

# Absorption and Excretion of Radioactive Vitamin B<sub>12</sub> in Diabetes

## A Study in Patients with and without Retinopathy

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Previous reports from this Institution have suggested that the metabolism of vitamin B<sub>12</sub> in patients with diabetes mellitus is different from that in nondiabetic controls. In 1953 Becker and others<sup>1</sup> reported a series of patients in which they showed that, following an *intramuscular* test dose of vitamin B<sub>12</sub>, diabetics with retinopathy excreted significantly more vitamin than nondiabetic controls, while diabetics without retinopathy excreted considerably less than nondiabetic controls.

It was postulated by the above investigators that diabetics with retinopathy lacked a hypothetical tissue-binding factor for B<sub>12</sub>, and consequently a higher urinary excretion of the intramuscular test dose resulted. It was felt that a difference in renal threshold or clearance of vitamin B<sub>12</sub> in the two diabetic groups was probably not an important factor, since it had been shown by Chow and others<sup>2</sup> that serum levels of vitamin B<sub>12</sub> in diabetics with retinopathy was significantly higher than in diabetics without retinopathy, a fact which is more compatible with a lack of a tissue-binding factor than with a reduced renal clearance.

The next problem which naturally presented itself was a consideration that if differences in the excretion of B<sub>12</sub> test doses between diabetics and nondiabetics were present, were there also differences in absorption of this vitamin? This report consists of a summary of B<sub>12</sub> absorption studies in diabetics and normal controls.

### METHOD

The procedure employed is essentially the one outlined by Schilling.<sup>3</sup> Two hours after the oral administration

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Presented in part at the meeting of Ophthalmic Biochemistry, Harvard University Faculty Club, Cambridge, Massachusetts, Feb. 12, 1956, and at the Meeting of the Wilmer Residents Association, April 4, 1957.

of 2 mcg. of Co<sup>60</sup>-labeled vitamin B<sub>12</sub>\* to the test subjects, one milligram (1,000 mcg.) of crystalline vitamin B<sub>12</sub>† in physiological saline solution was injected intramuscularly, and urine specimens were collected for twenty-four hours. Just prior to the test the patient was asked to void, and this specimen was discarded. An aliquot of each collection, equivalent to one-quarter of the urine collected, was evaporated in a beaker, containing 50 mcg. of vitamin B<sub>12</sub> as a carrier, and transferred to a previously graduated brown bottle (50 ml.-Merck). The radioactivity of the concentrate was measured with a scintillation counter, using a thallium-activated sodium iodide crystal. The radioactivity measured in this manner was corrected to the total volume of urine, and computed as a per cent of the oral dose excreted. The activity of the 2 mcg. dose measured in this manner was about 25,000 c.p.m. Only patients were included in this study who had not received any therapeutic vitamins or medications containing vitamins for a period of not less than one month.

### *Intrinsic Factor Used In This Study.*

Ten out of the forty subjects, studied by the above method, were given a second urinary excretion test one month after the first one. At the time of the second test they received 2 mcg. of labeled vitamin B<sub>12</sub> together with 50 mg. of an intrinsic factor concentrate‡ orally.<sup>4</sup>

### *Diabetic Patients.*

Forty subjects were studied. There were sixteen males and twenty-four females. Their ages ranged from fourteen to eighty-six. All subjects had been diagnosed as diabetics in the Diabetic Clinic of the Johns Hopkins Hospital. Twenty-nine of these patients were on insulin therapy, and eleven appeared controlled on diet alone.

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\* Kindly supplied to us by Merck and Company with specific activity of 180 microcuries per mg.

† Supplied by Merck and Company.

‡ Fifty mg. of this material together with 15 mcg. of crystalline vitamin B<sub>12</sub> constitute one U.S.P. unit. Kindly supplied by Lederle Laboratories, Division American Cyanamid Company, New York.

*Diabetics without retinopathy (eighteen)* were classified as such, after no retinal lesions whatsoever could be seen with the giant ophthalmoscope after mydriasis. *Diabetics with retinopathy (twenty-two)* were classified so after one or more capillary aneurysms, hemorrhages or waxy exudates were seen.

