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VITAMIN B_{12} *Excretion and* DIABETIC RETINOPATHY

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THERE IS increasing evidence to indicate that vitamin B_{12} plays an important role, directly or indirectly, in carbohydrate metabolism.[1,2,3,4] These findings, together with the fact that the pancreas retains a high concentration[5] of radioactivity following the administration of vitamin B_{12}[6] labeled with radioactive cobalt (Co^{60}), prompted this study of the urinary excretion of vitamin B_{12} by patients with diabetes mellitus. Data demonstrating a possible relationship between administration of cortisone or testosterone and vitamin B_{12} excretion in man and rats are also included and their significance discussed.

STUDIES IN MAN

Design of Experiments

All patients used in this study except the control subjects suffered from diabetes mellitus of different degrees of severity. The diabetic patients were selected on the basis of a clear-cut clinical diagnosis of this disease with hyperglycemia and glycosuria. The first few subjects were chosen from a hospital in Mexico City; all others were drawn from the Diabetic Clinic and The Wilmer Institute of The Johns Hopkins Hospital. Only the Johns Hopkins group of patients were examined for retinopathy.* When the diagnosis of diabetes had been established and the presence or absence of retinopathy recorded, all patients were injected with a single dose of 50 μg. or 65 μg. vitamin B_{12} by the intramuscular route. Insulin therapy was not interrupted during the vitamin B_{12} studies. All estimations of vitamin B_{12} in urine were made microbiologically. Recovery of added vitamin B_{12} to a basal urine specimen was essentially quantitative, thus demonstrating the absence of inhibitor. Radioactive vitamin B_{12} was administered to several subjects. Radioactivity was measured in the urine either by superimposing aliquots of urine samples on the planchets directly or by extracting the radioactivity with butanol.[7] Previous experience[8] with healthy individuals indicated that collections of urine beyond the eight hours immediately after injection were not necessary, since the extension of the period by an additional 16 hours seldom yielded more than 5 per cent of the amount excreted in the first 8 hours. In the present study, 24-hour urines from a number of subjects were collected in order to be certain that this conclusion held in tests with diabetics. This was found to be so, and therefore only 8-hour

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* All diabetic patients were examined with a giant ophthalmoscope following mydriasis. In this series those cases were included in which the fundi could be well visualized and in which no other ocular disease was present. Only the eyes with no evidence whatsoever of capillary aneurysms, exudates, or hemorrhages were classified as nonretinopathic.

TABLE I
Mexican Diabetics

Subjects	Male	Female	Average age	Vitamin B_{12} Excreted ($\bar{x} \pm SE$)*
			yrs.	μg.
Diabetics	3	2	37	4.9 ± 2.0
Controls	9	0	21	17 ± 1.7

* ($\bar{x} \pm SE$)—Mean and standard error.

not strictly comparable with respect to age and sex, prompted a more detailed study of vitamin B_{12} excretion in diabetics.

Work at The Wilmer Institute had indicated decreased adrenal cortical function in diabetics without retinopathy, and a possible causal relationship of relatively excessive adrenal activity to retinopathy and intercapillary glom-

TABLE II
U. S. Diabetics

Diabetics	Male	Female	Vitamin B_{12} excreted 25–45 yrs.	45–65 yrs.	65 yrs.
			μg.	μg.	μg.
Without retinopathy	2	11	3.3 (4)	5.5 (7)	1.3 (2)
With retinopathy	6	16	19 (6)	20 (9)	17 (7)

( )—Indicates number of subjects.

specimens were utilized during the rest of the series. After the measurement of urine volume, aliquot samples were kept at 2–5° C. under toluene until ready for assay by the procedure of Skeggs and Wright.[9]

The samples were identified by code numbers at the hospital and analyzed. Only after completion of the assay was the ophthalmoscopic diagnosis of the patient compared with the analytical results. This procedure was adhered to rigidly in order to minimize bias.

Results

In the first study, five diabetic persons hospitalized in the Hospital de Enfermedades de la Nutrición in Mexico City* were given 65 μg.† of crystalline vitamin B_{12} intramuscularly. For comparison, 9 healthy Mexican subjects‡ were also injected with a like amount of this vitamin. The results of determination of the microbial activity in the urine excreted by these two groups are given in Table I. They demonstrate that the diabetics excreted much less vitamin B_{12} than the controls. These data with a limited number of subjects,

erulosclerosis (the Kimmelstiel-Wilson renal lesion[10]). At the same time, some laboratory data from animal experiments became available, which indicated a possible interrelationship of the kidneys and adrenals with the metabolism of B_{12}. This combination of circumstances suggested the hypothesis that the urinary excretion of B_{12} by diabetics might be related to the presence or absence of retinopathy. To test this hypothesis, a total of 22 consecutive subjects with retinopathy and 13 without retinopathy were accumulated over a period of four months and each one given a vitamin B_{12} tolerance test. The distribution of the population with respect to age and sex is given in Table II.

