Alteration of Plasma Clearance of Vitamin B_{12} by Intravenous Hog Intrinsic Factor Concentrate.* (30556)

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Previous studies have demonstrated that the disappearance from the plasma of intravenously administered radiocobalt labeled vitamin B_{12} (0.5 μg) is most rapid in the normal subject, slowest in patients with chronic myelocytic leukemia, and intermediate in patients with pernicious anemia, total gastrectomy and those cases of malabsorption syndrome with a defective absorption of vit B_{12}(1,2,3,4). The rate of plasma clearance is considerably slower in patients with pernicious anemia in relapse than in the normal. Although Hall has reported(5) a normal clearance rate in pernicious anemia in complete remission, in our hands the plasma disappearance curve approaches but never reaches the normal(1).

The variation of the curve found by us in pernicious anemia in remission from that found in the normal has not been explained by any difference in tissue uptake, in urinary excretion of radioactivity, or in isotope dilution factors. The difference is not due to increased binding of the injected B_{12} by circulating globulin as is the case in chronic myelocytic leukemia(3) since the B_{12} binding capacity of pernicious anemia serum is normal(6,7,8), or possibly reduced(9).

It has been hypothesized that a factor similar to intrinsic factor is present in normal plasma whose function it is to facilitate transfer of circulating B_{12} to the tissues and that this plasma factor is diminished in pernicious anemia, total gastrectomy and certain cases of malabsorption syndrome(1). The present study was undertaken to examine the effect of coadministration of intrinsic factor on the fate of intravenously injected vit B_{12}.

Materials and methods. A. *Studies utilizing hog intrinsic factor concentrate (HIFC).* Two studies were performed on each patient. In one, vit B_{12} (0.5 μg) labeled with Co^{58} or Co^{60} (1 μg/1 μc) was sterilized by heating to 60°C for 2 hours, made up to a volume of 1 to 2 ml, and injected into an antecubital vein. In the other study, 0.5 mg of a highly purified hog intrinsic factor preparation† with an activity of 1 National Formulary intrinsic factor unit/0.5 mg, was incubated for 20 minutes at room temperature with labeled vit B_{12} (0.5 μg) and this combination (HIFC-B_{12}) was then injected into an antecubital vein.

Measurements were made of the plasma levels of radioactivity at regular intervals following injection, of the radioactivity excreted in the first 24-hour urine, and of the radioactivity appearing in the liver and other organs. The methods followed, the plasma, urinary, and *in vivo* measurements, as well as the standards prepared and the calculations utilized have been previously described(1). Microbiological assays of the "free" and total serum vit B_{12} concentrations utilizing *Euglena gracilis* as the test organism were performed following the method of Ross(10).

The two studies in each patient were conducted at intervals varying from one week to one year. The order in which the 2 tests were performed varied; *i.e.*, some patients received B_{12} alone in the first study while in others the study using the combination of HIFC-B_{12} was performed first.

B. *Studies utilizing intrinsic factor labeled with I^{131}.* Intrinsic factor was labeled with I^{131} and injected into 2 dogs and into 2 patients with pernicious anemia, one in relapse and the other in remission. Iodination of intrinsic factor was accomplished by the following method: 0.7 mg of the HIFC was dissolved in 2 ml of potassium phosphate buffer in a beaker and the beaker suspended in ice water. A solution containing 0.1 ml I^{131}

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(370 μc) in 0.2 ml KI_3 was added dropwise and the solution allowed to stand for 3 hours in the cold. This was then dialyzed twice (for 20 and 40 hours) against M/15 phosphate. Aliquots of the concentrate were then injected into the subjects and were used to prepare standards. Although our samples were not tested, I^{131}-labeled intrinsic factor prepared by L. Ellenbogen using the same method has been shown by him to retain intrinsic factor activity after iodination.

Subjects studied. Six patients with abnormally low serum concentration of vit B_{12} were tested in the hog intrinsic factor concentrate study. Four of these had pernicious anemia in relapse and 2 had malabsorption syndrome. One patient with schistosomiasis without liver damage and with no disorder of B_{12} was also studied.

The diagnosis of pernicious anemia or malabsorption syndrome was suggested in each patient by history, peripheral blood and bone marrow examinations and was subsequently established by means of the Schilling test (11).

