THE PLASMA DISAPPEARANCE OF INTRAVENOUSLY ADMINISTERED COBALT^{58} VITAMIN B_{12} *

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The combination of low serum content and low specific activity tracer material has presented a difficult technical barrier to the study of the plasma disappearance of tracer amounts of vitamin B_{12}. Three groups (1–3) have studied the rate of plasma disappearance of intravenous doses ranging from 0.5 to 4.0 μg. These doses were not and could not be considered to be of tracer magnitude since even 0.5 μg may equal the entire plasma content. Estren, Brody and Wasserman (3) in their review gave 2×10^{-4} to 9×10^{-4} μg per ml as the normal range of serum vitamin B_{12}. Thus, persons with plasma volumes of 3,000 ml would have a total plasma content of between 0.6 and 3 μg. All three groups referred to above found a rapid plasma loss of the labeled B_{12} in normal subjects. Patients with chronic myelogenous leukemia (1–3) retained more of the vitamin in the plasma, and it was assumed that the greater plasma-binding capacity of these patients prevented the escape of the vitamin.

If plasma binding determines the rate of loss from the circulation, the rapid loss described in normal subjects (1–3) may have been a consequence of the relatively large amounts of labeled material used. The measurement of the disappearance of truly tracer amounts of vitamin B_{12} appeared necessary for a proper understanding of the process.

The term "binding" is used here according to current concepts of vitamin B_{12} plasma binding. A detailed discussion is beyond the scope of this paper, but the many approaches which give similar but not identical values can be summarized under three categories: *1*) the use of dialysis or a related process for the separation of free and bound vitamin; *2*) the use of charcoal extraction for the same purpose; *3*) making use of the unavailability of bound vitamin to certain microorganisms until the serum is heated. Estren and associates (3) stated the unsaturated serum binding capacity to be on the order of 7×10^{-4} μg per ml. This amount can be bound in addition to that already present and in a bound form. A person with a plasma volume of 3,000 ml would then have a total unsaturated binding capacity on the order of *2* μg.

An important contribution to the problem of binding was recently made by Miller and Sullivan (4) who found a seromucoid with specific mobility on electrophoresis to be the binding site of native vitamin B_{12}. However, added B_{12} could be bound to other sites firmly enough to resist removal by dialysis, and it appears that binding as measured by current *in vitro* techniques may measure more than that vitamin attached to the native binding site. Too little is known about the relationships between the plasma binding of vitamin B_{12} and vitamin B_{12} metabolism to permit the assumption that binding at a site different from that of the native B_{12} does not have biological significance. This recently described more restricted concept of binding is presented here mainly to emphasize that while *in vitro* binding of B_{12} can be measured easily and precisely by charcoal extraction or dialysis techniques, one must be cautious in relating it to *in vivo* events until it is better understood. For the present study, bound vitamin was considered to be that vitamin which could not be removed by charcoal extraction.

Fortunately, a high specific activity cobalt^{58} vitamin B_{12} ($Co^{58}B_{12}$) has recently become available.[1] This development and the adaptation of more sensitive counting techniques made it possible to use very small amounts of labeled material in the present study. The smaller doses used were on the order of 1/50 to 1/250 of the entire

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plasma content and could be completely bound by 30 to 40 ml of plasma and serum prior to injection. The purpose of the study was to describe the plasma disappearance of such a dose and to study factors influencing it.

MATERIALS AND METHODS

Subjects. The subjects were hospitalized patients past age 50. With the exception of the two patients who received vitamin B_{12} mixed with large volumes of plasma, all of the control subjects had recovered from an acute illness. Patients with liver disease, alcoholism, malnutrition, gastrointestinal disease, neoplasms, leukemia, renal disease, diabetes, or endocrine disorders were excluded. All of the control subjects had normal serum vitamin B_{12} levels and normal serum binding capacities.

Radioactive vitamin B_{12}. All plasma and urine studies were made with two lots of $Co^{58}B_{12}$ of initial specific activities of 8.1 and 6.1 μc per μg. The body-counting studies were done with one lot of 9.7 μc per μg. The majority of the studies were performed within one month of receipt of shipment, a very few after two months. The vitamin B_{12} activity of each lot was checked by *Euglena gracilis* assay. One lot was checked for free Co^{58} by half-saturating a plasma with a known binding capacity and removing free Co^{58} by dialysis, and none was detected.

