Metabolic Turnover of Vitamin B_{12} in the Normal and Diseased Liver

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IT HAS been known for a long time that the liver is the main storage organ for vitamin B_{12}.[1-4] The mechanisms governing the metabolic turnover of vitamin B_{12} are not yet fully understood, however. Radioactive technics have now made it possible to study them in more detail. This paper deals with some of the data we have collected by means of isotope technics on the metabolic turnover of vitamin B_{12} in the normal and diseased liver.

MEASUREMENT OF HEPATIC UPTAKE OF RADIOACTIVE VITAMIN B_{12}

Several years ago, we developed a technic for counting radioactive vitamin B_{12} in visceral organs *in vivo*,[5] a technic based on the same principle as that involved in counting radioactive iodine in the thyroid.

In accordance with a recent modification of this method,[6] a tracer dose of 0.5 μg. of Co^{60}-vitamin B_{12} or Co^{58}-vitamin B_{12}, containing 0.4 to 0.5 μc. of radioactive cobalt, is given orally in the morning. Twenty-four hours later, 1 ounce of castor oil is administered and, the next morning, a cleansing enema is given. Forty-eight hours after administration of the tracer, counts are taken over the anterior and lateral projections of the liver, over two abdominal control areas, below and to the left of the umbilicus and over the posterior mid-area of the left gastrocnemius, which serves as body background. Counts are taken for five minutes each, with the scintillation counter in direct contact with the skin. After deduction of the body background, the hepatic uptake is then calculated in counts per minute and per 1 μc. of radioactive cobalt.

Following oral administration of radioactive vitamin B_{12}, the abdominal counts are initially higher than those over the liver. If a cathartic and enema are given on the third day, however, the abdominal radioactivity decreases below that of the liver, making it possible for the hepatic uptake of vitamin B_{12} to be measured as early as forty-eight hours after administration of the tracer.

In normal subjects, and in patients with simple hypo- or anacidity, the oral administration of radioactive vitamin B_{12}, alone or with intrinsic factor, results in an accumulation of radioactivity over the liver, as shown in Figure 1. With our technic, this amounts to from 400 to 1,200 counts per minute for each microcurie of Co^{60}-vitamin B_{12} ingested.

In pernicious anemia[5,6] or following total gastrectomy,[7] when intrinsic factor is absent, the absorption of vitamin B_{12} from the intestine is virtually abolished, and the hepatic uptake is either zero or present only in traces, as shown by the light squares in Figure 1. When an intrinsic factor preparation is added, intestinal absorption and hepatic uptake become normal, as shown by the black squares.

In most patients with sprue, vitamin B_{12} is not absorbed in the intestine and the hepatic uptake is either zero or present only in traces.[8-10] In contrast to its action in patients with pernicious anemia, however, intrinsic factor administration in patients with sprue does *not* improve the hepatic deposition of vitamin B_{12},[8] because this defect is due, not to the absence of intrinsic factor, but to a

