Vitamin B_{12} Absorption in Pyridoxine-Deficient Rats

Further Studies

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IN PREVIOUS REPORTS[1] from this laboratory it was demonstrated that pyridoxine deficiency results in an impairment of absorption of orally administered vitamin B_{12}. This phenomenon was demonstrated by urinary excretion and fecal excretion tests as well as by the measurement of radioactivity retained in certain organs of pyridoxine-deficient and pyridoxine-treated rats, orally fed with radioactive vitamin B_{12}. The impairment could be fully corrected by subsequent treatment with pyridoxine.

The manner in which the impairment occurs is not fully understood. The present report deals with a study to determine whether the impairment is due to the lack of secretion of adequate amounts of gastric juice—a source of intrinsic factor—or to the atrophy of some endocrine gland.

EXPERIMENTAL PROCEDURE

Preparation of Animals

Rats with Pyridoxine Deficiency. Young (six weeks old) as well as adult male rats (three months old) of the McCollum strain were used for this investigation. Animals were placed in individual screen bottom cages and kept on a casein-sucrose basal diet[2] with all known vitamins except pyridoxine. Half of the animals were given intraperitoneal injections three times a week of an aqueous solution of pyridoxine hydrochloride (1 mg in 1 ml). All animals were kept on the deficient diet until the untreated animals developed outward signs of deficiency, such as acrodynia, retardation of growth, and/or alteration in serum glutamic oxalacetic transaminase (SGOT) activity. Some biochemical data on our deficient and treated animals were also accumulated.

After Adrenalectomy. Two-month-old male rats were bilaterally adrenalectomized. They were allowed to recuperate from the operation and to rest for at least ten days before they were used. During this period, the animals were offered our stock diet and water containing 1 per cent sodium chloride. Intact male rats of the same age were used as controls.

After Hypophysectomy. Hypophysectomized male rats, purchased from the Charles River Breeding Laboratory, weighed 150 g at the time of experiment. According to the vendor these animals were 2–3 months old.

Materials

Adrenocorticotrophic Hormone (ACTH) and *Thyroid-stimulating Hormone* (TSH). The ACTH and TSH preparations were kindly supplied to us by Armour Research Laboratories.

Sources of Intrinsic Factor. Gastric juice from normal rats was obtained after histamine stimulation. Stomachs removed from stock animals were homogenized with three times its weight of a cold saline solution. These two preparations served as our sources of intrinsic

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Presented at the Symposium on Vitamin Interrelationships at the Medical College of Virginia, October 24, 1958, with the cooperation of The National Vitamin Foundation, Inc., New York.

factor. Only freshly prepared specimens were used for our study.

Chemical Procedures

The procedures used for the determination of SGOT activity[3] or vitamin B_{12} levels in sera[4] or vitamin B_{12} levels in liver[5] as well as for the estimation of vitamin B_{12}-binding capacity[6] have been published elsewhere in detail and will not be repeated here.

Gastric Secretion. The secretion of gastric juice of pyridoxine-deficient and treated animals in the fasting state was measured after ligating the pylorus under ether anesthesia. Gastric juice was collected with a syringe on the following morning, i.e. approximately 17 to 18 hours after ligation of the stomach. The total volume of gastric juice was estimated. Its pH and total vitamin B_{12}-binding power were measured.

Absorption of Vitamin B_{12}. The test animals were placed in individual metabolism cages. Feed and water were provided ad libitum. Each rat was force-fed with stomach tube 50 mμg of radioactive vitamin B_{12}-labeled with Co^{60} of specific activity of 1 μc/μg in 2 ml distilled water, with or without intrinsic factor preparation. Feces were collected in graduated bottles for 6 or 8 days, at the end of which time the animals were sacrificed under light anesthesia. The livers, kidneys, and gastrointestinal tracts of the rats were removed, cleaned with a normal saline solution, and finally solubilized by adding an aqueous solution of 30 per cent potassium hydroxide. All samples were then diluted to 50 ml for the measurement of radioactivity with a gamma scintillation counter.

RESULTS

In order to be certain that our animals are deficient in pyridoxine, body-weight changes and certain biochemical measurements of both pyridoxine-deficient and treated animals were compared. Results are given in Table I. Aside from the reported data on the decrease in the serum transaminase content, pyridoxine-deficiency resulted in low vitamin B_{12} levels in serum, but not in liver.