Results. Hog intrinsic factor study. Clearance of radioactive vit B_{12} from the plasma: The rate of plasma disappearance of vit B_{12} in 4 patients with pernicious anemia in relapse and 2 patients with malabsorption syndrome fell within the range of untreated pernicious anemia (Fig. 1). The serum vit B_{12} concentration just prior to intravenous administration of labeled vit B_{12} was low in each of these patients (Table I). These results are identical with those previously described (1).

When labeled vit B_{12} was incubated with hog intrinsic factor concentrate and injected intravenously into the same patients, the plasma disappearance rate increased to normal in 4 cases and approached normal in the other 2 (Fig. 2). The level of residual radioactivity in the plasma 24 hours after injection was not greater than 5.5% of the administered dose in any patient. This approaches the upper limits of the normal 24-hour radioactivity (5%) and is well below the lowest 24-hour radioactivity following injection of B_{12} alone (10.5%). Two of the patients with pernicious anemia and one with malabsorption syndrome were in hematologic relapse at the time of the test and the remaining 3 had normal serum B_{12} levels (Table I). One patient with schistosomiasis showed a similar (normal) plasma clearance rate following intravenous administration of B_{12}-HIFC.

TABLE I. Serum Vitamin B_{12} Concentrations at Onset of Each Test in the Hog Intrinsic Factor Concentrate (HIFC) Study.

	B_{12}			B_{12}-HIFC	
	Serum vit B_{12} Concentration ($\mu\mu$g/ml)			Serum vit B_{12} Concentration ($\mu\mu$g/ml)	
Curve (Fig. 1)	Free	Total	Curve (Fig. 2)	Free	Total
Pernicious anemia					
Q	0	45	Q′	10	300
R	12	80	R′	0	68
S	0	62	S′	0	64
T	0	40	T′	20	340
Malabsorption syndrome					
X	36	60	X′	8	690
Y	0	75	Y′	21	73
Normal			Z′	40	330

Tissue uptake of radioactivity: *In vivo* probe counting revealed that almost all of the measurable radioactivity appeared in the liver simultaneously with the drop in plasma radioactivity. Small increases in radioactivity above body background were also noted over the sacrum, heart and brain in the first 24 hours following injection.

Uptake in the liver was rapid in the first 3 to 6 minutes, slower in the next 30 minutes, and increased slowly during the next 3 to 5 days. There was a leveling off and/or gradual fall in liver radioactivity beginning on the third to tenth day. Liver radioactivity was detectable *in vivo* for at least one year after the tests.

It was not possible to quantitate the total amount of radioactivity in the liver and results are reported only as net counts per second. It should be noted, however, that in every patient the liver radioactivity was greater following injection of B_{12} combined with intrinsic factor than it was when B_{12} alone was injected (Fig. 3).

Urinary excretion of radioactivity (Fig. 4): There was somewhat greater 24-hour urinary excretion of radioactivity following