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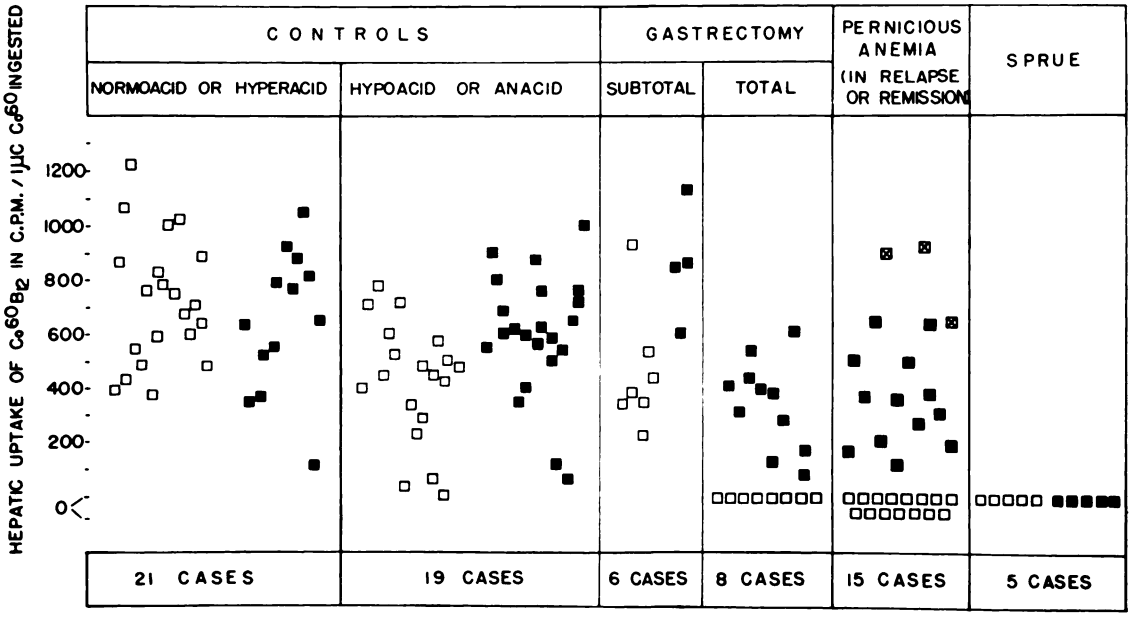

FIG. 1. Uptake of radioactive vitamin B_{12} by the liver in seventy-four persons following oral administration of Co^{60}-vitamin B_{12} alone (□) or together with potent intrinsic factor concentrate from the hog's stomach (■). (From: GLASS, G. B. J., PACK, G. T. and MERSHEIMER, W. L. *Gastroenterology*, 29: 666, 1955.[7])

defect in the absorptive mechanism of the small intestine.

DEPOSITION OF VITAMIN B_{12} IN THE LIVER IN HEPATIC DISEASE

Defective hepatic deposition of radioactive vitamin B_{12} may be due to hepatic disease as well. In about one-third of thirty-six patients with hepatic disease studied, the hepatic uptake of Co^{60}-vitamin B_{12} was below the lower limit of normal,[11] as shown in Figure 2. In three of them, i.e., in one patient with severe hepatitis, one with terminal liver cirrhosis, and one with advanced malignancy of the liver, the hepatic uptake was entirely abolished. No correlation could be observed, however, between the extent of damage to the liver, as shown by liver function tests, and the defective hepatic deposition of vitamin B_{12}.

The addition of intrinsic factor does not improve the hepatic uptake of radioactive vitamin B_{12} in hepatic disease. It seems reasonable to conclude, therefore, that the impaired hepatic uptake is due to damage to the liver cells, by which the ability of the liver cells to accept and deposit vitamin B_{12} has become impaired. This is supported by our other findings which indicate that the deposition of radioactive vitamin B_{12}, following parenteral administration, may be impaired in some of these cases as well.[11]

Vitamin B_{12} blood levels in advanced hepatic disease are, as a rule, high.[12–15] This has been correctly related to the liberation of hepatic vitamin B_{12} into the blood following destruction of liver tissue. Our findings suggest that high blood levels in some of these patients may also be due, in part, to the inability of the damaged liver cells to remove the vitamin from the circulation.

TURNOVER OF VITAMIN B_{12} IN DOGS

We next investigated the hepatic turnover rate of vitamin B_{12} in normal dogs.[16] These animals received a single injection of radioactive vitamin B_{12}, following which, for an observation period of as long as ten months, we determined the radioactivity of their livers by external recording. The tracings in the semi-logarithmic plot of Figure 3 represent the discharge of Co^{60} from dogs' livers, as determined by hepatic surface radioactivity. The arrows indicate the biologic half-life of radioactive vitamin B_{12} in the liver. This varied

