognition may depend on active search for subtle morphologic changes. In general, the severity of megaloblastic morphologic abnormality is proportional to the severity of the anemia, and early megaloblastosis may manifest only very mild changes.[2-4] Consequently, 6-lobed neutrophils may be absent, and only an increase of 4-lobed or 5-lobed neutrophils may be evident.[2] Partially or incorrectly treated megaloblastosis may also look relatively normal morphologically, although Herbert[5] has stated that incorrectly treated megaloblastosis never looks completely normal.

A normal, or even low, mean corpuscular volume (MCV)—often resulting from microcytes coexisting with macrocytes—and masking of abnormal red cell morphology in bone marrow and peripheral blood is not unusual with coexisting iron deficiency[2,6,7] though white cell changes are not thus masked. However, the MCV is otherwise a valuable screening tool and can occasionally uncover megaloblastosis in a patient whose hemoglobin level is still normal. Of course, macrocytosis occurs in nonmegaloblastic states also, but examination of cell morphology is often sufficient to differentiate those from megaloblastosis.

TABLE 1.—*Usual Serum and Red Cell Assay Results in Vitamin B_{12} and Folate Deficiency States*

Serum Vitamin B_{12}	*Serum Folate*	*Red Cell Folate*	*Status*
N	N	N	Normal
N	↓	N	Normal,* or early folate deficiency
N	↓	↓	Folate deficiency
↓	↓	↓	Folate deficiency, or combined folate and B_{12} deficiency
↓	N-↑	↓-N	Vitamin B_{12} deficiency

N = normal levels; ↓ = low levels; ↑ = elevated levels

*But usually with recent poor dietary intake.

Megaloblastic changes found in erythroleukemia, various acute and chronic leukemias, and some refractory anemias, and with antineoplastic drugs, may occasionally be impossible to differentiate from vitamin B_{12} or folate deficiency by morphology alone. The white cell changes may tend to be less classical (and in the leukemias and refractory anemias may be absent) and polyploidy may be more prominent in the conditions not associated with vitamin deficiency, but firm data on these aspects are lacking. It has also been suggested that vitamin-deficient megaloblasts tend to have a clockface chromatin pattern which is not seen in megaloblasts in some other conditions.[8]

Neutrophil hypersegmentation has been reported in iron deficiency[9,10] and renal failure.[3] Whether subtle folic acid or vitamin B_{12} abnormalities in fact coexisted in most such cases is unsettled. Congenital neutrophil hypersegmentation has also been described[11,12] and may possibly occur in 1 percent of the population.[13] Conversely, it has been claimed that neutrophil hypersegmentation may not necessarily accompany folate deficiency in pregnancy[14] and rare patients with the Pelger-Hüet anomaly presented with 3-lobed or 4-lobed neutrophils rather than 6-lobed ones when megaloblastic.[15]

2. Identification of the Specific Vitamin Deficiency

The development of specific vitamin assays has greatly simplified matters because vitamin B_{12} and folic acid deficiency can be differentiated neither morphologically nor, often, clinically. However, serum levels do not always reflect body stores and are affected by many factors. Further confusion may result from the interactions between serum vitamin B_{12} and folic acid levels which

TABLE 2.—*Misleading Serum Folate Results**

Misleadingly Low Levels

Apparently normal persons.[17]

Poor dietary intake, without actual folate deficiency.[4,18]

Improper handling of blood sample (such as storage without freezing).

Drugs affecting assay: antibiotics,†[19-23] 5-fluorouracil,†[24] and possibly others.

Drugs not affecting assay:‡ acetylsalicylic acid,[25,26] alcohol,[27] ? oral contraceptives (see reference 28 for review).

Abnormality of serum folate-binding proteins:§ may give low results in pregnancy, oral contraceptive use, uremia and the like, with some radioassay systems but not others.[29,30]

Radioisotopic contamination of serum§ (for example, tracer injection for gallium or technetium scans).[31]

Misleadingly Normal or Raised Levels

Ingestion of folate-rich food[13] or vitamin supplementation.

Hemolysis of blood sample (red cell folate content greatly exceeds serum content).

Coexisting vitamin B_{12} deficiency[16,32] ("methyltetrahydrofolate trap" hypothesis,[16] vitamin B_{12}-dependent entry of folate into red cells[33]).