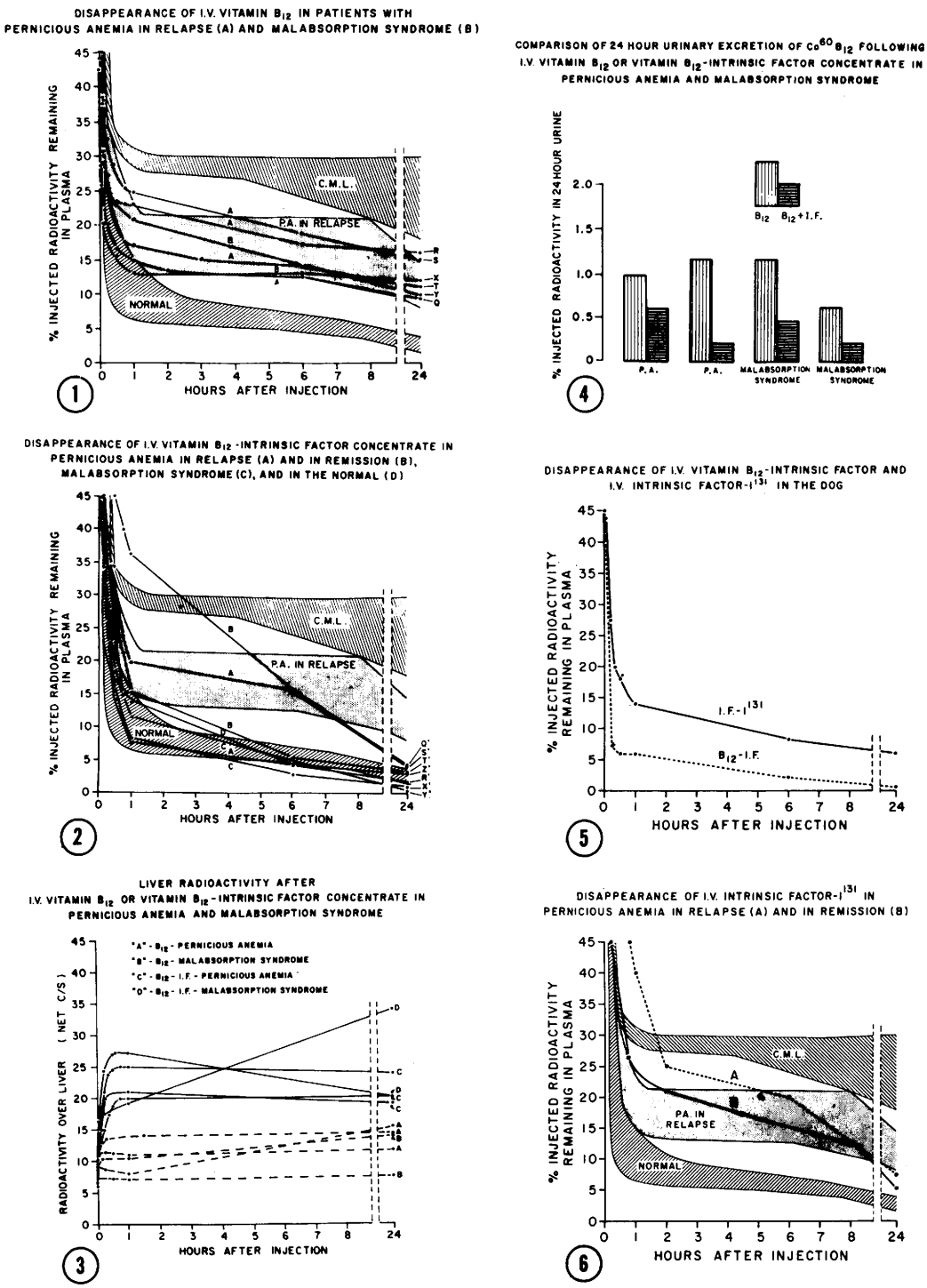

FIG. 1. In this Figure, as well as Fig. 2 and 6, shaded areas represent ranges of clearance of radiocobalt B_{12} in groups of patients with chronic myelocytic leukemia, pernicious anemia in relapse, or no disease ("normal"), respectively.

injection of B_{12} alone than of B_{12}-HIFC in all cases. It should be noted, however, that the 24-hour urinary excretion never exceeded 1.5% of the injected radioactivity and that the differences, although consistently in one direction, are of small magnitude.

Iodinated intrinsic factor: The plasma disappearance and tissue uptake following intravenous injection of I^{131}-labeled intrinsic factor (HIFC-I^{131}) into dogs and human subjects were compared with that following injection of HIFC-B_{12} and of B_{12} alone. The results indicate that intrinsic factor can be labeled with I^{131}, that the bond is a strong one, and that the I^{131} is not immediately removed by the thyroid.

The plasma disappearance curves in normal dogs demonstrated the same slope after the initial rapid fall, whether intrinsic factor was labeled with B_{12} or with iodine, but the overall rate of plasma clearance appeared to be slower when the iodine label was used (Fig. 5). The plasma disappearance curves following intravenous HIFC-I^{131} injections in the two patients with pernicious anemia (Fig. 6) were similar in slope to the curves following intravenous injection of B_{12} alone. Levels of plasma radioactivity at any given time after injection were also similar to the levels when B_{12} alone was injected, except for the 24-hour plasma radioactivity which was closer to the normal patient's levels. As in the dogs, the rate of disappearance of plasma radioactivity was significantly slower after HIFC-I^{131} injection (Fig. 6) than after HIFC-B_{12} injection (Fig. 2).

Hepatic uptake of HIFC-I^{131} was rapid at first and then declined slightly for the next 2 days in one of the 2 patients. Thyroid uptake was level in this patient for 8 hours, then rose moderately in the next 16 hours, with a marked rise in the following 24 hours. The second patient demonstrated a rapid liver uptake of the HIFC-I^{131} with a marked fall in hepatic radioactivity and a marked rise in thyroid radioactivity in 24 hours (Fig. 7).