Vitamin B_{12} assay. The *E. gracilis* Z strain method of Hutner, Bach and Ross (5) was used throughout. Serum B_{12} binding capacity was measured by the charcoal extraction method of Miller (6). Charcoal extraction was also used to determine the state of B_{12} in mixtures of $Co^{58}B_{12}$ and serum.

Sampling procedure. Thirty ml venous samples were taken at intervals and all measurements were performed on serum. Start and stop times of each sample were recorded, and the half-times were used in the calculations.

Counting procedures. The serum samples of 10 to 15 ml were counted in flat-bottomed vials fitting snugly in a large diameter (hole, 1 inch) well crystal. Urines were counted in volumes of up to 600 ml in a beaker so designed that a conventional sized well crystal protruded into the beaker from below. Both systems were shielded with 2 inches of lead. All counting was done with a Baird-Atomic model 513 scintillation spectrometer set to include only the 0.8 Mev Co^{58} peak in the window. The background with the conventional crystal ranged from 14 to 17 cpm and with the large diameter well, from 19 to 21 cpm. The following counts per minute per microcurie were representative. Urine beaker: 500 ml vol, 21,600; 100 ml vol, 43,000. Large well crystal: 10 ml vol, 157,000; 15 ml vol, 133,000.

Serum and urine standards were made up from each lot of material used, and the appropriate standards were counted with each set of samples. All samples from each experiment were counted on the same day without interruption or change in the spectrometer. When a series of samples required a long counting period, the standard was counted at intervals to check spectrometer stability. Almost all of the serum and urine counting was carried to an accuracy of within 10 per cent, and two-thirds of the serum samples were between 4 and 7 per cent. Some of the weaker serum samples (24 to 48 hours) could be counted to only 10 to 15 per cent accuracy without unreasonably long counting times.

Body counting. A dose of 0.17 μg (1.2 to 1.5 μc) in 1.0 ml was rapidly injected intravenously. One of two matched scintillation probes was placed over the fourth left intercostal space at the sternal border (precordium) and the other over the sixth right intercostal space at the midclavicular line angled at about 45° (liver). The collimators admitted radiation from an area 8.5 cm in diameter at 5 cm below the skin and from an area of 15 cm diameter at 15 cm depth. The ends of the collimators were in contact with the skin. Continuous recordings were made using a Baird-Atomic model 535 ratio analyzer. One probe recorded the liver counts and the other the ratio of liver/liver + precordium. Five minutes after injection the simultaneous and continuous recording was stopped, and interval body counts were continued with one probe and a scaler. The body counts using the latter setup were made over two liver sites, the sixth right intercostal space at the midclavicular line and the ninth right intercostal space at the midaxillary line; over the left kidney; and over the sacrum. The counting error with the latter setup was less than 5 per cent.

Calculations. The zero time concentration, as plotted in Figures 1, 2 and 5, was taken as microcuries injected/plasma volume. This time zero concentration was used as 100 per cent of plasma concentration in the text where plasma disappearance is discussed, in all tables, and in Figure 2. Plasma volumes were not measured but calculated to be

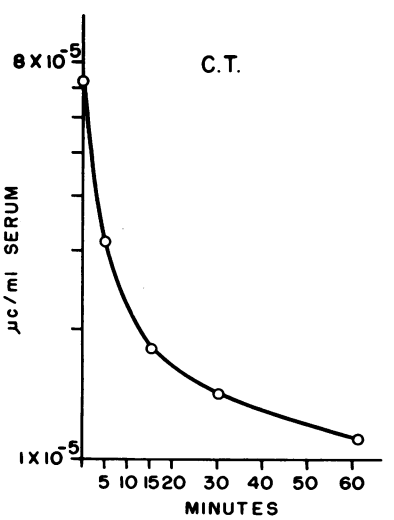

FIG. 1. PLASMA DISAPPEARANCE DURING FIRST HOUR OF A 0.043 μG INTRAVENOUS DOSE OF FREE $Co^{58}B_{12}$; CONTROL SUBJECT C.T.