The analytical results on the composition of gastric juice of pyridoxine-deficient and treated animals are tabulated in Table II. These data demonstrate clearly that pyridoxine deficiency results in slight decrease in the volume of gastric secretion and increase in pH of the gastric juice, but a marked decrease in the total vitamin B_{12}-binding power.

Intrinsic factor aids absorption of vitamin B_{12} by man[7,8] and by rats.[9,10] Our data show that pyridoxine-deficient animals secrete slightly less gastric juice and markedly less vitamin B_{12}-binding substance, which is believed to be an essential property of intrinsic factor. Therefore it was of interest to determine whether the coadministration of vitamin B_{12} with intrinsic factor either from gastric juice or stomach homogenate of rats will improve the absorption of vitamin B_{12} by pyri-

TABLE I

Comparison of Pyridoxine-deficient and Pyridoxine-treated Rats

Treatment	Weight increase (g)	Plasma transaminase (units)	Vitamin B_{12} levels: Liver (mμg/g)	Vitamin B_{12} levels: Plasma (μμg/ml)
Deficient	48 (6)	22.8 ± 4.5 (6)	161.0 ± 30.6 (9)	132.4 ± 12.8 (11)
Treated	124 (6)	87.0 ± 4.1 (6)	163.9 ± 24.6 (9)	194.7 ± 10.9 (11)

Eleven rats in each group were used at the beginning of the study. The number of specimens used for each measurement is indicated by the numbers in parentheses.

TABLE II

Some Properties of the Gastric Juice in Pyridoxine-deficient and Treated Rats

Treatment	Volume (ml)	pH	Total vitamin B_{12} binding power (mμg)
Deficient	4.7 (1.8–7.8)	1.65 (1.42–2.18)	10.7 (4.9–17.0)
Treated	6.5 (4.0–8.4)	1.49 (1.32–1.86)	34.4 (12.0–52.7)

Gastric juice was obtained 16 hours after ligation of pylorus. Vitamin B_{12} binding power was kindly assayed by Dr. Robert Davis, Veterans Administration Hospital, Baltimore, Md.

Four rats were used in each group for this study.

TABLE III
Absorption of Radioactive Vitamin B_{12}
Eight days' fecal excretion

Treatment	Radioactivity recovered as unabsorbed vitamin B_{12} (% of oral dose)
Deficient	74.9 ± 10.03 (4)
Treated	42.4 ± 2.00 (3)

Numbers in parentheses denote number of rats used.

doxine-deficient rats. The typical results of several such studies are summarized in Tables III, IV, and V. Table III demonstrates the impairment of the absorption of vitamin B_{12} due to pyridoxine deficiency. The pyridoxine-deficient animals absorbed on the average only 25 per cent of a 50 mμg dose of vitamin B_{12}, while the treated animals absorbed as much as 58 per cent. Although the magnitude of the difference in the absorption by these two groups of animals may vary somewhat from one experiment to another, consistent differences were observed in a series of many experiments.

In another study, pyridoxine-deficient and treated animals were divided each into three subgroups to receive 0, 1, and 3 ml of gastric juice of normal rats, respectively. The gastric juice was premixed with 50 mμg radioactive vitamin B_{12} and was then fed orally to the rats. Fecal excreta were collected for eight days, after which the animals were sacrificed and their livers, kidneys, and stomachs were removed. The radioactivity in all these specimens was measured. The results are tabulated in Table IV. There was no observable difference in the radioactivity in fecal matter or in the organs of the treated or deficient animals. Thus, feeding of intrinsic factor failed to improve the absorption of vitamin B_{12} by pyridoxine-deficient animals. Since the average body weight of pyridoxine-deficient animals was approximately one-half that of the treated animals, it is conceivable that a different conclusion might be drawn if our results were expressed in terms of millimicrograms of vitamin B_{12} per unit weight of organs. Nevertheless, such calculation likewise showed that the administration of gastric juice in quantities mentioned above resulted in no benefit to the pyridoxine-deficient or treated animals.