*Controls:* (forty) nondiabetic subjects whose ages ranged from nineteen to eighty-five with a mean age of forty-nine reported elsewhere<sup>5</sup> served as controls.

*Fecal Excretion*

Nine nondiabetic controls and five diabetics without retinopathy were given an oral test dose of 1,000 mmcgm. of radioactive vitamin B<sub>12</sub>. A five-day fecal collection was obtained, and the radioactivity of the total sample was determined.

RESULTS

In table 1 the individual urinary excretion data for the two diabetic groups are tabulated. A summary of the statistical analyses is given in table 2 and a graphic representation of data in figure 1. The results in table 2 indicate that, after an oral test dose of vitamin B<sub>12</sub>, diabetics without retinopathy excreted significantly less than diabetics with retinopathy. Furthermore, it is evident that diabetics with retinopathy, as a group, excrete essentially the same amount as nondiabetic controls.

TABLE 1

Urinary excretion of orally administered radioactive vitamin B<sub>12</sub> per 24 hours

Diabetics with Retinopathy (22)		Diabetics without Retinopathy (18)	
Initials	per cent of radioactivity excreted	Initials	per cent of radioactivity excreted
E.S.	10.7	G.H.	4.6
C.C.	9.5	P.S.	5.5
F.E.	5.4	E.W.	3.7
M.C.	22.2	E.Sh.	5.2
B.W.	6.3	J.C.	6.3
M.M.	7.9	L.B.	21.4
M.Mc.	11.7	E.A.	5.4
P.M.	6.7	L.G.	7.4
F.C.	8.3	E.Sw.	8.9
E.C.	13.9	J.J.	7.0
P.R.	5.7	E.Ha.	7.8
E.B.	13.6	B.Wa.	2.8
M.Ma.	13.7	M.W.	10.7
F.T.	7.3	S.D.	5.1
B.L.	8.8	E.Ba.	5.7
E.H.	19.5	S.Z.	3.8
B.A.	10.2	B.I.	4.7
E.He.	10.8	D.F.	3.6
H.O.	9.3		
C.J.	16.0		
F.Co.	12.3		
A.P.	9.1		

TABLE 2  
Percentage of orally administered radioactive vitamin B<sub>12</sub> excretion in twenty-four hours

	Diabetics without Retinopathy	Diabetics with Retinopathy	Nondiabetic Controls*
Mean per cent radioactivity excreted in urine	6.64 ± 0.99	10.86 ± 0.92	10.80 ± 0.68
Number subjects	18	22	40
S.D.	4.19	4.32	4.78
Mean age	48	54	49

p = 0.04

\*See reference 5.

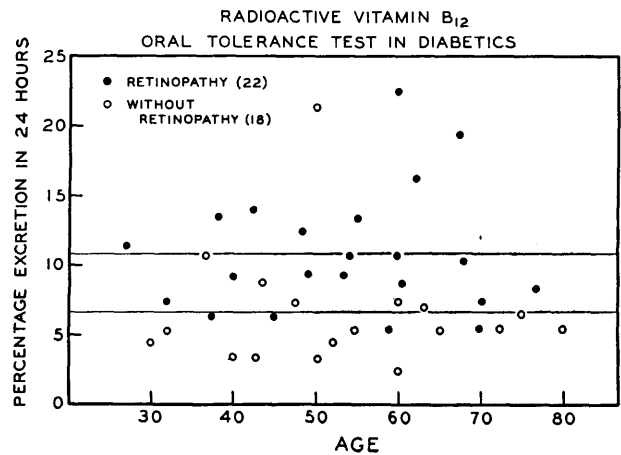


FIG. 1. Upper line represents mean for patients with retinopathy. Lower line represents mean for patients without retinopathy.

TABLE 3

Fecal excretion test for vitamin B<sub>12</sub> absorption after the oral administration of 1,000 mmcgm. of radioactive vitamin B<sub>12</sub>

Nondiabetic controls		Diabetics without Retinopathy	
RW	179 mmcgm.	NS	578 mmcgm.
DS	306	HH	644
AT	239	HHu	292
DG	99	CI	417
BC	318	ES	379
MT	179		
CC	300		
TS	106		
BF	114		

Mean and S.E. 204.4 ± 29.9      462.0 ± 58.0  
S.D. 87.7      129.9  
n 9      5

p < 0.01

In table 3 are tabulated the individual data obtained in fecal excretion studies after a single oral test dose. The average excretion of the diabetic group is approximately 46 per cent of the oral test dose, while the nondiabetics excreted approximately 20 per cent of the test dose.