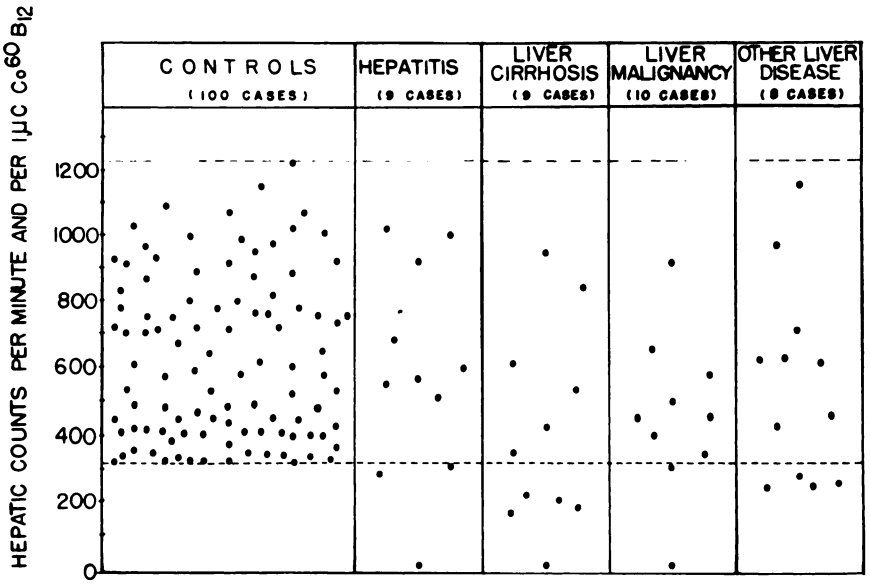

FIG. 2. Hepatic uptake of radioactive Co^{60}-vitamin B_{12} in 100 control subjects and thirty-six patients with hepatic disease. (From: GLASS, G. B. J., BOYD, L. J. and EBIN, L. *J. Lab. & Clin. Med.*, 52: 849, 1958.[11])