Beside gastric juice, stomach homogenate equivalent to one-half[9] of the rat stomach was administered together with vitamin B_{12} to pyridoxine-deficient and treated animals. The administration of stomach homogenate resulted in no difference in absorption of vitamin B_{12}.

Tauber[11] and associates demonstrated that the administration of histamine increases the absorption of vitamin B_{12} by the young subjects but not the old subjects. Since the latter group likewise show marginal pyridoxine-deficiency, it was, therefore, of interest to determine whether the administration of histamine to pyridoxine-deficient animals would increase the absorption of vitamin B_{12}. To this end, histamine or saline was injected to pyridoxine-deficient animals one hour prior to the oral feeding of radioactive vitamin B_{12}. To be certain that histamine effect lasted during the period when absorption of vitamin B_{12} remained critical, another dose of histamine was given four hours afterwards. Our results showed that the histamine-injected group absorbed 49

TABLE IV
Effect of Gastric Juice on Absorption of Radioactive Vitamin B_{12} and Residual Radioactivity in Organs[a]
Eight days' fecal excretion

Treatment	No. rats	Gastric juice (*ml*)	Radioactivity as vitamin B_{12}			
			Feces	Liver	Kidneys	Stomach
Deficient	4	0	56.8 ± 3.87	6.1 ± 0.71	5.3 ± 0.54	1.2 ± 0.33
	4	1	59.2 ± 2.84	7.1 ± 0.64	4.9 ± 0.57	1.4 ± 0.11
	4	3	52.9 ± 3.74	7.8 ± 0.71	5.9 ± 0.52	0.9 ± 0.16
Treated	3	0	52.7 ± 2.96	7.9 ± 0.42	5.3 ± 0.03	1.6 ± 0.05
	4	1	46.2 ± 2.57	8.7 ± 0.34	6.0 ± 0.35	1.1 ± 0.08
	4	3	42.9 ± 2.86	10.1 ± 0.64	7.1 ± 0.25	1.2 ± 0.06

[a] Percentage of oral dose.

per cent, and the saline injected group absorbed 45 per cent of the orally administered dose (50 mμg). Similarly, there was no difference in the radioactivity deposited in the target organs of the two groups of animals. Thus, histamine stimulation, under our experimental conditions, failed to enhance the absorption of vitamin B_{12} by pyridoxine-deficient rats.

The data presented above demonstrate that the prime factor in the impairment of absorption of vitamin B_{12} by the pyridoxine-deficient animals is not related to gastric juice secretion nor to any "deficiency" of intrinsic factor-like material. It remains, therefore, to determine whether pyridoxine deficiency may result in some endocrine disturbances which in turn affect vitamin B_{12} absorption. Consequently, the role of hypophyseal hormones on the absorption of vitamin B_{12} was examined. It was reported previously[12] that thyroid atrophy or removal can bring about a reduction in the absorption of vitamin B_{12}. However, there is no definite evidence that pyridoxine deficiency is accompanied by any atrophy of the thyroid. Efforts were made therefore, to determine whether other endocrine glands might cause the impairment of this absorption. Since adrenal insufficiency has been observed in connection with pyridoxine deficiency,[13,14] we investigated whether the adrenal may be involved in the absorption of vitamin B_{12} by pyridoxine-deficient animals. To this end, the absorption of vitamin B_{12} by adrenalectomized and stock animals was measured. Castrated and splenectomized rats were also included in this study in order to evaluate the relative specificity of adrenalectomy. The results tabulated in Table V demonstrate that the adrenalectomized rats absorbed less of the orally administered vitamin B_{12} than did the stock animals or the castrated rats. Splenectomy also reduced the absorption of vitamin B_{12}.

TABLE V

Effect of Adrenalectomy on Absorption of Radioactive Vitamin B_{12} by Stock Animals[a]

Treatment	Radioactivity as vitamin B_{12}		
	Feces	Liver	Kidneys
Adrenalectomy (6)	78.2 ± 2.04	3.7 ± 0.31	6.1 ± 0.50
Splenectomy (4)	75.0 ± 4.77	4.2 ± 0.37	2.9 ± 0.22
Castration (5)	41.1 ± 4.68	4.1 ± 0.58	5.5 ± 1.07
Control (5)	50.0 ± 4.78	3.6 ± 0.68	8.1 ± 1.38

[a] Percentage of oral dose.
Numbers in parentheses denote number of rats used.