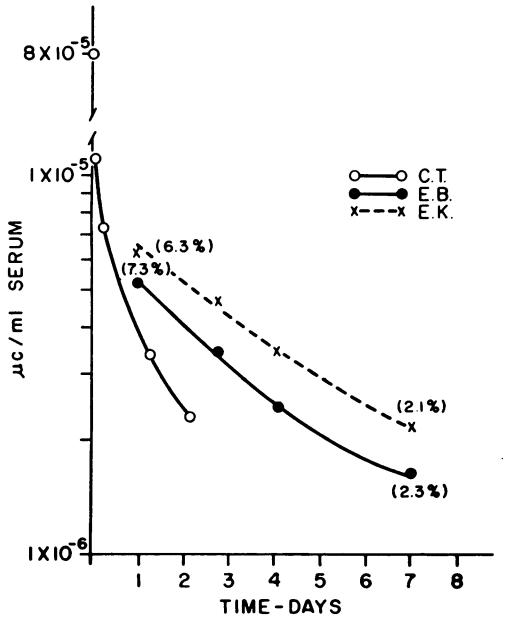

FIG. 2. PLASMA DISAPPEARANCE OF INTRAVENOUS FREE $Co^{58}B_{12}$, CONTROL SUBJECTS. Dose, C.T., 0.043 µg; dose, E.B. and E.K., 0.085 µg; % indicates the per cent of time zero concentration remaining at indicated times.

45 ml per kg body weight. As will become obvious on examination of the data, any increase in precision by actual measurement of plasma volume could have in no way altered the findings of the study. All radioactivity data were corrected for physical decay to the date that the samples were counted.

RESULTS

Disappearance of unbound intravenous dose. A dose of 0.043 µg (0.18 to 0.25 µc) of $Co^{58}B_{12}$ in 5.0 ml of saline was given rapidly (half-time, 10 to 13 seconds) to each of four subjects. The results, expressed as per cent of estimated time zero concentration remaining at intervals, are given in Table I. There was a period of very rapid loss followed by a much slower fall in radioactivity. A typical disappearance curve is given in Figure 1 and Figure 2 (C.T.). The curves of all four subjects were of similar configuration. In order to explore the period after 48 hours, two subjects were given doses of 0.085 µg, and samples were taken during the 1 to 7 day period. The results given in Figure 2 show that the slow rate of loss continued for several days.

TABLE I

*Vitamin B_{12} plasma disappearance, control subjects**

Subject	Per cent time zero concentration remaining at							
	5	15	30	1	6	12	24	48
	min			*hrs*				
E.S.	41	22	20	16	10	9.0	8.8	
L.S.	46	30	25	19	12	9.3	7.7	
H.G.	38	24	19	13	7.8	5.6	3.5	
C.T.	44	25	20	15	8.7	7.3	5.4	3.2
Mean	42	25	21	16	9.6	7.8	6.4	

* Dose in all cases was 0.043 µg $Co^{58}B_{12}$ in 5.0 ml of saline.

Disappearance of bound intravenous dose. Although in the first set of experiments the dose could have been bound by a small fraction of the total plasma volume, there was a possibility that some of the vitamin left the circulation in a free state. Doses of 7.0×10^{-3} to 1.2×10^{-2} µg (5.2×10^{-2} to 9.2×10^{-2} µc) were then mixed with various amounts and types of serum or plasma before injection. A complete set of samples was not taken in each case, but there were sufficient samples to determine the type of disappearance curve. The dose, when it was given in up to 35 ml of se-

TABLE II

Vitamin B_{12} plasma disappearance, control subjects

Subject	Dose	Dose added to:	Mixt. incub.		Binding of mixt.	Remaining in plasma at	
						15 min	1 hr
	µg		ml	min	%	%	
H.W.	0.012	Own serum	12	15		29	20
P.P.	0.007	Own serum	24	15	86	33	17
G.K.	0.012	Donor plasma	35	15	86	27	21
G.S.	0.012	Donor plasma	35	15	94	21	13
J.M.	0.012	Donor plasma	35	15	94	20	12
S.DiV.	0.010	Donor plasma	166	15	97	26	
O.S.	0.009	Donor plasma	190	15	96	24	
C.DuB.	0.007	Saline	16.5	120	80	37	20
		Own plasma	16.5				
J.Ma.	0.008	None				23	17

rum, was injected rapidly (half-time 16 to 21 seconds). When the dose was given in 166 to 190 ml of plasma, the half-times of injections were 3 minutes and 2 minutes, 30 seconds. The conditions of each experiment and the results are given in Table II. Although up to 97 per cent of the vitamin was bound prior to injection, the plasma removal was as rapid as that of the vitamin injected in a free state.