Table 4 represents the individual data of urinary excretion tests before and after the oral administration of intrinsic factor concentrate. No significant difference was found in this particular series.

TABLE 4

Percentage of radioactive vitamin B<sub>12</sub> excretion in ten diabetics before and after intrinsic factor administration\*

Initials	Before	After	Per cent change
G.H.	4.6	6.7	2.1
P.S.	5.5	3.5	-2.0
E.S.†	10.7	9.6	-1.1
E.W.	3.7	6.2	2.5
P.R.†	5.7	10.0	4.3
E.Sw.	8.9	4.1	-4.8
J.J.	7.0	10.1	3.1
E. A.	5.4	5.3	-0.1
A.P.†	9.1	10.1	1.0
S.D.	5.1	6.3	1.2
Mean and S.E.			+0.62±0.85
S.D.			±2.70
p <			0.50

\*50 mgm. of intrinsic factor concentrate.

†Retinopathy present.

DISCUSSION

The excretion test described by Schilling<sup>3</sup> was first employed in patients with pernicious anemia, and was found to be a reliable index of relative, though not absolute, gastrointestinal absorption of vitamin B<sub>12</sub>. Nevertheless, it is worthy of emphasis that this procedure does not merely test the absorption of the vitamin, but is dependent also on retention, metabolism and urinary excretion of the vitamin. This is a basic reservation which has to be kept in mind in all studies, using orally administered substances which are metabolized in the body.

Figure 1 reveals a considerable overlap of the excretion data between the two diabetic groups. This, however, is not surprising in view of the insidious onset and frequent remissions encountered in diabetic retinopathy.

A case of unusual interest is patient C. J. (table 1) who was referred to us through the courtesy of Dr. Frank B. Walsh. This patient was a juvenile diabetic with severe bilateral diabetic retinopathy. He was given an oral test dose, and his percentage of urinary excretion was 16.0 per cent which is higher than the average for nondiabetics. A week later the patient underwent hypophysectomy for his diabetic retinopathy. Two months postoperatively the patient was doing generally well, and his ocular status showed definite improvement. The urinary excretion test was repeated, and this time

revealed a decrease to 9.2 per cent excretion of the oral dose.

As was pointed out at the beginning of this paper Becker and others<sup>1</sup> found that, following an *intramuscular* test dose of B<sub>12</sub> diabetics with retinopathy excreted significantly more than normal controls, while diabetics without retinopathy excreted significantly less than normal controls. On the other hand, as can be seen from table 1, after an *oral* test dose diabetics without retinopathy also excrete less than normals, but diabetics with retinopathy excrete essentially the same as do normal controls. How can these findings be reconciled? One plausible explanation is that both the diabetics with and without retinopathy absorb less B<sub>12</sub> from their gastrointestinal tracts than do normals, and that the nonretinopaths are able to hold on to some B<sub>12</sub>, while the retinopaths, lacking a tissue-binding factor, cannot do likewise, and thus their urinary excretion after an oral test dose is essentially the same as that of normals. The fecal excretion studies (table 3) lent some support to the view of a reduced absorption of a B<sub>12</sub> test dose in diabetics.

Another factor which deserves emphasis is the fact that in this study we are dealing with B<sub>12</sub> test doses the absorption of which may not necessarily reflect accurately the absorption of the daily dietary intake of vitamin B<sub>12</sub> in these patients.

The intrinsic factor study is too preliminary to warrant any conclusions; a much larger series is needed to evaluate the effect of intrinsic factor in diabetics.

Lastly, it is becoming increasingly apparent that reduced absorption of daily dietary B<sub>12</sub>, if such there is, may not merely be contributory to some of the complications of long-standing diabetics, but is more probably itself a consequence of the abnormal metabolic state which we call diabetes.