There was an uneven distribution with respect to the number of subjects in each group and a preponderance of females in both groups. In spite of this heterogeneity, the urinary excretion of vitamin B_{12} by all subjects fell into two distinct groups, as illustrated in Figure 1. In this figure, the urinary microbial activity of vitamin B_{12} in micrograms is plotted against age of the patient. It is clearly demonstrated that over the entire age range, there was a uniformly greater excretion by subjects with retinopathy than by those without. The two exceptions were subjects who were originally diagnosed as diabetics without retinopathy, but were found to have (or to have developed) early retinopathic changes on subsequent re-

* The authors wish to express their appreciation for the collaboration of Dr. Bernardo Sepulveda and his associates for this phase of our work.

† Squibb crystalline vitamin B_{12} solution with 0.5% phenol (65 μg. per cc.) was used.

‡ The authors wish to express their appreciation for the collaboration of Dr. Santiago Castro Estrada of E. R. Squibb and Sons, Mexico, in this phase of our work.

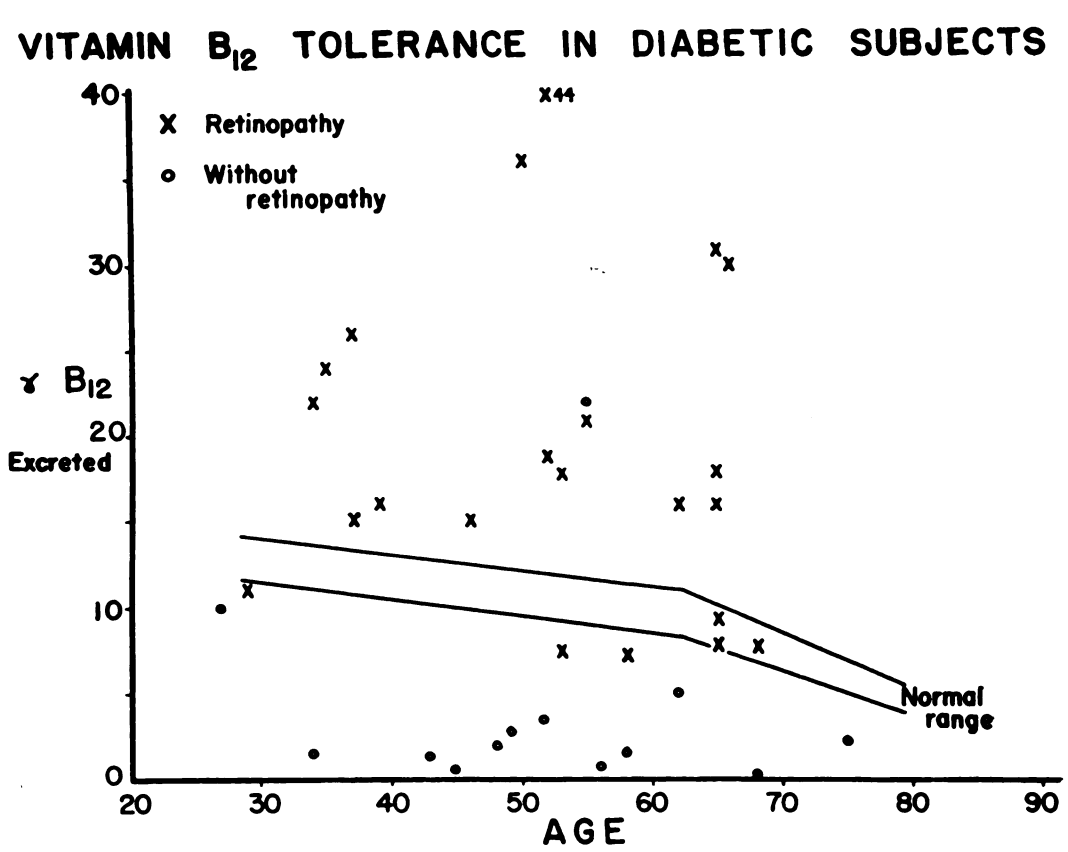

Fig. 1. The urinary excretion after intramuscular injection of vitamin B_{12}.

examination. However, in our statistical analyses (Table III) only the original diagnoses were considered. The urinary excretion of normal subjects was included for comparison. The data show that the diabetics with retinopathy excreted an average of approximately 19 μg. of vitamin B_{12}, and subjects without retinopathy excreted only 4.2 μg.; whereas the normal subjects excreted 9.6 μg. Statistical analyses indicate a significant difference, not only between the two groups with this disease, but also between either one of them and the healthy subjects.

