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Vitamin B_{12} Symposium

The Disappearance of Intravenously Administered Vitamin B_{12}

Studies in Normal Subjects and in Patients with Pernicious Anemia

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THE STUDIES to be described were designed to follow the fate of a small dose of radioactive vitamin B_{12} after its intravenous injection into "normal" subjects and patients with a variety of disorders. Following such injection, it is possible to plot the rate of disappearance of radioactivity from the plasma, the rates of uptake in the liver and the bone marrow, and the excretion in the urine. The pattern of disappearance was found to differ in certain disease states. We shall describe this pattern in the normal subject, in patients with chronic myelocytic leukemia and in patients with pernicious anemia in relapse or in remission.

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MATERIAL AND METHODS

A standard dose of 0.5 μg. (rarely, 1.0 μg.) of Co^{58}-vitamin B_{12} or Co^{60}-vitamin B_{12} was rapidly injected intravenously into a subject. Specimens of blood were collected in heparin at frequent intervals after the injection: one, three, five, fifteen, thirty and sixty minutes, and then at two, six or eight hours, and twenty-four hours. At the same time, *in vivo* radioactivity was measured over the liver and over the sacral bone marrow. The radioactivity present in an aliquot of plasma at each point in time was measured in a scintillation counter and converted to total plasma radioactivity by estimating the plasma volume of the patient, and this total plasma radioactivity was then expressed as a percentage of the injected dose.

The radioactivity excreted in the twenty-four-hour urine sample following the injection was similarly determined and was also ex-

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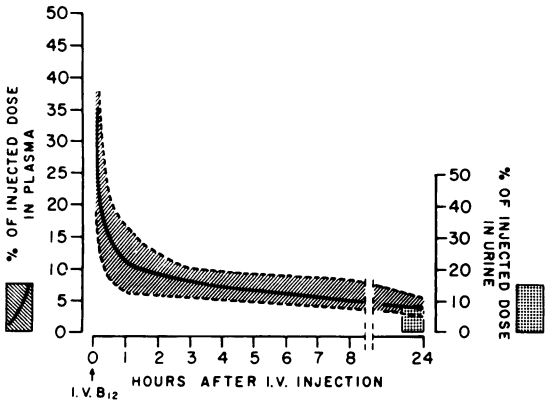

FIG. 1. Pattern of disappearance following the intravenous administration of radiocobalt vitamin B_{12} in the normal subject.

pressed as a percentage of the injected dose. No radioactivity appeared in the stool or in the circulating red cells. The simultaneous *in vivo* counting showed slight but definite radioactivity over the sacral marrow, and marked radioactivity over the liver. We have not, however, found a satisfactory method of quantitating these *in vivo* counts in terms of the injected dose.

In addition, microbiologic assay of vitamin B_{12} was performed on each specimen of plasma by means of Euglena gracilis.

RESULTS

The pattern of disappearance in the normal subject* is shown in Figure 1. If the first specimen of plasma is obtained early enough, within one minute, the radioactivity remaining in the plasma may be as high as fifty to sixty per cent of the injected dose. Within three to five minutes, seventy per cent of the injected radioactivity has already disappeared from the plasma. In one hour the residual radioactivity is ten to twelve per cent; in six to eight hours, between five and ten per cent; and in twenty-four hours, five per cent or less. The twenty-four-hour urinary excretion never exceeds five to eight per cent of the injected dose. Thus, after twenty-four hours, five per cent of the dose is still in the plasma, five per cent has appeared in the urine, and ninety per cent is as yet unaccounted for.

* Our normal subjects had no evidence of hematologic disease, hepatic disease or renal disease.

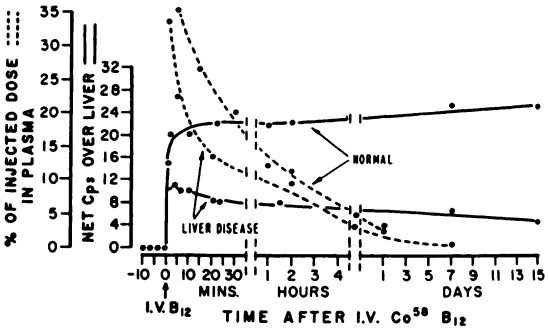

FIG. 2. Plasma disappearance and hepatic uptake of injected radiocobalt vitamin B_{12}.

Much of this ninety per cent can be assumed to be present in the liver. Thus, in Figure 2, disappearance of radioactive vitamin B_{12} from the plasma is plotted together with hepatic uptake of radioactive vitamin B_{12} (expressed as net counts per second) in a normal subject and a patient with severe liver disease. As seen by the normal curves, there is an instantaneous rise in hepatic radioactivity, i.e., in the uptake of vitamin B_{12} from the plasma by the liver. That the persistence of this rise is related at least in part to the ability of the liver to bind vitamin B_{12} normally, is suggested by the curve of the patient in Figure 2. This patient had hepatic disease and showed considerably less uptake of radioactivity than did the normal subject.