TABLE VI

Effect of Adrenalectomy on the Absorption of Radioactive Vitamin B_{12} by Pyridoxine-deficient Animals[a]

Treatment	Radioactivity as vitamin B_{12}			
	Feces and gastrointestinal tract	Liver	Kidneys	Spleen
Adrenalectomy (4)	78.5 ± 4.49	8.4 ± 0.23	3.0 ± 0.56	0.38 ± 0.09
Control (4)	65.0 ± 4.05	10.9 ± 1.43	7.8 ± 0.56	0.50 ± 0.03

[a] Percentage of oral dose.
Numbers in parentheses denote number of rats used

The results given in Table VI demonstrate that adrenalectomy of pyridoxine-deficient animals reduces to a small extent the absorption of vitamin B_{12} as indicated by the fecal excretion and by the retention of radioactivity in various organs such as liver, kidneys, and spleen.

If the adrenal function of the rat can affect the absorption of vitamin B_{12}, it may be expected that hypophysectomy would also impair the absorption of vitamin B_{12}. To this end, hypophysectomized rats were divided into three subgroups and given treatments subcutaneously, as follows for ten days: group A, a normal saline solution; group B, 2 U.S.P. units of ACTH; and group C, 0.25 U.S.P. units of TSH. Our stock animals, group D, served as control. Approximately two weeks after treatment with hormone preparations, the vitamin B_{12} absorption test was conducted. The results tabulated in Table VII demonstrate that hypophysectomy results in poor absorption which can be improved by the injection of ACTH and TSH. Since commercial preparations of ACTH may be contaminated with TSH

TABLE VII

Effect of Hypophysectomy on Absorption of Radioactive Vitamin B_{12}[a]

Group	Treatment	Radioactivity as vitamin B_{12} Feces	Liver	Kidneys
A	Hypox[b]-saline (6)	80.1 ± 7.0	2.2 ± 0.36	2.2 ± 0.42
B	Hypox-ACTH (7)	54.3 ± 7.3	7.8 ± 2.62	7.5 ± 1.63
C	Hypox-TSH (5)	50.5 ± 13.5	7.6 ± 2.82	6.2 ± 1.96
D	Stock diet (8)	60.7 ± 1.4	4.8 ± 0.82	4.2 ± 0.58

[a] Percentage of oral dose.
[b] Hypox = hypophysectomy.
Numbers in parentheses denote number of rats used.

or other pituitary hormones, or vice versa, an examination of the weights of the thyroids and adrenals of the animals in the four groups indicated, however, that the specimens were relatively free from contamination (Table VIII). It can then be concluded that ACTH and TSH both play roles in controlling the absorption of vitamin B_{12}.

Since atrophy of the adrenal cortex may decrease the secretion of many hormones by the adrenals, it was of interest to determine whether the impairment of vitamin B_{12} absorption is due to the lack of cortisone. This hormone was injected into pyridoxine-deficient and stock animals. The absorption was measured by the fecal excretion test and by the measurement of radioactivity in different organs following the oral administration of radioactive

TABLE VIII

Weights of Thyroid and Adrenal Glands of Hypophysectomized Rats under Various Treatments

Group	Treatment	Thyroid Average (mg)	Thyroid Range (mg)	Adrenals Average (mg)	Adrenals Range (mg)
A	Hypox[a]-saline (6)	4.8	3–6	8.4	7–10
B	Hypox-ACTH (7)	5.1	3–7	15.6	13–19
C	Hypox-TSH (5)	8.6	5–11	4.5	3–6
D	Stock diet (8)	12.3	9–14	29.8	22–50

[a] Hypox = hypophysectomy.
Numbers in parentheses denote number of rats used in each group.

TABLE IX

Effect of Cortisone on Vitamin B_{12} Absorption[a] in Pyridoxine-Deficient Rats[b]

Treatment	Radioactivity as vitamin B_{12} Feces	Liver	Kidneys
Cortisone (3)	44.8 ± 3.80	7.2 ± 0.58	18.8 ± 1.40
Saline (3)	60.8 ± 2.10	5.6 ± 0.75	15.7 ± 3.20

[a] Percentage of oral dose.
[b] Vitamin B_{12} supplied in the ration.
Numbers in parentheses denote number of rats used.

vitamin B_{12}. The results tabulated in Table IX demonstrate that the injection of cortisone improves the absorption of vitamin B_{12}. These data taken as a whole, suggest a definite role of adrenal glands on absorption of vitamin B_{12}, mediated possibly by 11-oxycorticosteroids.