Radioactive vitamin B_{12} studies revealed similar differences between the two groups of diabetics (Table IV). There was no correlation, however, between the severity of the retinopathy and the B_{12} excretion.

TABLE III

Urinary Excretion after Injection of Vitamin B_{12} (50 μg.)

Subjects	No. of subjects	Vitamin B_{12} excreted ($\bar{x} \pm SE$)*
		μg.
Diabetics without retinopathy	13	4.2 ± 1.7
Diabetics with retinopathy	22	19 ± 2.1
Healthy controls	6	9.6 ± 1.4

* ($\bar{x} \pm SE$)—Mean and standard error.

TABLE IV

Vitamin B_{12} Excretion and Diabetic Retinopathy

Group	No. of subjects	Vitamin B_{12} by radioactivity	Microbiological activity
		C. p. m.	μg.
With retinopathy	7	4150	17.5
Without retinopathy	4	1100	4.5

Endocrine Studies

Testosterone has been used in the management of diabetics with retinopathy.[11] It was, therefore, of interest to ascertain the effect of this hormone on the excretion of vitamin B_{12}.

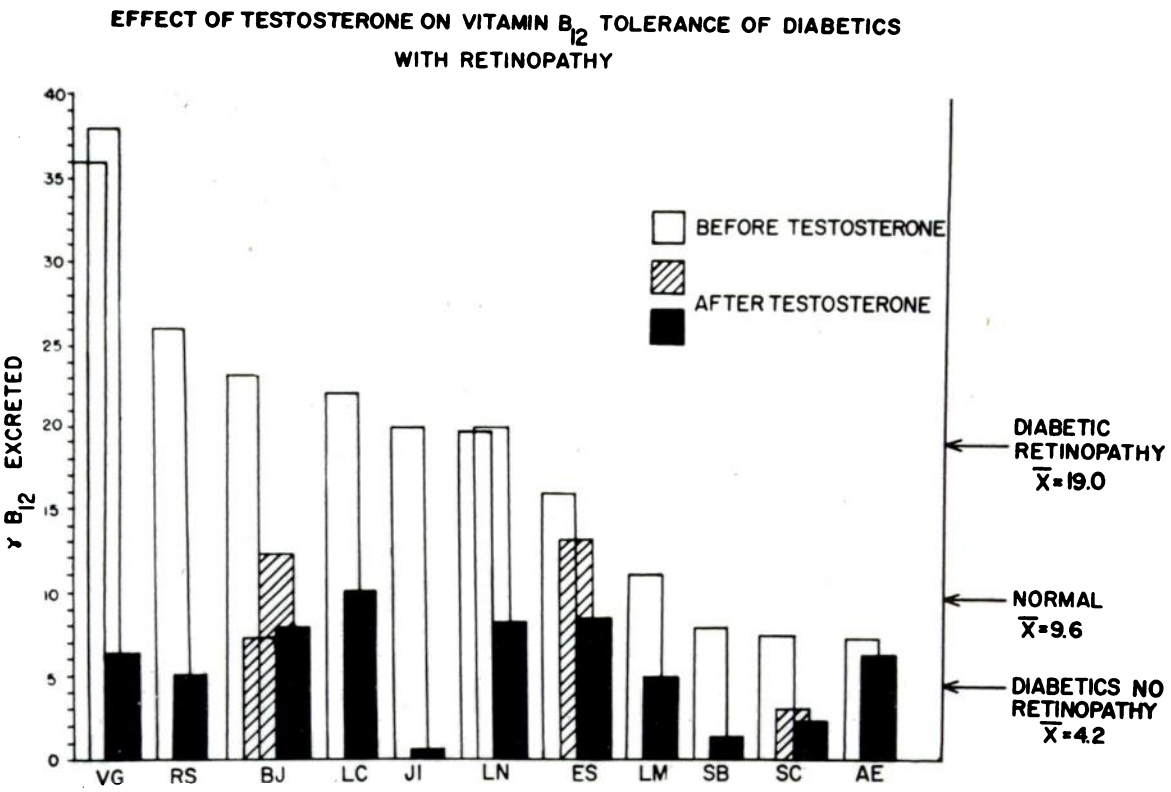

Fig. 2. The effect of testosterone on the urinary excretion following intramuscular injection of vitamin B_{12}.