Quantitation of the percentage of the injected dose taken up by the liver and the bone marrow has not been possible. Postmortem studies have suggested, however, that approximately fifty per cent of the injected dose is taken up by the liver, with smaller amounts by kidney, pituitary, bone marrow and other tissues.

Simultaneous microbiologic assay in normal persons (Figure 3 is typical) similarly shows that, within the first few minutes, there is a rapid rise of plasma vitamin B_{12}, and that this rise is accounted for by a rise in the bound vitamin. The total vitamin B_{12} level then falls rapidly to the preinjection level, the fall in the bound fraction paralleling the fall in total vitamin B_{12}. The microbiologic curve parallels the radioactive disappearance curve.

In attempting to determine why the curve of vitamin B_{12} disappearance has its particular

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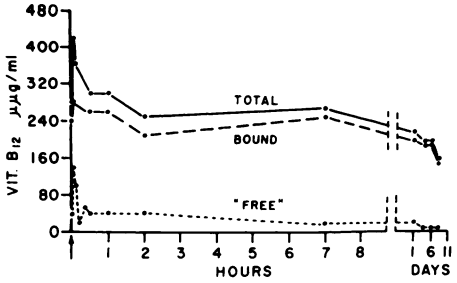

FIG 3. Disappearance of intravenously injected vitamin B_{12} in the normal subject, measured by microbiologic assay. Arrow indicates time of injection.

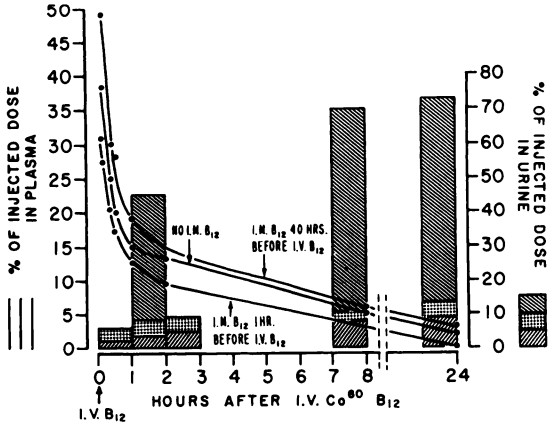

FIG 4. Effect of presaturation with 1,000 µg. vitamin B_{12} (intramuscularly) on the disappearance curve in the normal subject. The highest urinary excretion (diagonal shading to the right) corresponds to presaturation one hour before the intravenous dose. See text.

shape, we studied an artificial situation in which a normal person was given an injection of 1,000 µg. of vitamin B_{12} intramuscularly just prior to the intravenous injection of radiocobalt vitamin B_{12}. The resulting curve is shown in Figure 4. This curve is displaced downward as compared with the normal curve and, at the same time, the urinary excretion of radioactivity, which is normally 5 to 8 per cent, becomes 75 per cent. Presaturation with vitamin B_{12}, in other words, presumably so saturates the serum and other body proteins that, when additional (radioactive) vitamin B_{12} is injected, little additional (radioactive) vitamin B_{12} can be accepted by these proteins and plasma radioactivity rapidly disappears from the blood because of its ready excretion in the urine.

In contrast to this artificial situation is the curve of the patient with chronic myelocytic leukemia. This curve is raised above the normal (Fig. 5). In other words, at any given time, there is more residual radioactivity (i.e., vitamin B_{12}) in the plasma of patients with this disorder than in the normal person. This fact cannot be accounted for by the radioactivity excreted in the urine, which is essentially the same as in the normal subject. The curve is unique, is apparently found only in patients with chronic myelocytic leukemia and is related to the proteins in this disease. Although chemical and ordinary electrophoretic methods have shown no difference between the plasma proteins of normal subjects and those of patients with chronic myelocytic leukemia, special studies (in which the amount of vitamin B_{12} present in each protein fraction was determined) have shown increased binding of vitamin B_{12} to the alpha-globulins in the leukemic plasma. Apparently, an abnormal protein in the alpha-globulin region is present in patients with this disease, which has the property of binding increased amounts of vitamin B_{12}.[1]

This concept is confirmed by the extremely high serum vitamin B_{12} levels known to be present in this disease. It is further confirmed when the test dose of 0.5 µg. of radiocobalt vitamin B_{12} is incubated with plasma from a patient with chronic myelocytic leukemia prior to its injection into a normal subject (Fig. 6). The resulting disappearance curve is not normal but resembles that seen in the leukemic patient. This result is found only with chronic myelocytic sera, and is not found after preincu-