Urinary excretion. The 24-hour urinary excretion was measured in nine subjects. In some cases urine samples were taken at intervals throughout the 24 hours. The 24-hour excretion ranged from 0.1 to 1.9 per cent (mean 0.7) and almost all of the loss occurred in the first 1 to 2 hours. The dose was given in saline in four subjects, and bound to serum in five. There was no correlation between manner of administration and amount excreted.

Other body fluids. It was considered that the initial rapid loss from the serum may have been due to a rapid mixing with all extracellular fluid. Two subjects were then given 0.017 μg doses 1 hour and 3 hours, respectively, prior to a pneumoencephalogram. No radioactive vitamin was found in the cerebrospinal fluid removed, although simultaneous serum measurements showed the expected concentration. An identical dose was given to each of two subjects with cirrhosis and ascites, 2.5 and 7.5 hours prior to paracentesis. No radioactivity was found in the ascitic fluid.

Surface counting of organs. Four subjects were given an intravenous dose of free $Co^{58}B_{12}$ followed by simultaneous and continuous counting over the precordium and liver. Although absolute counting rates varied among the subjects, the distribution of the radioactivity, the time relationships, and the general proportions of activity between counting sites were the same. A typical organ uptake pattern is given in Figure 3. The hepatic radioactivity rose rapidly during a period when the precordial level was falling and reached a plateau between 1 and 2 minutes after the appearance of hepatic activity. At 5 minutes the simultaneous and continuous recording was discontinued, and counting of other organ sites at intervals was started. In Figure 3 there is an interval of 10 minutes between the plots of continuous and interval counting. From other cases it was known that little or no increase in hepatic activity would be expected during this interval. The hepatic activity of Subject A.D. rose slowly from the 15 minute level to a peak 5 days later. The activity at this peak was between 60 and 70 per cent greater than at the 2 minute level. Thus the liver took up more than half of its ultimate radioactivity in less than 2 minutes, and the remainder slowly over several days. There was also an uptake of the

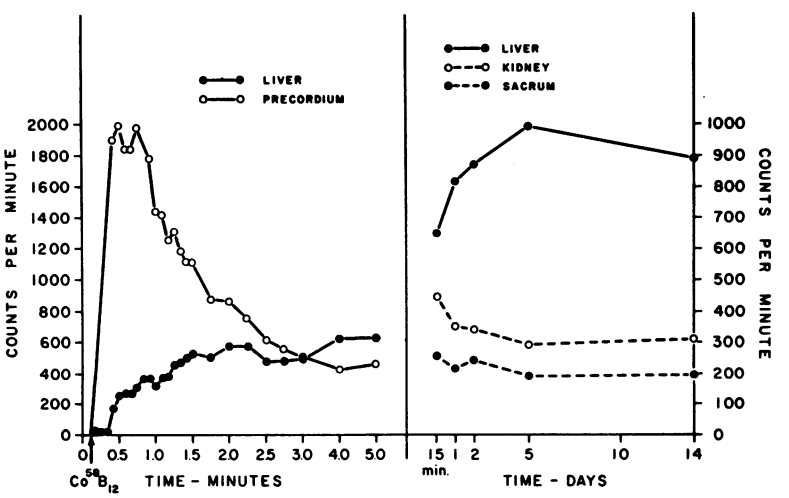

FIG. 3. BODY COUNTING, SUBJECT A.D., 0.17 μg I.V. DOSE. Section on left from continuous, simultaneous recording between 0 and 5 minutes. Section on right interval counting over three organs, 15 minutes to 14 days. Note change in vertical axis. Because of change in equipment, absolute counts are not strictly comparable between the two sections.