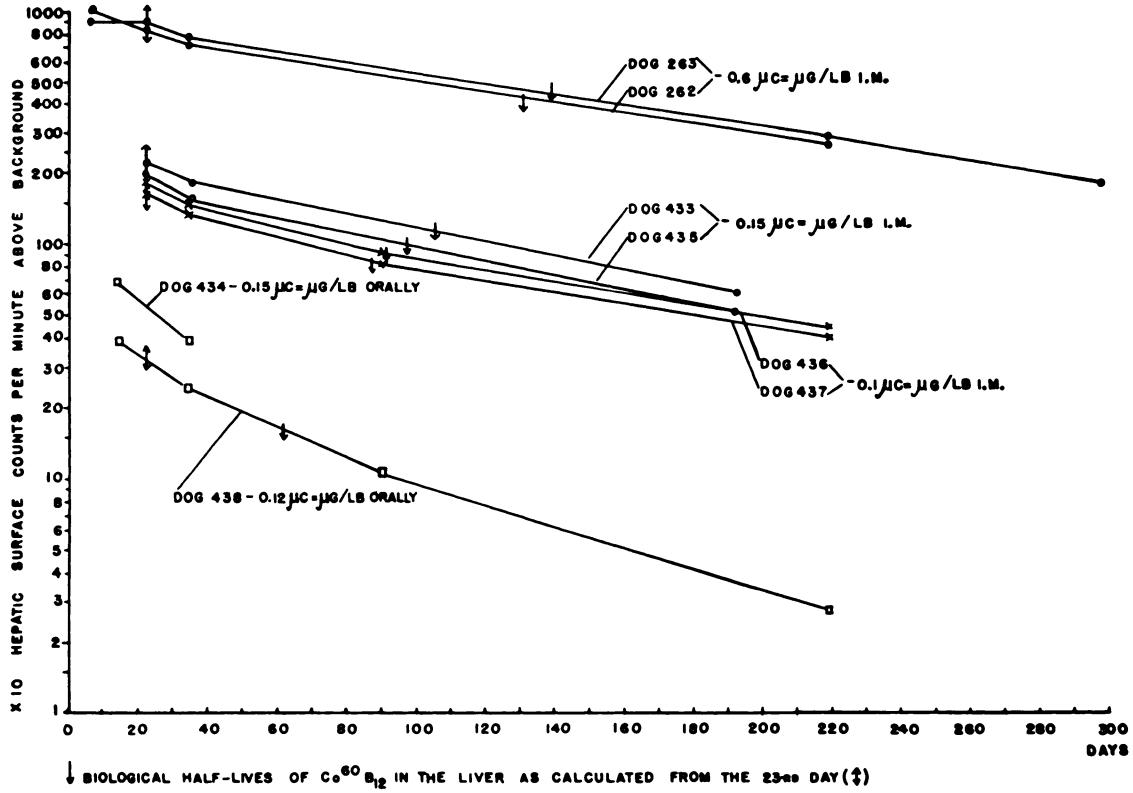

FIG. 3. Hepatic surface counts in eight dogs followed up to ten months after parenteral or oral administration of radioactive Co^{60}-vitamin B_{12}. (From: GLASS, G. B. J. and MERSHEIMER, W. L. *J. Lab. & Clin. Med.*, 52: 860, 1958.[16])

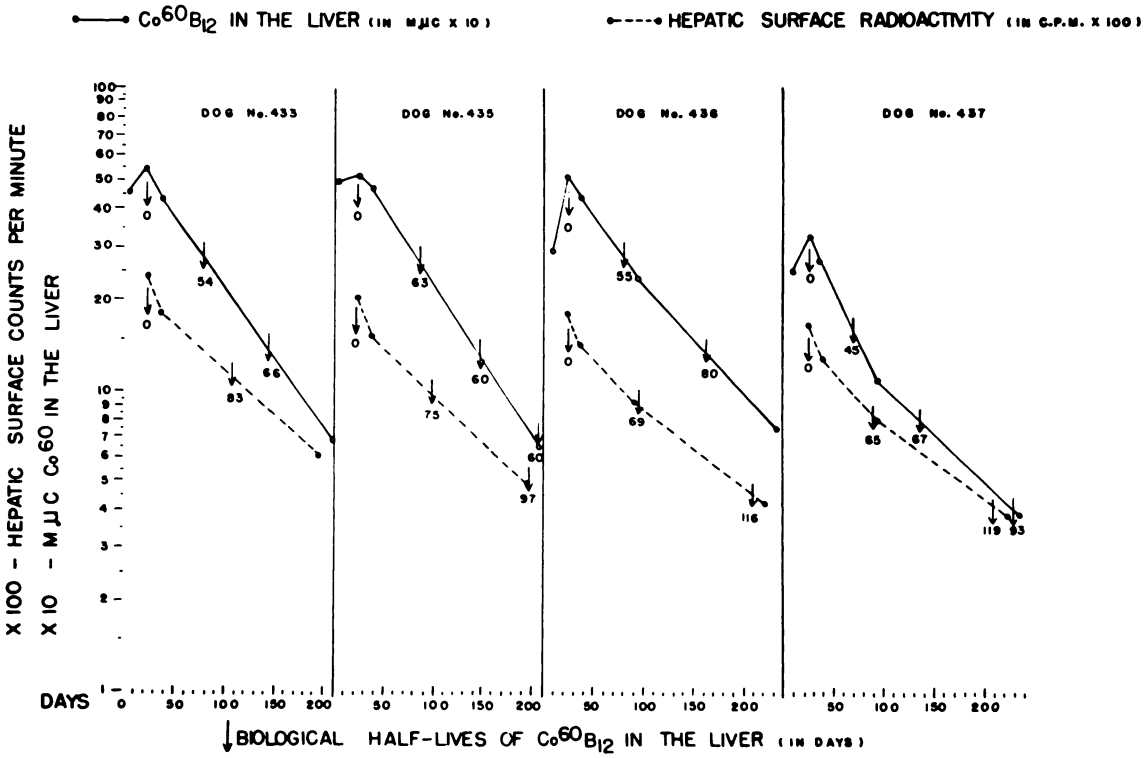

FIG. 4. Hepatic storage of Co^{66}-vitamin B_{12} and its discharge from the liver, as determined by surgical biopsies and hepatic surface scanning. (From: GLASS, G. B. J. and MERSHEIMER, W. L. *J. Lab. & Clin. Med.*, 52: 860, 1958.[16])

from six to twelve weeks in our dogs, depending on the dose injected. The discharge of vitamin B_{12} from the dog's liver proceeds much faster than it does in human beings, probably because of a more rapid general metabolic turnover.