Disturbances in folate metabolism due to some drugs.[34]

Inborn errors of folate metabolism.[35]

Presence of methotrexate in serum.§[24,36]

*This information has been established for microbiological assay. Unless otherwise specified, it is assumed to be true for radioassay results also, but in some instances that assumption is untested.

†True for microbiological assay only.

‡The mechanisms of the drug effects are unknown, nor does true folate deficiency apparently result.

§True for radioisotopic assay only.

reflect in good part their metabolic interactions.[16] Sometimes overlooked too is the coexistence of both vitamin deficiencies. Therefore, determination of both vitamins simultaneously is often desirable. Table 1 shows the usual results of this approach and illustrates the occasional usefulness of red cell folate assay. Nevertheless results can obviously be inconclusive even with all three tests combined.

Serum Folate

The possible pitfalls in interpreting serum folate results are outlined in Table 2. Most of the accumulated experience has been with the microbiologic assay, the most common using Lactobacillus casei.[20] Because of the cumbersomeness of microbiologic assay and the inhibitory artifact induced by antibiotic therapy, many radioisotopic assays have been developed and marketed commercially as kits. Unfortunately, great differences exist among the isotopic methods, and many clinical laboratories have prematurely adopted and even modified kits without adequate testing.[37] The effect and significance of many variables remains controversial, and sufficient experience in comparing results to the standard microbiologic assay and to clinical data is lacking. Nevertheless, while the routine clinical use of folate radioassay is regrettable, the ultimate advantage of a reliable isotopic assay is clear.

The microbiological assay too is subject to many technical variables and results differ among laboratories.[32,38] In fact, in some cases the "falsely" low radioisotopic assay folate level due to serum folate binder abnormality may possibly reflect the true folate status more accurately than does the normal microbiologic assay level.[29] The current status of radioisotopic folate assay has been recently reviewed.[37,39]

It bears emphasizing that low serum folate levels need not, and often do not, mean actual folate deficiency and should be interpreted cautiously. Low levels have been found in apparently healthy control subjects.[17] Too, an early rapid fall in serum folate, appearing not to reflect true depletion of body stores, has been described in subjects on a folate-poor diet.[4,18] The mechanism for the fall appears to be the cessation of exchange between tissue stores and absorbed folate, and may at least partially explain the unusually great incidence of low serum folate levels in patients in hospital.[32] Such patients apparently require only reinstitution of a normal diet, but will progress to true folate deficiency if the poor diet is continued. Identifying the level of depletion may sometimes be difficult, and much obviously depends on how one chooses to define deficiency.

Red Blood Cell Folate

Red cell folate assay usually reflects tissue stores more accurately than does serum assay,[32] is less subject to influence by extraneous factors, and correlates with severity of anemia and megaloblastosis whereas serum folate does not.[40] Its greater specificity may be particularly useful when the existence of megaloblastosis is in question. An additional benefit is its helpfulness even after therapy has been started—thereby flooding the serum with folate—in the few days before folate-repleted cells are released from bone marrow in significant numbers. Because almost 10 percent of all serum specimens submitted for assay to my laboratory were drawn after the patient was given folic acid (unpublished data), this benefit may have great clinical utility. Obviously, too, samples

need not be obtained in the fasting state. Finally, antibiotic use has little effect on red cell folate assay.[21]

Table 3 outlines the various problems of evaluating red cell folate levels. The chief drawbacks of the test are its nondiscrimination between folate and vitamin B_{12} deficiency[40] due to the apparent dependence of folate entry into cells on vitamin B_{12},[33] and to its insensitivity to very early deficiency states.[4] The former drawback may be minimized by calculating red cell-to-serum folate ratios, which should be normal or high in folate deficiency but low in vitamin B_{12} deficiency,[40] but such ratios obviously require assay of both red cells and serum. As with serum folate, radioisotopic red cell folate assays are being developed[39,42,43] but are not fully tested yet.