TABLE III

Influence of large amounts of nonradioactive ("cold") B_{12} on plasma removal of 0.043 μg Co^{58} B_{12}

Subject	"Cold" B_{12}	Route	Injection time* "cold" B_{12}	$Co^{58}B_{12}$ remaining at Minutes 5	15	30	60	Hours 6	24	24-Hour urinary excr. of $Co^{58}B_{12}$
	μg			% dose						% dose
S.N.	1,000	I.M.	2.5 min	40	31	23	18	7.2	2.8	40
A.R.	60	I.V. Infusion	1–33 min	45	34	31	20	11	7.7	
D.R.	60	I.V. Stat dose	−15 sec	34	21	14	11	3.9	1.3	
W.H.	89	I.V.	Simultaneous†	34	19	16	12	4.3	2.1	51

* Injection time is time "cold" B_{12} given in relation to time $Co^{58}B_{12}$ given.
† $Co^{58}B_{12}$ and "cold" B_{12} were well mixed prior to injection.

kidney and sacral areas followed by a decline in activity between Days 0 and 5, a period of rising hepatic activity. The dose of 0.17 μg, which was larger than that used in the studies of plasma disappearance, was necessary to give adequate counts for continuous recording. Interval organ counts were made on three patients receiving smaller doses of 0.017 to 0.043 μg, and the same prompt hepatic uptake of half or more of the ultimate level was observed.

The plateau of hepatic uptake at 1 to 2 minutes could be considered as evidence of saturation of hepatic receptors. This possibility was investigated by giving one subject the same dose of $Co^{58}B_{12}$ (0.17 μg) mixed with 80 μg of nonradioactive B_{12}. The 5 minute and ultimate levels of hepatic activity were on the order of one-half that of the subjects receiving only $Co^{58}B_{12}$, a difference which could be accounted for by the urinary loss known to result from a dose of 80 μg (Subject W.H., Table III). There was no evidence of a hepatic saturation phenomenon.

Effect of large doses of nonradioactive B_{12}. The effect of a high plasma concentration of vitamin B_{12} on the plasma disappearance curve was studied by the following series of experiments. The in-

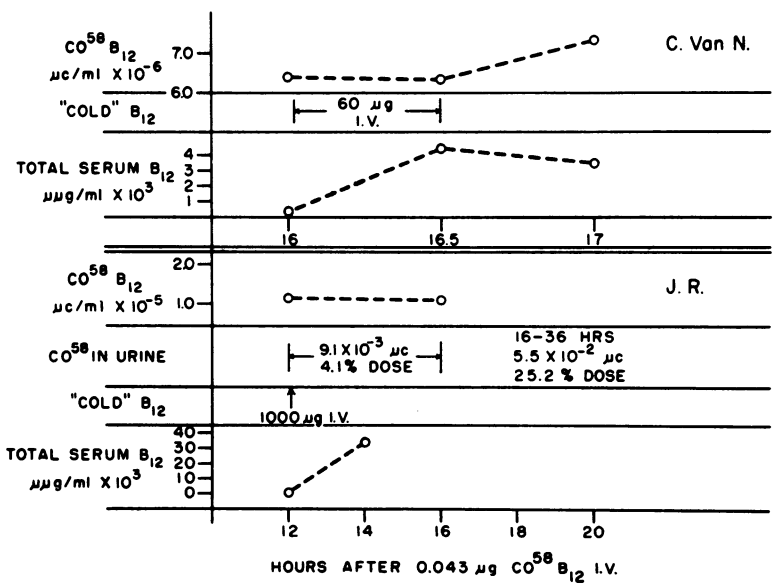

FIG. 4. THE EFFECT OF LARGE AMOUNTS OF NONRADIOACTIVE VITAMIN B_{12} ("COLD" B_{12}) ON THE $Co^{58}B_{12}$ REMAINING IN THE PLASMA 12 AND 16 HOURS AFTER I.V. ADMINISTRATION; CONTROL SUBJECTS, C. VAN N. AND J.R.

travenous dose of $Co^{58}B_{12}$ was 0.043 μg in 5.0 ml of saline in all instances.

1. In order to test the stability of the plasma B_{12} at the region of the curve where fall-off was slow, an intravenous infusion of 60 μg of nonradioactive ("cold") B_{12} was given 16 hours after $Co^{58}B_{12}$. The results given in Figure 4 (Subject C. Van N.) indicate that in spite of a rise in plasma B_{12} level to 10 times the pre-experiment level, there was no fall in the $Co^{58}B_{12}$ concentration. The rise after discontinuing the infusion was of borderline significance. In a second similar experiment (Subject J.R.), 1,000 μg was given intravenously at 12 hours (Figure 4). There was no fall in the $Co^{58}B_{12}$ plasma level over a 4-hour period although there was a rise in the total B_{12} plasma level of 70 times that of pre-experiment. Four per cent of the dose of $Co^{58}B_{12}$ was excreted in 4 hours. Had all of the excreted $Co^{58}B_{12}$ come from the plasma, there would have been a fall in $Co^{58}B_{12}$ plasma level to 65 per cent of the starting level, which did not happen. The vitamin in the urine either came from the plasma and was exactly replaced with tissue vitamin B_{12}, or it moved from tissue to urine without measurable change in plasma level.