At the bottom of Figure 3 is the tracing of the hepatic radioactivity following oral administration of Co^{60}-vitamin B_{12}. This is much lower than that obtained after a similar dose of radioactive vitamin B_{12} given intramuscularly, shown in the middle of this figure. This indicates the limited range of intestinal absorption of vitamin B_{12} in dogs, a limitation similar to that found in human beings.[17,18]

In four dogs, in addition to the measurement of surface radioactivity, we determined the radioactivity in the liver directly, by serial surgical biopsies of the liver (Fig. 4). These were repeated at intervals of several weeks for the duration of the observation period. The measurements of vitamin B_{12} in specimens obtained by surgical biopsies correlated well with the concentration of radioactive vitamin B_{12} in the entire homogenized organ.

The upper tracings of Figure 4, shown by continuous lines, represent the radioactivity of the liver as determined by serial surgical biopsies. These tracings are compared with those of hepatic surface radioactivity, shown below by intermittent lines. The consecutive biologic half-lives of radioactive vitamin B_{12} in the liver are again shown by arrows. The correlation obtained by both technics was close, and the tracings obtained were grossly parallel. The determination of the biologic half-lives of vitamin B_{12} in the liver by both methods gave similar values, in the range of from six to twelve weeks.

The distribution of Co^{60}-vitamin B_{12} in the viscera of two dogs was studied postmortem, several months after a single injection of Co^{60}-vitamin B_{12}. The concentration of radioactivity in the organs was highest in the kidneys, next highest in the liver, and followed in descending order, by the pancreas, spleen, small intestine and lungs. This is in line with the observations of Willigan et al.[19] who showed that the kidney of the dog contains a

TABLE I

Content of Radioactivity in the Liver and Kidneys of Five Dogs Sacrificed 205 to 232 Days After Intramuscular Administration of Co^{60}-Vitamin B_{12}

Dog	Dose of Co^{60}-Vitamin B_{12} Injected in μc./lb. Body Weight	Day after Administration	Radioactivity in mμc./gm. Tissue and per μc./lb. Body Weight Injected	
			Liver	Kidneys
1	0.10	232	0.30	0.37
2	0.10	232	0.37	0.35
3	0.15	205	0.20	0.35
4	0.15	205	0.27	0.25
5	0.60	218	0.14	0.20

higher concentration of vitamin B_{12} than does the liver.

The total vitamin B_{12} content in the viscera is highest in the liver, which contains more of the vitamin than all other abdominal organs combined. The kidneys are the second main storage depot, followed by the pancreas, small intestine, spleen and lungs.

The data in Table I show that larger parenteral doses of vitamin B_{12} result in much higher losses and less efficient retention in the organs. We calculated the radioactivity in the liver and kidneys per gram of tissue and per 1 μc. of Co^{60}-vitamin B_{12} injected per pound of the animal's weight. The larger the injected dose, the less, on a percentage basis, of the material injected will be stored in the liver and kidneys. In other words, one can "load" the liver with vitamin B_{12} much more effectively if one gives the same dose in small fractional portions at repeated intervals rather than as a single, massive dose. This has been thought to be so for a long time, but our data now give it experimental support.

RADIOACTIVE VITAMIN B_{12} IN THE LIVER

The data contained in textbooks on isotopes and in the publications of the Atomic Energy Commission mention a figure of 68 per cent as the total radioactivity of the body stored in the liver as the target organ, after administration of Co^{60}.[20] We believe that this figure is incorrect, not only for dogs, but for human beings as well. At the peak of hepatic radioactivity, as determined by biopsy of the liver, the amount of radioactivity found in the liver of dogs (after intramuscular administration of 0.1 to 0.15 μg. of radioactive vitamin B_{12}, which corresponds to about 15 μg. in man) is from 18 to 27 per cent of the administered dose. This figure is much less than the 68 per cent just mentioned. Our belief is that the same is true as well for human beings.