Recently Bookman and others<sup>6</sup> have reported a series of diabetic patients in whom urinary excretion studies were performed after an intramuscular test dose of vitamin B<sub>12</sub>. They conclude that their results do not confirm Becker's<sup>1</sup> original observations. If one scrutinizes their data carefully, one finds that under "results" they state that ". . . no P values of less than 0.1 were found, indicating a total lack of significance . . ." If one, however, recalculates their P values by the student t test on the basis of their figures in table 1, one arrives at a value of P=0.05 for the diabetics with and without retinopathy under "lactobacillus" and a value of P<0.1 for the similar group under "ochromonas," both P values being definitely smaller than 0.1. Bookman's statistical analyses therefore are open to some questions.

SUMMARY AND CONCLUSIONS

1. Forty diabetic patients were tested with an oral radioactive vitamin B<sub>12</sub> test dose, and the urinary excretion was determined. It was found that diabetics without retinopathy excreted on the average a significantly smaller amount of the test dose than did nondiabetic controls, whereas diabetics with retinopathy excreted the same amount as the controls. Some overlap between the excretion data of the two diabetic groups was found.

2. An interpretation of these findings in light of previous studies, using intramuscular vitamin B<sub>12</sub> test doses, is presented.

3. Fecal excretion studies in six diabetics without retinopathy revealed a significantly higher fecal excretion of an oral test dose when compared with nondiabetic controls.

4. Assuming that the oral tolerance test employed in this study does not measure absolute absorption, the data suggest a relative impairment of absorption of vitamin B<sub>12</sub> test doses in diabetics.

5. Intrinsic factor concentrate had no significant effect on the absorption of oral test doses in a series of ten diabetics.

6. The results provide further support for the view that vitamin B<sub>12</sub> is metabolized differently in diabetic and nondiabetic subjects. Only further studies will show whether a relatively reduced absorption of vitamin B<sub>12</sub> is contributory to some of the complication of long-standing diabetes, or if it is a consequence of the deranged metabolic conditions which exist in diabetic patients.

SUMMARIO IN INTERLINGUA

*Absorption e Excretion de Radioactive Vitamina B<sub>12</sub> in Diabeticos Con e Sin Retinopathia*

1. Quaranta patientes diabetic esseva testate con un oral dose experimental de radioactive vitamina B<sub>12</sub>, e le excretion urinari esseva determinate. Esseva trovate que le excretion medie del vitamina constitueva significativamente plus parve porcentages del dose experimental in le diabeticos sin retinopathia que in nondiabetic subjectos de controllo. Diabeticos con retinopathia exerceva le mesme quantitates como le subjectos de controllo. Le datos del excretion in le duo gruppos diabetic monstrava un certe area de coincidentia.

2. Es presentate un interpretation de iste constatationes in le lumine de previe studios con doses experimental de

vitamina B<sub>12</sub> intramuscular.

3. Studios del excretion fecal de vitamina B<sub>12</sub> oral in sex diabeticos sin retinopathia revelava significativamente plus alte porcentages del dose experimental que in nondiabetic subjectos de controllo.

4. Si nos admitte que le test de tolerantia oral que esseva usate in le presente studio non effectua un mesuration del absorption absolute le datos indica in omne caso un defecto relative del absorption de vitamina B<sub>12</sub> administrate in doses experimental a patientes diabetic.

5. Concentrato de factor intrinsec habeva nulle effecto significative super le absorption de oral doses experimental de vitamina B<sub>12</sub> in dece diabeticos.

6. Le resultados apporta supporto additional pro le vista que vitamina B<sub>12</sub> es metabolisate differentemente in diabeticos e in subjectos nondiabetic. Studios additional es requirite pro determinar si un relative reduction del absorption de vitamina B<sub>12</sub> es un factor contributori in le causation de certes del complicationes de diabete de longe durantia o si illo es un consequentia del disturbate conditiones metabolic que existe in patientes diabetic.

ACKNOWLEDGMENT

This study was supported by the Grant-in-Aid of the United States Public Health Service, No. A-435-C-2.

We are indebted to Dr. Ernest Brown of the Diabetic Clinic of The Johns Hopkins Hospital for referral of diabetic patients.

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