2. Three subjects received nonradioactive vitamin B_{12} at various times in close relation to the $Co^{58}B_{12}$ (Table III). When given after the $Co^{58}B_{12}$, it had no measurable influence on the $Co^{58}B_{12}$ curve except possibly after 6 hours (Subject S.N.). Nonradioactive B_{12} given before the $Co^{58}B_{12}$ (Subject D.R.) resulted in speeding up of removal of the $Co^{58}B_{12}$, but the change was slight.

3. Subject W.H. was given both $Co^{58}B_{12}$ and nonradioactive B_{12} simultaneously in the form of 7.0 ml of a previously prepared mixture in saline. He received a total of 89 μg of vitamin B_{12} (injection half-time 6 seconds). There was some deviation from the type of curve obtained with small doses, but the changes were surprisingly minor (Table III) and could have been produced solely by the 51 per cent loss in the urine.

DISCUSSION

The present study is primarily concerned with the description of plasma vitamin B_{12} disappearance in the subject without disturbance of vitamin B_{12} metabolism. Mathematically, the vitamin B_{12} disappearance curves obtained above could be analyzed in more than one way, but the concept of a multi-compartment closed system (7) seemed to best fit the data. The equation for such a system of four compartments is:

$$C = ae^{-k_1 t} + be^{-k_2 t} + ce^{-k_3 t} + de^{-k_4 t} + f,$$

where C = the plasma concentration; a, b, c, d, and f are constants; and k_1, k_2, k_3, and k_4 are the summation of constants expressing the fractional change per unit time for separate processes. All of the curves could be broken down into four such components. For example, the curve of Subject C.T. was analyzed by the procedure of Veall and Vetter (7) to give the following values of k as the fractional part removed per hour: $k_1 = 7.4$; $k_2 = 0.80$; $k_3 = 0.10$; $k_4 = 0.017$. The biological significance of this analysis is a matter of conjecture, but it is useful in discussing the curves. The rapid component of the disappearance curve was similar following doses ranging from 7×10^{-3} to 89 μg, although the data do not permit the conclusion that rates or physiological mechanisms were identical. With the doses of 0.043 μg and less, all of the $Co^{58}B_{12}$ in the serum was in the bound form. In the study using an 89 μg dose, almost all was in the free form, but similar curves were obtained in both studies. In the pre-binding studies, 80 to 97 per cent pre-injection binding was achieved, but the vitamin was still no better retained in the circulation. These findings do not support the concept that the greater the binding capacity of the plasma, the slower the loss from the circulation.

The distribution of the initially rapidly disappearing tracer dose could be only partially determined. It was not excreted and was not detected in cerebrospinal fluid or ascitic fluid. There was evidence of rapid accumulation in the liver and kidney. Liver accumulation then continued at a slower rate after the plasma $Co^{58}B_{12}$ had fallen to a low level. Possibly, that vitamin rapidly taken up by tissue was intracellular but not in its ultimate intracellular form. Evidence of such a concept is found in the recent work of Strength, Alexander and Wack (8). They observed a fall in rat kidney homogenate B_{12} and supernatant B_{12} but a rise in the mitochondrial content over the period of 0.5 day to 12 days to 30 days after the last

of five doses of 1.0 μg. The rat liver homogenate B_{12} rose over the same period, and while the B_{12} content of both the supernatant and mitochondrial fractions increased, the latter showed the greater increase.

The initial phase of disappearance was virtually complete in an hour or less. The events following immediately were less well defined. A substance which moves among several compartments, as in the type of system being used as a model in this discussion, both enters and leaves a compartment. Re-entry into a compartment such as the plasma could not be detected if the total rate of loss was continually greater, and no evidence of re-entry into the plasma was detected in the control subjects. The two experiments made 12 and 16 hours after the injection of $Co^{58}B_{12}$ indicate that by this time the vitamin was in a state differing markedly from that shortly after administration. The level of $Co^{58}B_{12}$ did not fall as large amounts of non-radioactive B_{12} passed through the plasma, but $Co^{58}B_{12}$ was still available from some source for urinary excretion and hepatic uptake.