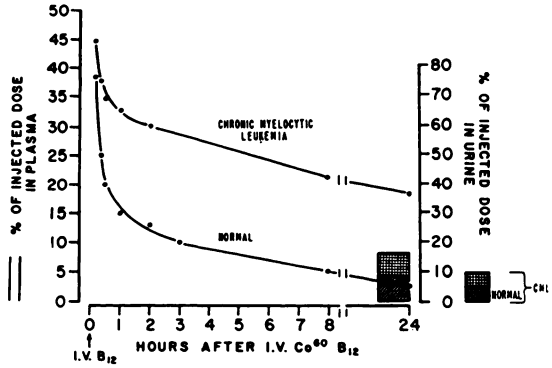

FIG. 5. Disappearance of injected radiocobalt vitamin B_{12} in the patient with chronic myelocytic leukemia.

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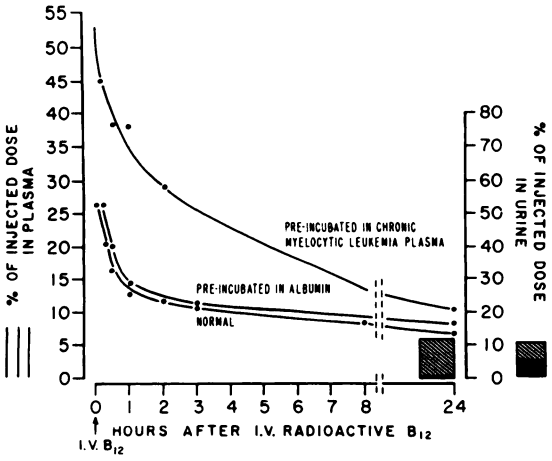

FIG. 6. Effect of preincubation with radiocobalt vitamin B_{12} in plasma of patients with chronic myelocytic leukemia, on the disappearance curve after subsequent injection into the normal subject.

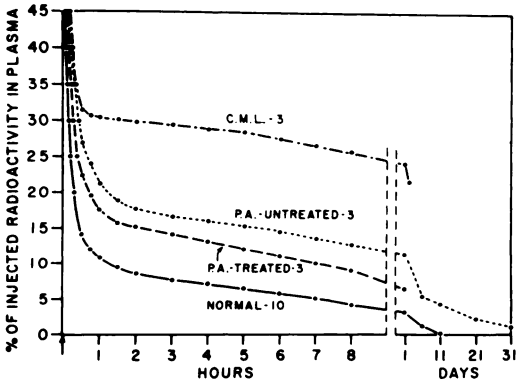

FIG. 7. Disappearance of injected radiocobalt vitamin B_{12} in patients with pernicious anemia in relapse and in remission. Arrow indicates time of injection.

bation with other sera (e.g., normal, lymphosarcoma) or with human serum albumin.

With this background information we became interested in studying the disappearance test in patients with pernicious anemia. We explained the normal curve by initial binding of injected vitamin B_{12} by serum protein, followed by rapid uptake of the plasma vitamin B_{12} by the liver and other tissues. We explained the presaturated curve, with its artificially rapid disappearance, on the basis that the serum (and tissue) proteins were already saturated by vitamin B_{12} before the radioactive material reached them, so that they could not accept the additional vitamin B_{12}. We explained the leukemic curve, with its abnormally slow disappearance, by the increased capacity of the serum proteins in this disease to bind vitamin B_{12}.[2]

In patients with pernicious anemia, the serum proteins are normal in their binding ability, and the tissues, which are depleted of vitamin B_{12}, might be expected *a priori* to take up avidly large amounts of the injected vitamin. We therefore anticipated that the disappearance of injected vitamin B_{12} might be more rapid than normal, so that the curve would be displaced downward. The actual results, however, were entirely different, and demanded more profound analysis. Thus (Fig. 7), patients with untreated pernicious anemia showed a displacement of the curve upward as compared with the normal subjects; i.e., disappearance was slower than in the normal person, although not as slow as in patients with chronic myelocytic leukemia. Even more unexpected was the finding that patients with pernicious anemia in remission still showed a disappearance curve displaced upward from the normal curve, i.e., disappearance was less rapid than in the normal subjects, although not as slow as in patients with pernicious anemia in relapse.

Studies of liver and urine function failed to explain these findings: Hepatic uptake of radioactivity did not differ from that in the normal subject, and urinary radioactivity was the same in patients with pernicious anemia as in the normal subject. Simultaneous microbiologic studies showed that, as in the normal subject, there was an initial rise in total vitamin B_{12}, due to a rise in bound vitamin B_{12}, and that, as the total vitamin B_{12} level fell, the bound material closely paralleled its fall.