DISCUSSION

The importance of the status of the adrenal gland in the absorption of vitamin B_{12} is demonstrated by the diminution of absorption after adrenalectomy and by the improvement of absorption upon the injection of cortisone. The latter compound affects not only pyridoxine-deficient animals but also our stock animals. The impairment of the absorption of vitamin B_{12} after hypophysectomy can be corrected by the administration of ACTH as well as by the administration of TSH. However, the injection of ACTH alone apparently brings the vitamin B_{12} absorption to normalcy. If thyrotropic hormone, which is likewise essential for the absorption of vitamin B_{12}, plays a role independent of that of ACTH, it should not be expected that the administration of ACTH alone should correct the absorption almost completely, unless our ACTH preparation might be contaminated with thyrotropic hormone in an amount sufficient to improve the absorption of vitamin B_{12} but inadequate to stimulate hypertrophy of thyroid of the hypophysectomized rats.

Involvement of adrenals in pyridoxine deficiency was suggested by electrolyte imbalance[14,17] increase in adrenal size and cholesterol content in pair-fed rats,[14] and histochemical changes.[13] These alterations suggest some impairment of adrenal cortical function in pyri-

doxine-deficient animals. Furthermore, most of the changes can be readily corrected by administration of cortisone but not by desoxycorticosterone acetate. Our findings at least in part suggest this impairment of vitamin B_{12} absorption can be the result of the adrenal insufficiency.

According to a report of Strauss and Brokaw,[18] adrenocortical function in patients with addisonian anemia is impaired. It is interesting to note that Addison originally described adrenal insufficiency and pernicious anemia as closely related conditions.[19] Prednisone was recently reported to enhance vitamin B_{12} absorption in one patient with addisonian anemia.[20] Cortisone can also enhance vitamin B_{12} absorption in tropical sprue.[21,22] Whether this enhancement of absorption of vitamin B_{12} following cortisone administration is only specific for vitamin B_{12} or other nutrients as well has not been explored.

The influence of adrenal cortical hormones on gastric secretion has received considerable attention. Adrenalectomy produces an increase in pH and mucin content of fasting gastric juice, but a reduction in the pepsin and renin activities, even under the stimulus of histamine and cholinergic drugs. Administration of cortisone or ACTH brings about an increase of pepsin and basal and nocturnal secretion, with augmentation in acidity, pepsin activity, and total volume of gastric juice both in normal subjects and in patients with duodenal ulcer.[23,24] Our pyridoxine-deficient rats may well be suffering from certain degrees of adrenal dysfunction followed by secondary changes in gastrointestinal tract. The latter effect may play a more direct role in the impairment of absorption of vitamin B_{12}.

SUMMARY

Pyridoxine deficiency in rats results in an impairment of absorption of orally administered vitamin B_{12}. The manner in which the impairment occurs is not fully understood. In this present study, data are presented to determine whether the impairment might be due to the insufficiency of secretion of gastric juice or the atrophy of some endocrine glands. It was found that pyridoxine-deficiency resulted in a lesser secretion of gastric juice and a marked reduction of vitamin B_{12}-binding power. However, the coadministration of vitamin B_{12} with intrinsic factor, either from stomach homogenate or from rat gastric juice, failed to improve the absorption of orally administered vitamin B_{12}.

Studies were conducted to examine the possibility of endocrine dysfunction as a cause of the impairment of vitamin B_{12} absorption. It was found that in addition to the thyrotrophic hormone the adrenal cortical hormones can affect the absorption of vitamin B_{12}. Thus, hypophysectomy or adrenalectomy impairs the absorption of orally administered vitamin B_{12}. This absorption can be improved by the administration of cortisone to pyridoxine-deficient and stock animals or by injection of ACTH or TSH to hypophysectomized animals. These data taken as a whole indicate that pyridoxine deficiency results in adrenal atrophy, which in turn brings about a decrease in the absorption of vitamin B_{12}.

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