To this end, a group of diabetics with retinopathy were first given one or occasionally two tests with vitamin B_{12} (50 μg.) to establish excretion levels before androgen therapy. After intramuscular injections of 100–200 mg. testosterone cyclopentylpropionate (Depo-Testosterone®)* every two to three weeks, these subjects were again given one or sometimes more tests for vitamin B_{12} excretion. Successive tests, when made, were at intervals of at least four weeks. Only after the completion of the microbiological assays for vitamin B_{12} activity in urine was the schedule of testosterone administration compared. The results of such a study are presented in Figure 2 as a bar graph. It was noted that among the 11 subjects studied there was in every instance a significant decrease in vitamin B_{12} excretion following the testosterone administration, although the magnitude of this decrease varied from one individual to another. Whether testosterone will likewise affect the excretion of vitamin B_{12} in nondiabetics, or in diabetics without retinopathy, remains to be established.

*The Depo-Testosterone® used in this study was generously supplied by The Upjohn Company, Kalamazoo, Michigan.

Animal Studies

Experimental Procedure

To further explore the interrelationships of vitamin B_{12} and adrenal cortical function it was of interest to ascertain, if possible, whether the excretion of vitamin B_{12} can be affected by administration of cortisone or of testosterone. To this end, a series of experiments was performed. In a typical experiment, three groups of female adult rats (4 each) raised in our colony, weighing about 220 Gm., were employed. The duration of the therapy in various experiments ranged from one week to one month. No marked differences in the results to be described below were observed at the different times of treatment. Group A received daily (except Sundays) 5 mg. of cortisone suspension per rat per day for three weeks. Group B received on the same days

an injection of 1 cc. of an isotonic saline solution instead of cortisone. These animals served as controls. Group C was given 0.2 cc. of a solution containing 50 mg./cc. of testosterone in peanut oil by subcutaneous injections every third day for two weeks. After this pretreatment, each animal in all three groups was given a test dose of one microgram of radioactive vitamin B_{12}.* Urinary specimens collected 24 hours before as well as 24 and 48 hours after injection were assayed for radioactivity and for microbiological activity of vitamin B_{12}. Forty-eight hours after the injection of vitamin B_{12} the animals were sacrificed and tissues such as liver, kidneys and 5.0 Gm. of muscle from the thigh were removed. Radioactivity in these samples was determined after wet ashing.†

Discussion

The low microbiological activity in the urine of diabetics without retinopathy might be explained on the basis of the presence of an inhibitor. This argument, however, loses its force since, in the studies in which radioactive vitamin B_{12} was administered, there was a similar marked difference in the urinary excretion of radioactivity by diabetics with and without retinopathy (Table IV). However, the complications arising from the unlabeled vitamin B_{12} reserve in the tissues of test subjects, as discussed in a previous report,[12] makes it unwise to interpret the data beyond a suggestion of a qualitative difference in the radioactivity in the urine specimens of these two groups of diabetics after administration of radiovitamin B_{12}. No efforts have been made to ascertain whether the microbiological activity excreted by the two types of diabetics is due to the presence of more than one form of this vitamin or of vitamin B_{12}-like compounds.

TABLE V

Radioactivity in Urine and Tissues

Groups	Treatment	Vitamin B_{12} in urine		Vitamin B_{12}* bound/gram tissue		
		R	M	Muscle	B Liver	Kidneys
				μg.	μg.	μg.
A	Cortisone	0.72	0.77 ± 0.03	0.11	5.7	167
B	Saline	0.44	0.48 ± 0.03	0.18	11.0	244
C	Testosterone	0.62	0.57 ± 0.04	0.11	7.7	118

R—Micrograms of vitamin B_{12} as measured by the radioactivity in the 24-hour urine specimen.
M—Micrograms of vitamin B_{12} as measured by the microbial activity in the 24-hour urine specimens.

Results

The results of a typical experiment, given in Table V, demonstrate that the vitamin B_{12} activity in the 24-hour urine specimens of cortisone-treated animals was almost twice that of the control animals, as measured either by the microbiological activity or by radioactivity. The testosterone-injected animals, however, showed no significant difference from the controls under these experimental conditions. The results of tissue analyses demonstrate that in every instance the organs of the cortisone-treated animals, and to some extent those receiving testosterone, retained less radioactivity than saline-injected controls.