We were therefore unable to explain why the disappearance of injected vitamin B_{12} from the plasma was abnormally rapid in patients with pernicious anemia in relapse and, more significant, why it remained abnormally rapid even after adequate vitamin B_{12} therapy had caused complete clinical and hematologic remission. On purely theoretical grounds, one might list those factors which regulate the disappearance of injected vitamin B_{12} from the normal plasma (Table I). These would include loss to the outside (urine) and loss to the inside

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TABLE I

Factors Which May Regulate Disappearance of Injected Vitamin B_{12} from Normal Plasma

Loss via
Urinary excretion
Tissue (liver) uptake
Mechanism of tissue uptake
Ability of liver to retain vitamin B_{12} (in hepatic disease, liver binding falls, urine vitamin B_{12} rises)
? serum factors which transfer vitamin B_{12} from serum protein to liver protein

TABLE II

Factors Which May Decrease Disappearance of Injected Vitamin B_{12} from the Plasma

Chronic myelocytic leukemia
Increased binding by serum proteins
Serum proteins bind B_{12} more than liver proteins, or
Lack of "B_{12}-transferase" as well as increased binding by serum proteins
Pernicious anemia
Total serum vitamin B_{12} and vitamin B_{12}-binding capacity are same as in normal
Hence
Binding by serum proteins is greater than normal, or
Binding by liver proteins is less than normal, or
"B_{12}-transferase" is lacking

(uptake by body tissues, especially the liver). The functional capacity of the liver is related to the curve since, in a number of patients with hepatic disease, vitamin B_{12} uptake by the liver seemed to be diminished. In addition, however, the question arises as to whether there might also be substances in the serum which aid the transfer of vitamin B_{12} from its location in the serum (alpha) globulins to the beta-1 globulin which is said to bind vitamin B_{12} within the liver.

Table II lists factors which might help explain the two groups of patients with diminished rates of plasma disappearance, chronic myelocytic leukemia and pernicious anemia. In patients with chronic myelocytic leukemia, the serum proteins bind more vitamin B_{12} than do serum proteins and liver protein in normal subjects. This fact adequately explains the resultng curve, although the possibility of diminution of serum "B_{12}-transferase," in addition to the increased binding by serum proteins, theoretically exists.

In patients with pernicious anemia in remission, however, it is known that the total serum B_{12} and the proportion of the bound vitamin are the same as in the normal person. In addition, it is known that the capacity of patients with pernicious anemia serum to bind vitamin B_{12} is the same as that of normal subjects. Although the capacity of the serum in patients with pernicious anemia in relapse to bind injected vitamin B_{12} is increased above normal by virtue of the fact that the circulating serum vitamin B_{12} is virtually zero, this state no longer holds when remission has occurred, so that the abnormally slow disappearance in patients with pernicious anemia in remission cannot be explained by this "unbound binding capacity." The slow disappearance thus is not the result of increased binding of the injected vitamin B_{12}, since there is no increased binding. It might be explicable by a diminished ability of liver protein to accept vitamin B_{12}, but there is no evidence for any such diminished ability. The slow disappearance in patients with pernicious anemia in remission might also be explained by a lack of the hypothetical "serum B_{12}-transferase" mentioned before.

At this point, all discussion becomes quite theoretical, since we do not have data to support any of this speculation. It is generally accepted that the dietary vitamin B_{12} which goes into the lumen of the gastrointestinal tract requires intrinsic factor to allow its transfer to the intestinal mucosa: this intrinsic factor is lacking in patients with pernicious anemia, whether in relapse or in remission. How vitamin B_{12} passes from intestinal mucosa to the plasma is not, to our knowledge, known. This passage might be mediated by an enzyme, or perhaps by some substance such as intrinsic factor.[3] How, once vitamin B_{12} is present within the plasma and attached to serum globulin, it then passes from the plasma to the tissue (liver) proteins, is also unknown. This step might also be due to the same intrinsic factor in the blood, or to a transferring enzyme of another kind. The patient with pernicious anemia, known to lack intrinsic factor within

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the gastrointestinal tract, would then be expected to lack intrinsic factor within his serum.

We should like to suggest that the abnormal pattern of disappearance of vitamin B_{12} from the plasma following its intravenous injection into patients with pernicious anemia can best be explained, especially in complete remission, by the postulate of "serum B_{12}-transferase" which, present in normal amounts in normal sera, is diminished or absent in patients with pernicious anemia, whether in relapse or in remission.

Whether this postulate will withstand the test of further experimentation remains, of course, to be determined.

SUMMARY

The fate of radioactive vitamin B_{12} following its intravenous injection was determined in normal subjects and in patients with chronic myelocytic leukemia and pernicious anemia.

The resulting plasma disappearance curves were abnormal patients with chronic myelocytic leukemia and pernicious anemia.

The abnormally slow plasma clearance of vitamin B_{12} found in pernicious anemia persisted even in patients in complete hematologic and clinical remission.

The concept of a circulating "serum B_{12}-transferase" is suggested to explain these findings.

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