Serum Vitamin B_{12}

Serum vitamin B_{12} is assayed by radioisotope dilution almost universally now. Results are comparable to microbiologic assay results, although slightly lower values have been reported with the latter assay, particularly in patients with folate deficiency and after gastrectomy.[44] It is not clear which result is "correct," and comparisons have suggested that the microbiologic assay identifies vitamin B_{12} deficiency more reliably.[45] A recent preliminary report[46] suggests that falsely-normal radioassay results may occasionally occur in vitamin B_{12} deficiency where microbiologic assay gives appropriately low values. Hall[47] has observed occasional falsely normal radioassay results also, as have I, but also mentions occasional falsely low vitamin B_{12} radioassay results where microbiologic assay levels are normal. The numerous published versions of the radioisotopic assay attest to the fact that an ideal technique does not yet exist. Many commercial kits have been marketed. Several of them unfortunately give higher results than others.* Therefore, 330 pg per ml is the lower limit of normal with some kits, whereas over the years microbiologic and most radioisotopic assays have established 150 to 200 pg per ml as the lower limit. The possible confusion resulting from this disparity is both obvious and deplorable. (I have recently seen a case where vitamin B_{12} deficiency was overlooked because of just this problem). Furthermore, each laboratory must establish its own range of normal values even when using commercial kits and not rely on manufacturers' claims (see last sentence of footnote).

Table 4 lists the possible interpretive errors with serum vitamin B_{12} levels. As with folate, serum levels only indirectly reflect body stores of vitamin B_{12} and its metabolic and transport sufficiency. A particularly important problem is that

TABLE 3.—*Misleading Red Cell Folate Results*

Misleadingly Low Levels

More than half of patients with vitamin B_{12} deficiency.[40]
Improper handling of specimen.
? oral contraceptives (see reference 28 for review).

Misleadingly Normal or High Levels

Early folate deficiency.[4]
Blood transfusion.
Significant reticulocytosis (since reticulocytes are relatively richer in folate than older cells).[16,40]
Iron deficiency.[41]

TABLE 4.—*Misleading Serum Vitamin B_{12} Results*

Misleadingly Low Levels

Folate deficiency.[49-51]
Pregnancy, near term[50,52,53]—particularly seen with microbiologic assay.[54]
Drugs: oral contraceptives,[55]
large amounts of vitamin C (probably by affecting assay).[56]
Idiopathic: aplastic anemia,[57] multiple myeloma,[58-60] cancer.[61]
Poor dietary intake in vegans (without true deficiency).[62]
Transcobalamin I deficiency.[63]
? Iron deficiency.[50,64]
? Old age.[65-67]
Artifact of radioassay.[47]
Contamination of serum with other radioisotopes in patients undergoing scans and uptake studies.[31]

Misleadingly Normal or Raised Levels

Vitamin administration before blood drawing.*
Artifact of radioassay.[46,47]
Serum vitamin B_{12}-binding protein abnormality coexisting with vitamin B_{12} deficiency: chronic myelogenous leukemia,[68,69] ? polycythemia vera.[70]
? Liver disease.[71]
Transcobalamin II deficiency.[72,73]
The generally higher results with some assay kits may be misinterpreted (must know normal range for the assay used).†

*Manufacturers of kits giving the higher results include Pharmacia Laboratories Inc., Piscataway, N.J. (Phadebas B_{12} Test) and Diagnostic Biochemistry Inc., San Diego, Calif. The more usual normal range is claimed for kits made by RIA Products Inc., Waltham, Mass.; Diagnostic Products Corp., Los Angeles Calif.; Clinical Assays Inc., Cambridge, Mass.; Schwartz-Mann, Orangeburg, N.Y.; Bio-Rad Laboratories, Richmond, Calif., and Curtis-Nuclear, Los Angeles, Calif. However, one study[48] reports that the last-named kit produced higher results than stated by the manufacturer.

*However, this is not as common as with folate since oral administration usually has no significant effect except in the rare patients with dietary vitamin B_{12} deficiency. I have found vitamin B_{12} injection to have contaminated at least 2.5 percent of sera submitted for vitamin B_{12} assay (unpublished data).

†For example, vitamin B_{12} assays for the Los Angeles County-University of Southern California Medical Center have in the past few years been done by several different laboratories, and three distinctly different normal ranges have therefore been operative at one time or another. A level of 150 pg per ml could be normal, borderline or clearly low, depending on the assay in use.

folate deficiency causes a decrease in serum vitamin B_{12} level in approximately half the cases,[49-51] sometimes to a level in the deficient range. While coexisting vitamin B_{12} deficiency has been suggested in some of these cases by *in vitro* studies,[74] the vitamin B_{12} level usually rises spontaneously following folate therapy alone.[49-51] Consequently, it is often difficult to establish whether a low serum vitamin B_{12} level in a folate-deficient patient represents a secondary phenomenon or coexisting true vitamin B_{12} deficiency. The problem then must be resolved by repeating vitamin B_{12} studies shortly after giving folic acid alone, or by assaying urine for methylmalonic acid. In my laboratory, 8 percent of patients with low serum folate had low levels of vitamin B_{12} also (unpublished data).