Discussion. The presence of circulating intrinsic factor has been suggested by various studies including: The *in vitro* increased uptake of radioactive vit B_{12} by rat liver homogenate or rat liver slices(12,13) as well as

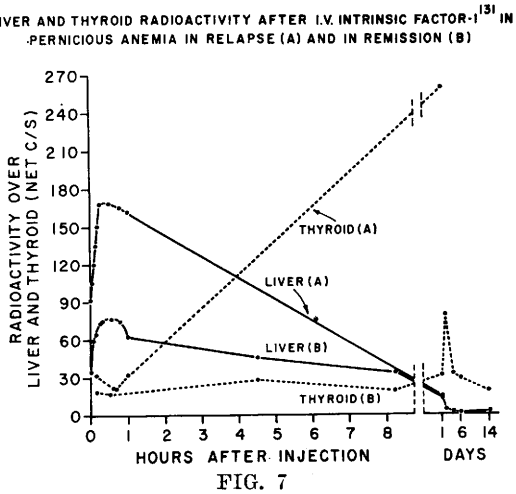

FIG. 7

in the perfused rat liver(14) in the presence of HIFC; the enhanced hepatic uptake *in vivo* of radioactive vit B_{12} in the rat when HIFC was introduced into the blood stream (15); the possible presence of intrinsic factor in rat and human bile(16,17); increased uptake *in vivo* in the human liver of vit B_{12} in the presence of human gastric juice (HGJ)(18,19) or HIFC(20). These studies suggest the possibility of a circulating intrinsic factor-like activity which functions to facilitate the transfer of B_{12} from the plasma to the liver.

Addition of HIFC to vit B_{12} in the present study resulted in alteration of the abnormal pernicious anemia-malabsorption syndrome plasma clearance to normal, associated with increased hepatic deposition of B_{12}. These results are consistent with the concept of intrinsic factor being absorbed with B_{12} from the normal small intestine and then acting to transfer B_{12} to the liver. According to this concept, in pernicious anemia and malabsorption syndrome intrinsic factor is not absorbed and is thus not present in the plasma, resulting in a decreased plasma clearance of B_{12} with a decreased deposition of B_{12} in the liver. This approaches normal when HIFC is added to the intrinsic factor-deficient plasma.

B_{12}-binding substances other than intrinsic factor are present in gastric juice and in body fluids. The results reported above may be explained by the presence of nonspecific

binders in either HGJ or HIFC, such as the R-fraction of Simons(21) or the Transcobalamin II of Hall(22,23). However, preliminary studies have also been done on the plasma clearance of B_{12} following intravenous injection of radiocobalt B_{12} combined *in vitro* with inactive HGJ (*i.e.*, HGJ without IF activity). Such inactive human gastric juice did not return the plasma B_{12}-clearance to normal in one patient with pernicious anemia in relapse and in one patient with malabsorption syndrome. Opposite results have been found by Rosenthal and Glass in at least some pernicious anemia gastric juices which behaved in rats similarly to normal gastric juice(24). This may be related to a different method of preparation of the gastric juice.

Heller *et al*(8,9) speculated that a diminished capacity to bind B_{12} might exist in the tissues of B_{12}-deficient individuals and that this lack of tissue receptors could account for the slow disappearance of intravenously injected B_{12} from the circulation in such individuals.

The possibility also exists that the "normalizing" effect of HIFC on the slow plasma B_{12} disappearance is not a specific transferring effect of intrinsic factor or of a non-intrinsic factor binder, but a nonspecific removal of a foreign substance (HIFC) from the blood by the reticuloendothelial system of the liver. It was to test this possibility that HIFC was labeled with I^{131}. In each instance the plasma clearance of HIFC labeled with I^{131} was slower than that of HIFC labeled with radio-B_{12}. If the radio iodine label is on the same molecule as the radio-B_{12} label, then the more rapid disappearance from the plasma of HIFC-B_{12} than of HIFC-I^{131} suggests a specific mechanism for the deposition of circulating B_{12} in the liver.

Summary. 1. Studies of the fate of intravenously injected vitamin B_{12} bound to hog intrinsic factor concentrate have been performed in patients with pernicious anemia and malabsorption syndrome. 2. The abnormal plasma disappearance curves of vit B_{12} in pernicious anemia and malabsorption syndrome became normal after incubation of B_{12} with hog intrinsic factor concentrate. 3. Plasma clearance of radioactivity in pernicious anemia is slower following injection of intrinsic factor labeled with I^{131} than following injection of intrinsic factor labeled with radiocobalt-B_{12}. 4. A specific effect of intrinsic factor in transferring circulating vit B_{12} to the liver is discussed.

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