The fourth component of the equation which predominated after 24 hours may be significant in relation to total body turnover. This component is evident by inspection of all curves carried to 24 to 48 hours but is best seen in the curves of the two patients followed beyond 48 hours (Figure 2). The k_4 for Subject E.B. was 8.0×10^{-3} per hour with a half-time of 82 hours. Assuming that the daily plasma turnover of vitamin B_{12} equals the fraction removed per day times total plasma B_{12} (micromicrograms per milliliter) times plasma volume (milliliters), Subject E.B. turned over 0.18 μg per day between Days 1 to 7. There are too many unknown factors to permit the conclusion that this figure represents the plasma turnover of normally metabolized B_{12}, but the order of magnitude is in keeping with the general concept that body B_{12} turns over slowly.

A significant point is brought out by comparing the 48 hour $Co^{58}B_{12}$ level of a subject receiving 0.043 μg (C.T.) with that of the subject receiving 89 μg (W.H.). C.T. bound 3.2 per cent of the dose in his plasma, or less than 1.0 $\mu\mu$g per ml. W.H., who lost 50 per cent of the dose in the urine, bound either 0.50 per cent or 2.4 per cent, depending upon whether the serum level was measured by radioactivity or bioassay, which was on the order of 1,000 times that bound by C.T. Obviously the amount of B_{12} bound in the plasma at 48 hours (body distribution was relatively fixed by this time) was determined by a process more complex than a simple addition of vitamin B_{12} to a plasma with unsaturated binding sites.

The present work should also be discussed in terms of the more restricted concept of binding presented by Miller and Sullivan (4). Subject C.DuB. (Table II) received 7.0×10^{-3} μg of B_{12} previously mixed with 16.5 ml of his own plasma and incubated for 2 hours. Using 650 $\mu\mu$g per ml as the binding capacity of the native binding protein (BP) (4), this subject could bind 460 $\mu\mu$g per ml in addition to that already present (650 $\mu\mu$g per ml minus the bound native B_{12}, 190 $\mu\mu$g per ml). Since 420 $\mu\mu$g was added per ml, all could have been bound to the native BP. However, actual determination of the binding site is necessary in such experiments, for possibly other binding sites preferentially bind the B_{12} *in vitro*. Handling of plasma prior to *in vitro* binding may alter the BP, although this did not appear to be the case with the chronic myelogenous leukemia sera studied by Miller and Sullivan. Applying the same type of calculation, Subject J.Ma. (Table II), who received the dose in saline, would be able to bind all of the dose in 29 ml of plasma. Even Subject C.T. (Table I), who received a larger dose in saline, would be able to bind the dose completely with 112 ml of plasma. It would then appear that these two subjects could have promptly bound the added vitamin B_{12} to the BP *in vivo*. One cannot assume that such a binding does take place. Actual measurements of binding site must be made and compared with the binding resulting from intramuscular and oral administration. It is not known whether intravenous administration simulates natural intake. Orally induced vitamin may bind to BP immediately on entry into blood or it may bind to another site and shift to BP either within the circulation or within a specific tissue.

If adequate techniques can be developed, further studies of the chemical state of the B_{12} in plasma would also answer the question of whether the Co^{58} in the plasma various times after a dose remains in the form of $Co^{58}B_{12}$. Such a question is crucial in the interpretation of the present study. The amounts in plasma in the present study were

too small to permit analysis. However, several investigators studying dynamics of larger doses have used various direct or indirect means to determine whether labeled B_{12} remains as such after administration. No one has presented any evidence of the *in vivo* separation of the radioactive cobalt from the B_{12}.

SUMMARY

The plasma removal of intravenous doses of 7.0×10^{-3} to 4.3×10^{-2} μg of cobalt^{58} vitamin B_{12} was studied in control subjects. Initially the vitamin was removed rapidly, less than 20 per cent remaining in 1 hour. This loss did not represent excretion but was due to entry into tissue. Complete *in vitro* serum or plasma binding of the vitamin prior to administration did not influence the rate of disappearance. An increase in dose of 2,000-fold did not significantly change the fraction removed per unit time during the initial phase of loss. Twelve to 16 hours after administration the vitamin in the blood was more firmly fixed than some of the tissue B_{12}. After 24 hours a slow decline in plasma activity continued for several days.

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