Renal Clearance of Intravenously Administered Vitamin B_{12}. (26583)

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Previous studies have shown greater excretion of parenterally administered vit. B_{12} among young subjects than old. Furthermore, the decrement in vit. B_{12} excretion with age seemed to parallel agewise decrements observed in various renal functions(1). Since no direct experiments were performed to estimate whether rates of removal of vit. B_{12} from the plasma by the kidneys were reduced in older individuals, the following studies were carried out.

Materials and methods. Simultaneous clearances of B_{12} and inulin were measured in 2 groups of ambulatory subjects selected by clinical and laboratory tests as free from renal or hepatic disease as previously described(2). In the 6 subjects of Group A and the 10 of Group B, constant plasma levels of B_{12} and inulin were maintained following initial priming doses* by continuous infusion at a rate held constant by a Bowman pump. After 30 minutes of equilibration, 4 or more 15-minute clearance periods were run on each subject. All subjects were catheterized; their bladders were washed out with distilled water and air at the beginning and end of each period.

In Group A, the plasma was heated with buffer solution to liberate bound B_{12} from protein(3). Protein was removed by centrifugation and the supernatant assayed for B_{12} activity. This technic measured total B_{12}; *i.e.*, both free and that bound to plasma protein. In Group B, the plasma was dialyzed and the assay run on the dialysate, so that only free B_{12} was measured.

* The priming dose of inulin was calculated in the usual manner(2). The B_{12} primary dose was calculated for each subject as sufficient to provide the desired plasma level assuming that shortly after administration B_{12} was uniformly distributed in the extracellular fluid space as calculated from body weight.

Triplicate microbiological assays using *Lactobacillus leichmannii 4797*(4) were averaged to estimate plasma and urine levels of B_{12}. Mendelsohn and Watkin(5), using an *L. leichmannii* assay, have reported recoveries of added B_{12} from sera averaging 98.6 $\pm$ 3.13%. Experiments were conducted to determine the reproducibility of the vit. B_{12} assay. The variability due to technical error can be conservatively estimated to be within $\pm$20% of the true value. Although no information on the over-all accuracy of B_{12} clearances is available, it is entirely possible that the error of individual portions of the clearance determination may be additive leading to a total error as great as 20-30%.

Inulin was determined in plasma and urine by the method of Harrison(6). In this laboratory, Davies and Shock(7) reported standard deviations about the mean inulin clearance of 4.7 to 7.0% between successive 10 minute periods during the same test.

Results. Investigations of B_{12} clearances at endogenous plasma levels of B_{12} (0.2 to 0.3 mμg/ml) in 3 normal subjects revealed no measurable quantities of B_{12} in the urine. The minimum amount of B_{12} which could be determined by this method was 0.010 mμg. In the 6 subjects of Group A, total B_{12} plasma levels were increased to concentrations ranging from 0.3 to 2.4 mμg/ml by continuous intravenous infusion. Glomerular filtration rates ranged from 29 to 136 ml/min in these subjects.

At B_{12} plasma levels below 1 mμg/ml practically no B_{12} appeared in the glomerular filtrate or urine (Fig. 1). At levels above 1.5 mμg/ml large variations existed between plasma level and urinary B_{12}/ml of glomerular filtrate. However, at these low levels (0.4-2.4 mμg/ml) the error in estimating B_{12} is sufficiently large to warrant the inference that

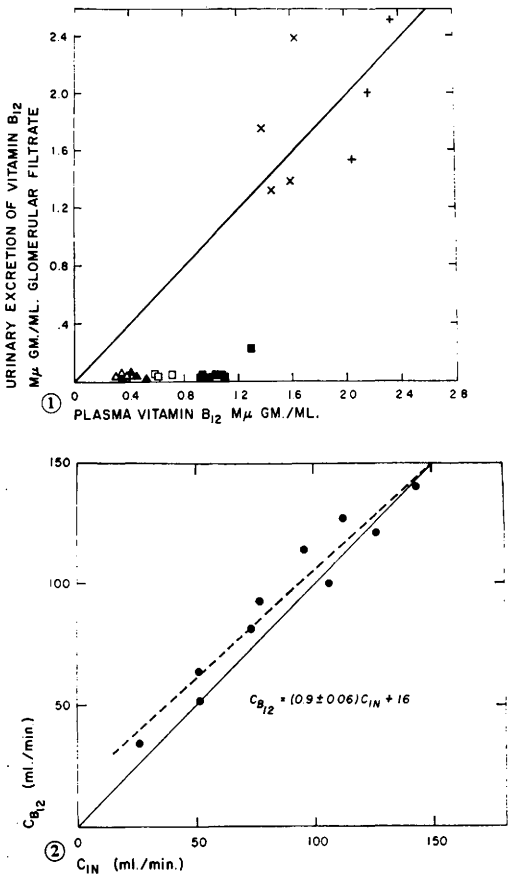

FIG. 1. Relationship between plasma level and urinary excretion of vit. B_{12} at low plasma levels. Individual clearance periods are plotted for 6 subjects as indicated by different symbols. At plasma levels less than 1.2 mμg/ml of B_{12}, excretion of B_{12} was very small and did not increase with plasma concentration.