Recently[13] a difference was reported in the excretion of vitamin B_{12} by young and old healthy subjects following injection of a test dose. This phenomenon may be attributed to the decrease of kidney or adrenal functions with age. A difference[12] in the rate of loss of radioactivity from the kidneys of young and old rats following subcutaneous injection of labeled vitamin B_{12} was also observed. These results suggest likewise a possible role of renal function in the excretion of vitamin B_{12}. Friedenwald and his associates[14] showed a correlation of Kimmelstiel-Wilson renal lesions with diabetic retinopathy. It is therefore plausible to attempt to explain the observation of the difference in vitamin B_{12} excretion by

* The radioactive vitamin used in all these experiments was kindly supplied by Merck and Company, Incorporated, on allocation from the Isotope Division of the United States Atomic Energy Commission.

† Marked hypertrophy of the uterus was observed at the time of necropsy.

these two groups of diabetics on the basis of the difference in the renal function. However, clinical tests of renal function did not correlate with vitamin B_{12} tolerance in this series of diabetic patients. The effects of testosterone on patients with diabetic retinopathy make this thesis even less likely. Nevertheless, the possibility of subclinical renal involvement affecting the vitamin B_{12} tolerance test requires further study.

Observations on the effect of cortisone on urinary excretion of rats suggest a possible explanation for the increased excretion of vitamin B_{12} in diabetic retinopathy. This hypothesis receives support from preliminary data demonstrating a high concentration of oxysteroids related to, but not identical with, cortisone in the urine of diabetics with retinopathy as compared to that of patients without retinopathy. In this communication it is merely intended to report the difference in the excretion of a test dose of B_{12} between these two groups of diabetics. The possible influence of the adrenal cortex, in so far as it is demonstrated by the increase in the excretion of vitamin B_{12} by rats treated with cortisone, deserves further investigation.

Such findings provide us with some of the necessary background information to study the biochemical and physiological defects in diabetics and may throw light on the metabolic function of vitamin B_{12}.

Summary

Following an intramuscular test dose of vitamin B_{12}, diabetic subjects with retinopathy excrete significantly more of the vitamin than non-diabetics; while diabetics without retinopathy excrete considerably less than the non-diabetics.

Administration of testosterone to diabetics with retinopathy decreases the urinary excretion of a test dose of vitamin B_{12}. Cortisone increases the test dose excretion of vitamin B_{12} in rats.

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Resumen

Excreción de vitamina B_{12} y retinopatía diabética

La vitamina radioactiva B_{12} no rotulada se administró por via parentérica a pacientes afectos de diabetes mellitus con y sin retinopatía. Se halló que los diabéticos con retinopatía excretaron alrededor del doble, y aquellos

sin retinopatía la mitad de la cantidad de la vitamina inyectada excretada por los sujetos sanos. La administración del testosterón a los diabéticos con retinopatía resultó en una disminución de la excreción urinaria de vitamina B_{12} al ser sometidos posteriormente a un test de tolerancia vitamínica.

En ratas normales, el tratamiento con cortisona por una a tres semanas resultó en un aumento de la excreción urinaria de la vitamina B_{12} inyectada. Estos datos sugieren una posible interrelación entre la excreción de vitamina B_{12} y las funciones de las glándulas suprarenales en los dos tipos de diabéticos. Esta opinión se halla aun más reforzada por el hecho de que las orinas de los diabéticos con retinopatía contienen una mayor concentración de oxisteroides relacionados pero no idénticos con la cortisona que las de los pacientes sin retinopatía.

The Child's Changing Appetite

"It is amazing that the average mother does not understand that her child has a normal desire for variation in the type of food he consumes. The mother will readily admit that at times she would prefer sandwiches and iced tea to a full meal, and she will admit that there are some days when she wants to eat dessert first and some meals when meat and potatoes simply do not taste good. However, she will refuse to agree that her child might have these same variations in preference for foods."
—P. Williamson. *GP* 8: 57, 1953.

Philosophy of the First-Rate

"There is a philosophy which gives standards of value and judgment that apply to all spheres and activities of human life. It is a very simple one, and no one would reject it. I would call it the philosophy of the First-rate. Ask a doctor or a surgeon if he is ignorant of or indifferent to the best methods in his profession. Would he answer yes? Or would he consider that there are no differences of quality in art, literature, music, architecture, and that it does not matter whether a picture or a sonata or a play or a building is first-rate or third-rate? Or, taking the inquiry into a still more important province, do we think that when we apply the words good or bad to human character and conduct we are drawing meaningless and unimportant distinctions? In all fields of life all people, whatever their creed or race, admit what I called the philosophy of the first-rate. I have already quoted John Morley's saying that an educated man knew when a thing was proved and that an uneducated man did not know. I would prefer to say that an educated man knows, and an uneducated man does not know, what is first-rate, and that the best-educated man is he who knows the first-rate in the most important human activities."
—Sir Richard Livingstone. *British Medical Journal* 4834: 454, 1953.