Urinary Methylmalonic Acid Excretion

In view of the occasional unreliability of serum vitamin B_{12} levels, determination of methylmalonic acid excretion may sometimes be clinically useful. This test would probably have wider utility were it more readily available and less demanding technically. Excretion of this intermediate is elevated in vitamin B_{12} deficiency but not in folate deficiency,[75] though oral loading with valine or isoleucine may sometimes be required to stress the metabolic pathway.[76] Furthermore, excretion results often remain abnormal for some time after vitamin B_{12} therapy,[77] making the test diagnostically useful in inadvertently treated patients. However, abnormal excretion also occurs in children with methylmalonic aciduria, a rare inborn error of metabolism, one variety of which is vitamin B_{12}-responsive,[78] and has been reported in advanced cirrhosis[79] and in a few elderly persons.[80] Misleadingly normal results in vitamin B_{12}-deficient subjects occur sometimes even with valine or isoleucine loading,[81] a reflection of the somewhat limited sensitivity of the procedure.

Other Tests

Beyond the above tests, few others are clinically useful. Red blood cell vitamin B_{12} assay is not generally available and lacks specificity;[82] low levels are encountered in folate deficiency,[83,84] iron deficiency[85] and polycythemia vera,[83,86] in addition to vitamin B_{12} deficiency.

Urinary excretion of formiminoglutamic acid is increased in folate deficiency[87] but is also increased in vitamin B_{12} deficiency.[88] This lack of specificity combined with its inconvenience makes the determination of urinary excretion of formimino-glutamic acid a test of limited clinical applicability.

The "deoxyuridine suppression" test[89,90] has been advocated as a very sensitive test of megaloblastosis, which identifies depletion, transport abnormality or metabolic block of either folate or vitamin B_{12}. Suitable *in vitro* maneuvers can frequently identify the specific defect. However, the test involves *in vitro* bone marrow culture, and is therefore only of research interest currently. Furthermore, coexisting iron deficiency can produce falsely normal deoxyuridine suppression,[91] much as red cell morphology can be masked in such a situation.

Even with the availability of the many laboratory tests mentioned above, evaluation of hematologic response to specific therapy with vitamin B_{12} or folic acid[5] may occasionally be clinically useful in some patients. Though the therapeutic trial is not necessary or feasible in most cases, its diagnostic validity requires the use of minimal doses of the specific vitamin to avoid the well-known response of vitamin B_{12} deficiency to massive doses of folic acid and to a lesser extent vice versa.

3. Identification of the Specific Disease Entity

Once the deficient vitamin has been identified, the underlying disease must be sought. The differential diagnosis has been well covered in many reviews[92-94] and textbooks, and is outside the scope of this discussion. Therapy can usually be started without interfering with this part of the evaluation, though certain precautions must be observed (see below).

Vitamin B_{12} Deficiency

Attention is directed primarily at establishing intrinsic factor lack (usually pernicious anemia) versus intrinsic factor ineffectiveness (usually intestinal disorders). Dietary deficiency is rare, though not unheard of, in the United States.[5,94] Even when the diet is unequivocally vitamin B_{12}-deficient, coexisting absorptive abnormality should still be considered since even vegans do not usually develop overt vitamin B_{12} deficiency.[95,96]

Gastric studies. Direct assay of gastric juice for intrinsic factor content is conceptually the ideal test. However, this assay is not widely available, even though it is a relatively simple, rapid method.[97,98] Its unavailability is particularly re-

TABLE 5.—*Misleading Results of Immunologic Assay for Intrinsic Factor Content in Gastric Juice*