We attempted to determine whether the prolonged hepatic radioactivity following administration of Co^{60}-vitamin B_{12} is, in fact, due to hepatic storage of radioactive vitamin B_{12}, or whether it is due, rather, to the deposition of the radioactive cobalt atom in the liver.*

A slurry of the liver of a dog sacrificed seven months after injection of radioactive vitamin B_{12} was processed into a cyanocobalamin concentrate by the standard cyanide-nitrite treatment, followed by extraction by benzyl alcohol-ethyl ether and methanol. The usual recovery of cyanocobalamin by this procedure is in the range of 60 to 70 per cent of the total content of vitamin B_{12} in the liver. The recovery of radioactivity was in a similar range, 60 to 62 per cent. This shows that most of the radioactivity which accumulates in the liver after administration of radioactive vitamin B_{12} passes into the cyanocobalamin extract, and also indicates that the radioactivity was in association with the vitamin.

The cyanocobalamin extract was then submitted to descending chromatography in secondary butanol on large channelled sheets of filter paper for thirty hours. The papers were then cut into horizontal strips, one inch wide. Some strips were eluted, and the vitamin B_{12} content in the elutes was determined by Lactobacillus leichmannii and Euglena gracilis assay. In the other cut strips, the radioactivity was measured in a well counter.

Only small amounts of microbiologic activity and radioactivity accumulated in the zone of origin, the factor-B zone, and in the intermediate area. In contrast, about 85 per cent

* This work was done in association with Drs. F. H. Wolf and R. H. Weston of Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania.

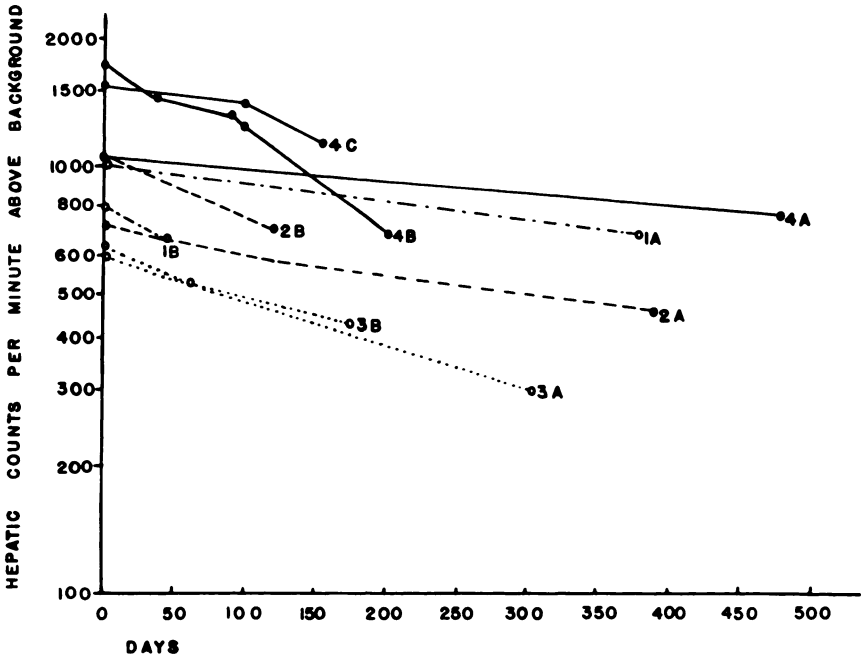

FIG. 5. Biologic decay of radioactivity over the liver in one patient with nutritional anemia (No. 1) and in three patients with pernicious anemia (Nos. 2 through 4), as determined by surface scintillation counting. (From: GLASS, G. B. J. *J. Lab. & Clin. Med.*, 52:875, 1958.[27])

of the microbiologically active vitamin B_{12}, and up to 63 per cent of the total radioactivity, were located in a single zone, that corresponding to the usual area of cyanocobalamin.