FIG. 2. Relationship between inulin clearance and vit. B_{12} clearance in 10 subjects. Each point is the average of 4 clearance periods determined at constant plasma levels of B_{12} in each subject. Dotted line represents regression line calculated by the method of least squares. The regression equation is: Clearance of B_{12} (ml/min) $= (0.9 \pm 0.06)\ C_{In} + 16$. The experimental regression line (------) does not differ significantly from the regression (——) based on the theoretical identity of B_{12} and inulin clearances.

urinary clearance of B_{12} would be equivalent to inulin clearance if the free B_{12} content of the plasma could be accurately estimated.

In the 10 subjects of Group B, B_{12} plasma levels ranged from 12 to 26 mμg/ml. Glomerular filtration rates ranged from 25 to 160 ml/min (Table I). In Fig. 2, values obtained by averaging clearance data for B_{12} and inulin from 4 periods in each individual in Group B are presented. These average values indicate that B_{12} clearance is approximately that of inulin over a wide range of glomerular filtration rates at plasma levels ranging from 12 to 26 mμg/ml. Calculation of the regression of B_{12} clearance on inulin clearance based on data from 40 individual clearance periods in the 10 subjects yielded the following equation: Clearance of $B_{12} = (0.9 \pm 0.06)\ C_{In} + 16$. This is shown by the dashed line in Fig. 2. The slope of the regression does not differ significantly from 1.0.

Discussion. At low plasma levels (0.3 to 1.1 mμg B_{12}/ml), no B_{12} was detected in the urine. An explanation for this observation is that at low plasma levels vit. B_{12} is bound to plasma protein and therefore not filtered at the glomerulus. Indeed, *in vitro* experiments have indicated that at B_{12} levels of 0.5 to 1.0 mμg/ml, the B_{12} is bound to the alpha globulins(8-10). Furthermore, the low level of free B_{12} that may exist could not be detected by the assay method used. When concentration of vit. B_{12} was increased to 1 mμg/ml, the binding capacity of alpha globulin was exceeded and measurable amounts of the vitamin were found in the urine. The data obtained on the second group of subjects (Group B), in which vit. B_{12} level was raised to insure greater accuracy of determination and the plasma was dialyzed to separate the free from the bound B_{12}, showed that vit. B_{12} clearance was indistinguishable from glomerular filtration rate.

The impaired glomerular filtration rate observed in older subjects results in an elevated plasma level of vit. B_{12} for a longer period of time than that in younger individuals. Although the mechanism by which vit. B_{12} is presented to and retained by the tissues is unknown, this may provide a greater opportunity for the various tissues of the body to retain the vitamin in the older subjects than in the young.

Conclusions. 1. At plasma levels ranging from 0.3 to 1.5 mμg/ml urinary excretion of B_{12} is a function of degree of B_{12} binding by plasma protein. 2. At constant plasma levels between 12 and 26 mμg/ml of vit. B_{12}, urinary excretion of B_{12} in a given individual is proportional to plasma level and glomerular fil-

TABLE I. Simultaneous Inulin and B_{12} Clearances in 10 Subjects by Individual Periods at Plasma Levels of 12-26 mμg B_{12}/ml.

Subject	Age	Clearance, ml/min.	Period I	II	III	IV	Mean clearance
Bn	17	Inulin	110.3	114.9	107.9	113.2	111.6
		B_{12}	122.5	121.4	133.8	131.6	127.3
Df	35	Inulin	131.1	134.4	122.3	114.7	125.6
		B_{12}	99.6	124.1	115.8	143.5	120.8
Cn	51	Inulin	160.5	140.7	128.7	141.5	142.8
		B_{12}	147.8	127.4	130.7	154.5	140.1
Ti	52	Inulin	51.9	49.7	56.1	47.2	51.2
		B_{12}	63.7	55.3	58.3	76.6	63.5
Le	53	Inulin	103.3	108.0	107.3	104.7	105.8
		B_{12}	86.4	115.0	83.0	117.3	100.4
Po	59	Inulin	73.4	76.1	68.5	76.1	73.5
		B_{12}	85.0	80.4	78.3	82.9	81.6
Ro	64	Inulin	99.5	98.5	94.7	89.5	95.6
		B_{12}	134.2	107.2	98.7	116.6	116.7
Sm	75	Inulin	50.8	50.8	51.5	53.1	51.6
		B_{12}	46.1	56.9	61.8	44.7	52.4
Fv	77	Inulin	66.6	84.1	78.3	80.1	77.3
		B_{12}	86.9	88.6	94.4	100.9	92.7
Mu	88	Inulin	25.8	26.8	25.5	25.9	26.0
		B_{12}	34.4	37.5	38.6	27.4	34.5

tration rate. 3. No relation of renal clearance of B_{12} with advancing age was noted except for that resulting from the gradual reduction in glomerular filtration rate with age.

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Partial Purification of Erythropoietin by Alcoholic Fraction. (26584)

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A number of methods for concentration of the erythrocyte stimulating factor or erythropoietin in the plasma of anemic rabbits have been devised. Chemical methods of varying complexities have been employed and assays have been performed on both normal and abnormal animals(1). The method to be described utilized simple fractionation procedures and all assays were performed on normal adult albino rats. A preliminary report was made in 1958(2).

Methods. Production of anemia. Adult