Misleadingly Normal Results
Low intrinsic factor secretion which, though detectable, is insufficient for maintaining normal vitamin B_{12} absorption.[99]
Steroid therapy.[100-103]
Nonfunctional, but immunologically detectable, intrinsic factor molecule.*[104]
Misleadingly Abnormal Results
Incomplete gastric juice collection (most commonly improper placement of gastric tube or inadequate suctioning).
Failure to stimulate with histamine, betazole or pentagastrin.[105]
Improper processing of sample—for example, delay in depepsinizing sample may destroy intrinsic factor.[97]
Diminished amounts of intrinsic factor are occasionally found in patients who nevertheless absorb vitamin B_{12} adequately.[99]
Intrinsic factor secretion may be decreased secondarily in malabsorption syndromes such as sprue.[106-109]

*Can be identified by *in vitro* tests of mediation of vitamin uptake by gut mucosa.

grettable since patients with megaloblastic anemia often undergo gastric analysis anyway for the much less diagnostically specific purpose of determining gastric acid secretion. Intrinsic factor assay could decrease or eliminate the need for subsequent exposure of many such patients to the radioactivity of absorption tests. Possible artifacts of the assay are presented in Table 5, the most common being related to inadequate patient preparation and specimen collection.

Testing acid secretion, long a part of the evaluation of pernicious anemia, is not a specific test since achlorhydria often occurs in patients without pernicious anemia, particularly in elderly patients. As an illustration of its potential to mislead, in 28 consecutive *vitamin B_{12}-deficient* patients with achlorhydria I found five in whom adequate amounts of intrinsic factor actually were secreted and who therefore did not have pernicious anemia (unpublished data). Furthermore, reversible gastric atrophy has been reported to result from vitamin B_{12} deficiency itself[110] and from malabsorptive disorders.[106,107,109] Therefore, if gastric intrinsic factor assay is available, testing for acidity is an unnecessary procedure in evaluating vitamin B_{12} deficiency. However, because intrinsic factor assay generally is not available, determination of acid secretion is useful as a confirmatory but never as a diagnostic test. Exceptions to the rule that normal acid secretion rules out pernicious anemia include the rare entity of congenital pernicious anemia[111,112] and patients with adult pernicious anemia receiving steroids.[101,102]

Radioactive vitamin B_{12} absorption tests. The most commonly used of the various types of tests available is the Schilling test.[113] If properly done and analyzed, these tests are reliable. Reliability may be further enhanced by combining the Schilling test with plasma determinations of absorption.

However, a result indicating an intestinal absorption defect may be misleading since it is often a transient complication of pernicious anemia itself.[114,115] Furthermore, vitamin B_{12} deficiency can decrease absorption of other substances also.[114,115] Usually such malabsorption is quickly reversible but occasionally the Schilling test must be repeated after several months of vitamin B_{12} therapy. Nevertheless, delaying the Schilling test for this reason more than a few weeks is probably not necessary in most cases. It is also worth interjecting that while the prolonged reversible intestinal defect has been generally ascribed to vitamin B_{12} depletion of the intestinal cells, this explanation may not be entirely correct. Bone marrow normalization with therapy occurs within a few days, and intestinal cells have equally, if not more, rapid turnover. Therefore, if the absorptive defect takes months to reverse, mechanisms other than vitamin B_{12} deficiency per se may be implicated. For example, Lindenbaum and associates[115] found bacterial overgrowth requiring antibiotics in two such cases of pernicious anemia with prolonged vitamin B_{12} malabsorption. To further complicate matters, Brody and associates[116] found pernicious anemia and various primary intestinal diseases coexisting independently in several patients; in those cases the malabsorption was not transient. A much less common interpretative problem may be one arising in Diphyllobothrium latum infestation, where the abnormal Schilling test may or may not be correctable by exogenous intrinsic factor.[117] Obviously, being able to rule pernicious anemia in or out by examining gastric secretion of intrinsic factor by direct assay would be invaluable in the various situations mentioned above. However, as long as intrinsic factor assay remains generally unavailable, the Schilling test is still the cornerstone in investigating the cause of vitamin B_{12} deficiency despite its artifacts (Table 6).