This clearly shows that, seven months after administration of a single dose of radioactive vitamin B_{12}, at least 60 per cent of the hepatic radioactivity emanates from the microbiologically active, labeled vitamin B_{12} stored in the liver.

DISCHARGE OF RADIOACTIVE VITAMIN B_{12} FROM THE LIVER IN MAN

When a single dose of labeled vitamin B_{12} is injected intramuscularly into a human subject,[21] the radioactivity over the site of the injection decreases rapidly with the result that, four hours later, not more than about 1 per cent of the initial activity remains. A few minutes after injection some radioactivity appears over the cutaneous projections of the liver, spleen and kidneys and this gradually increases during the next few hours.

Subsequently, the radioactivity over the liver continues to increase over the next five to seven days, while that over the kidneys and spleen levels off or diminishes.[21] This is probably due to a relocation of vitamin B_{12} at this time from other tissues to the liver. After seven to ten days, however, the hepatic radioactivity levels off, and then for a period of months decreases only slightly.[21] The human liver is thus shown to retain vitamin B_{12} most tenaciously, like the animal's liver,[1–4] a finding in man first reported by Smith[22] and since confirmed by many other investigators.[21,23–26]

Table II summarizes our results in eighteen subjects studied for a period of forty-five to 878 days.[27] The data show that only about 5 per cent, on the average, of the hepatic stores of vitamin B_{12} are discharged each month from the liver of both normal subjects and patients with pernicious anemia in remission. Large individual variations are observed in this figure however; the range in various subjects extending from 1.5 per cent to about 10.5 per cent per month.[27] The slopes of the tracings

TABLE II

Rate of Discharge of Co^{60}-Vitamin B_{12} from the Liver in Ten Control Subjects and Eight Patients with Pernicious Anemia in Remission

Group	No. of Cases	No. of Observations	Duration of Observation Period (days)			Decrease in Hepatic Surface Radioactivity per Month (%)			Presumptive Biologic Half-Life Time of Co^{60}-Vitamin B_{12} in the Liver (mo.)		
			Range	Mean	Median	Range	Mean	Median	Range	Mean	Median
Control subjects	10	11	45–377	139	101	2.4–10.5	5.5	4.1	4.8–20.8	11.1	12.1
Patients with pernicious anemia in remission	8	12	64–878	297	214	1.5–10.3	4.8	4.9	4.8–33.3	14.5	11.9
Totals	18	23	45–878	222	169	1.5–10.5	5.1	4.2	4.8–33.3	12.8	12.0

are not parallel, however, which suggests individual differences among various subjects in the rate of hepatic discharge (Fig. 5). It is to be noted, moreover, that the rate of discharge from the liver may change at various times in the same person.

CONCLUSIONS

Hepatic surface radioactivity is a true measure of the content of radioactive vitamin B_{12} in the liver. This assay technic can also be applied successfully to the measurement of the intestinal absorption of vitamin B_{12} and to the diagnosis of pernicious anemia and sprue in relapse and remission.

In one-third of patients with hepatic disease, the ability of the liver cells to take up vitamin B_{12} is impaired. This may be a contributory factor in causing the high vitamin B_{12} blood levels in some cases of hepatitis, liver cirrhosis and malignant infiltration of the liver.

The liver is the main storage organ for vitamin B_{12} in man and the dog. At least 60 per cent of the radioactivity found in the liver long after the administration of a single dose of radioactive vitamin B_{12} is due to the microbiologically active labeled vitamin B_{12} which has been stored in the liver.

The rate of discharge of the vitamin from the liver of the dog is about five times faster than it is in human beings. The rate of discharge from the liver in man is from 1.5 to 10 per cent per month, which assumes that the biologic half-life of vitamin B_{12} in the human liver is, on the average, one year, with individual variations of from five to thirty-three months.

This remarkably prolonged period of hepatic storage of vitamin B_{12} in man explains the long latent periods which precede the development of clinical vitamin B_{12} deficiencies.

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