Some question has been raised about the physiologic validity of an absorption test using crystal-

line cyanocobalamin rather than food vitamin B_{12} and of testing in the absence of the gastric stimulation normally occurring with food ingestion.[96,137-139] Modifications have therefore been proposed to more closely simulate the actual digestive and absorptive process[137-140] but a definitive answer is not available. These problems may be particularly pertinent in chronic pancreatitis,[121,122] postgastrectomy states[137,139] and gastric achlorhydria.[96,139]

Various technical modifications of the standard Schilling test have been introduced, but should be adopted cautiously. One such modification, the use of intrinsic factor in capsules, has been recently reported to give low results.[141] A commercial kit now also allows the simultaneous performance of the Schilling test with and without intrinsic factor by using two different cobalt isotopes,[142] but raises not only the question of exchange between the two simultaneously given doses of vitamin B_{12} but also of a possible 25-fold increase in radiation dose from the cobalt 58 isotope.[143] This radiation plus the unnecessariness of a second test in the many patients who turn out to have normal absorption without intrinsic factor probably outweigh any advantage in time saved that the test provides.

Alternatives to the Schilling test exist which, instead of measuring urinary excretion of radioisotope, determine radioactivity in plasma,[119,144,145] feces,[146] whole-body counting[147] or hepatic uptake.[148] These tests enjoy the advantages of not require "flushing" injections of vitamin B_{12} or urine collection, but are subject to many of the other problems listed in Table 6 for the Schilling test. Since most of these alternative tests are not widely used, their specific problems will not be considered. The only test that is clinically important is the plasma absorption test which is rapid and simple, and bypasses the need for patient reliability and compliance. However, it is subject to other technical sources of possible error, there is overlap between normal and abnormal results[149] and results may be falsely elevated if renal impairment coexists.[119] Nevertheless, the plasma absorption test appears to be useful as a supplement to the Schilling test, particularly since the two tests can be carried out concurrently with the same dose of radioactive vitamin B_{12}.

Due to the many unavoidable, minor variations in procedure—such as the actual dose of radioactive vitamin B_{12} given—it is particularly important for each laboratory to establish the normal range for their test as done by them, rather than relying on published normal ranges.

Serum antibodies. More than half of all patients with adult pernicious anemia have "blocking" (type I) antibody to intrinsic factor circulating in their blood streams.[150] Assay is simple[97] and clinically useful because presence of this antibody is virtually diagnostic of pernicious anemia. Falsely positive antibody results[151-153] are rare, but assay artifact may occur if serum is drawn less than 24 to 48 hours after vitamin B_{12} injection.[97] The sole drawback to this test is the fact that absence of antibody is diagnostically meaningless: nearly half of all patients with adult pernicious anemia and all patients with congenital pernicious anemia do not have circulating anti-intrinsic factor antibody.

Parietal cell antibody is frequently found in the serum of patients with the adult but not the congenital form of pernicious anemia,[154] but is totally nonspecific. The antibody is found in so many patients without pernicious anemia as to make the

TABLE 6.—*Misleading Schilling Test Results*

Misleadingly Normal Results

Contamination of urine by other radioisotopes or by feces containing unabsorbed radioactive vitamin B_{12} (such as due to diarrhea, especially in women).

Patients with pernicious anemia taking steroids.[100-103,118]

Misleadingly Low Results

5 percent or more of normal subjects may have abnormal results.[119,120]

Vitamin B_{12} deficiency may produce transient malabsorption pattern.[114,115]

? Chronic pancreatitis.*[121,122]

? Folate deficiency.[123,124]

Drugs:† para-aminosalicylic acid,[121,122] colchicine,[127] neomycin,[128] phenformin,[129] metformin,[130] anticonvulsants,[131,132] potassium chloride,[133] potassium citrate,[134] alcohol.[135]

Incomplete urine collection.

Renal failure[92]—may often be circumvented by extending urine collection period to 48 or 72 hours.[88]

Vitamin B_{12} injection within 24 hours preceding the test.[97]

Vomiting or diarrhea.

Omitted or inappropriately timed "flushing" dose of vitamin B_{12}.[5]

A defective intrinsic factor preparation.

? Serum vitamin B_{12}-binding protein abnormality.[136]

*Schilling test results are frequently abnormal in chronic pancreatitis but the question raised about this entity is whether or not the test abnormality reflects true malabsorption.

†These are misleading only insofar as the drugs are rarely taken long enough to cause vitamin B_{12} deficiency, even though they apparently cause true, though transient, vitamin B_{12} malabsorption. The effect of many of these drugs, as had been shown with metformin, is dose-related.

test of doubtful value clinically, other than as an indicator of gastritis.[154] This test is therefore rarely helpful diagnostically.

Folate Deficiency

Determination of the cause of folate deficiency is more difficult than with vitamin B_{12} deficiency, and in fact is usually not even attempted. A major problem is the lack of a reliable, widely available test for folate malabsorption. The various methods, reviewed elsewhere,[155] include measuring serum folate levels or urinary excretion of folate following a standard oral dose and tests resembling the radioactive vitamin B_{12} absorption tests in which isotopic folates are given. All require great expertise in carrying out and in interpreting results. A diagnosis of folate malabsorption, if made at all, is therefore usually established only indirectly by showing the presence of intestinal disease. However, in all truly deficient patients without very obvious dietary cause of deficiency, further general investigation must be done for malabsorption. Drug effect or possibly increased utilization of folate (the last named, incidentally, has never been conclusively shown as a sole cause of folate deficiency) should also be considered.

4. Reevaluation After Therapy

This phase is unfortunately often neglected, especially in patients discharged from hospital shortly after institution of therapy. Correctly treated, uncomplicated megaloblastic anemia should respond fully, and the maximal reticulocyte response by seven to ten days is well known. Equally important, the hemoglobin level should return completely to normal within two months. It may also be useful to recheck the serum vitamin B_{12} level in a folate-treated patient if it was borderline or low initially. The serum vitamin B_{12} level should rise spontaneously in folate deficiency correctly treated with folate;[49] further decline suggests vitamin B_{12} deficiency which coexisted or was previously misdiagnosed as folate deficiency.[156]

Lack of proper reticulocyte and red blood count response indicates incomplete correction of deficiency as outlined above, or coexistence of other causes of anemia, most often iron deficiency, inflammatory disease or alcohol abuse. Since any suboptimal response therefore calls for reinvestigation in the patient, it is essential to follow each case carefully to determine that full response has occurred.

Conclusion

Megaloblastic anemia should be suspected in any patient with macrocytosis and is confirmed by peripheral blood and bone marrow morphology. However, morphologic changes may be subtle, especially in early vitamin deficiency or with coexisting iron deficiency.

Identification of the specific vitamin deficiency is usually accomplished by assay of serum levels even though these do not always reflect body stores. Assay for both vitamin B_{12} and folate should be done together in most cases regardless of which vitamin deficiency is suspected clinically. This is so because the serum levels of the two vitamins interact (for example, low serum vitamin B_{12} levels have been reported in 10 percent of folate-deficient subjects) and because in some patients, particularly those with malabsorption, deficiencies of both vitamins may coexist. Even excluding artifacts and laboratory error, the specific vitamin deficiency may sometimes be hard to establish. Misleadingly low serum levels are particularly common. Folate stores may be more reliably assessed by red cell folate assay, but low levels do not distinguish between folate and vitamin B_{12} deficiency; vitamin B_{12} status may also be assessed by urinary methylmalonic acid excretion. Both red cell folate and urinary methylmalonic acid excretion assays are particularly useful in patients seen shortly after "shotgun" therapy in whom morphology and serum vitamin levels are no longer diagnostically reliable.

Identification of the specific disease entity must be pursued once the specific vitamin deficiency is identified. Tests of vitamin B_{12} absorption are widely available and generally reliable. A major interpretive problem is the transient intestinal malabsorption, ostensibly due to vitamin B_{12} deficiency itself, which often accompanies pernicious anemia. Although absorption tests usually need not therefore be delayed beyond a few weeks, other approaches obviously may be useful. Direct assay of gastric juice for intrinsic factor, ideally the single best test for establishing or ruling out pernicious anemia, is unfortunately not widely available. Assaying for serum antibody to intrinsic factor is also a useful test because a positive result

in a vitamin B_{12}-deficient patient is virtually diagnostic of pernicious anemia.

Identification of the cause of folate deficiency is more difficult since tests of folate absorption are not widely available. However, in any patient with definite folate deficiency not obviously due to poor diet, studies should be done for malabsorption or other causes.

Reevaluation in patients after treatment has important diagnostic implications as well. If appropriate hematologic response has not occurred, reinvestigation is indicated.

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Use of Propranolol in Diabetic Patients

PROPRANOLOL CAN CAUSE a really severe problem in a diabetic patient, especially one taking insulin, [because it] can mask the symptoms of hypoglycemia. Physicians must watch carefully for this and warn diabetic patients about it if propranolol is to be used.

—A. RICHARD CHRISTLIEB, MD, *Boston*

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