

# The Vitamin B<sub>12</sub> Content of Human Liver Tissue and Its Nutritional Significance

## A Comparison Study of Various Age Groups

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THE INTRODUCTION of methods for measuring vitamin B<sub>12</sub> absorption using the Co<sup>60</sup> labeled form<sup>1-3</sup> has resulted in the accumulation of evidence that absorption of this vitamin is greatly reduced in certain clinical conditions where functioning of the gastrointestinal tract is impaired. Lack of absorption of vitamin B<sub>12</sub> has been associated with pernicious anemia,<sup>1</sup> total gastrectomy,<sup>4</sup> sprue,<sup>5</sup> and the blind intestinal loop syndrome,<sup>6</sup> as well as with old age.<sup>7</sup> When the nutritional status of vitamin B<sub>12</sub> is considered in those individuals where absorption has been reduced, one of the first questions to arise is this: how much time must elapse before a change in the absorption pattern produces symptoms of a vitamin B<sub>12</sub> deficiency? In answering this question, of paramount importance is the extent of vitamin B<sub>12</sub> storage in the liver and possibly other organs of the body in terms of vitamin requirement. There is some clinical evidence that vitamin B<sub>12</sub> tissue stores may be considerably greater than is the case with other B vitamins. It has been reported that in pernicious anemia, when therapy is discontinued, a relapse may not occur for periods of as long as three years.<sup>8</sup> In a survey report<sup>9</sup> of 46 cases of total gastrectomy, a macrocytic anemia indicative of a vitamin B<sub>12</sub> deficiency developed in 12. In 9, the anemia appeared four years or more after surgery.

The present investigation was undertaken as a survey study to obtain definite information regarding the extent of vitamin B<sub>12</sub> body stores by actual measurement of the vitamin content of liver tissue. Since it has been reported that blood levels of vitamin B<sub>12</sub> are lower in old age<sup>10</sup> and suggested that a decreased absorption of the vitamin may occur in individuals in the older age group, it was decided to compare the liver stores of vitamin B<sub>12</sub> according to age groups to determine whether there was any evidence of depletion in old age.

### METHODS

The liver tissue used for vitamin analysis was obtained at autopsy from a total of 132 cases, all males. Clinical records were examined and it was realized that many factors such as cause of death, duration of illness, and state of nutrition could conceivably influence the vitamin B<sub>12</sub> liver stores. Moreover, these factors might vary with the age group and therefore experimental conditions were not ideal. However, it was decided to consider separately only one cause of death as recorded clinically and this was cirrhosis since disease of the

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TABLE 1.—Vitamin B<sub>12</sub> in Liver Tissue Assayed at Various Time Intervals

Time	Vitamin B <sub>12</sub> (γ per Gm. wet tissue)
6 hours	.80
24 hours	.80
48 hours	.88
72 hours	.92
7 days	.86

TABLE 2.—Vitamin B<sub>12</sub> Content of Liver

Age in Years	No. of Cases	Vitamin B <sub>12</sub> (γ per Gm wet tissue)	
		Range	Average
20 to 40	12	0.46-1.09	0.72
40 to 60	31	0.17-1.40	0.73
Over 60	79	0.20-1.41	0.71
Cirrhosis	10	0.06-0.46	0.26

liver itself would presumably have a direct effect on vitamin storage and metabolism. Cases having a history of vitamin B<sub>12</sub> administration for therapeutic purposes were excluded from the study only if the vitamin was given parenterally. There is evidence that orally administered vitamin is absorbed in extremely limited amounts.<sup>11, 12</sup>

In the assay procedure, the liver tissue was homogenized (Potter-Elvehjem technic), placed in pH 4.5 buffer at 1 to 100 dilution, and autoclaved 15 minutes. The filtered extract was assayed for vitamin B<sub>12</sub> using *L. leichmannii*.<sup>4, 9</sup> Since all of the microbiologic activity was alkali-labile, it was assumed that the value obtained was a true measure of "vitamin B<sub>12</sub> activity" and did not represent any of the nucleic acid derivatives which cause a growth response of *L. leichmannii* when present in considerable concentration.<sup>13</sup>

## RESULTS

It was first determined that the study was feasible from the point of view of the stability of vitamin B<sub>12</sub> in liver tissue. Table 1 shows that vitamin B<sub>12</sub> values for liver tissue remained constant over a time interval of several days when kept at icebox temperature.

In table 2 the values obtained in the study expressed as γ of vitamin B<sub>12</sub> per gram of wet tissue are recorded and grouped according to the age of the subject. For the age group under 40, of which there were thirteen cases, the range was from 0.46 to 1.09 γ of vitamin B<sub>12</sub> per Gm. of liver tissue with an average of 0.72 γ. The 40 to 60 year age group of 31 cases had a wider range, from 0.17 to 1.40 γ per Gm. with an average of 0.73 γ. Range and average values almost identical with these figures were obtained in the age group over 60. The average values for all age groups are considerably higher than the average value of 0.26 γ per Gm. obtained in ten cases of cirrhosis where it was expected that evidences of a vitamin B<sub>12</sub> deficiency might be found. When actual values obtained for each case were plotted in a scattergram (fig. 1) there was no apparent tendency for higher or lower values to be associated with a particular age group. The lowest value, other than for the cirrhosis cases, was obtained in a 56 year old man and the highest value in an individual 79 years of age.

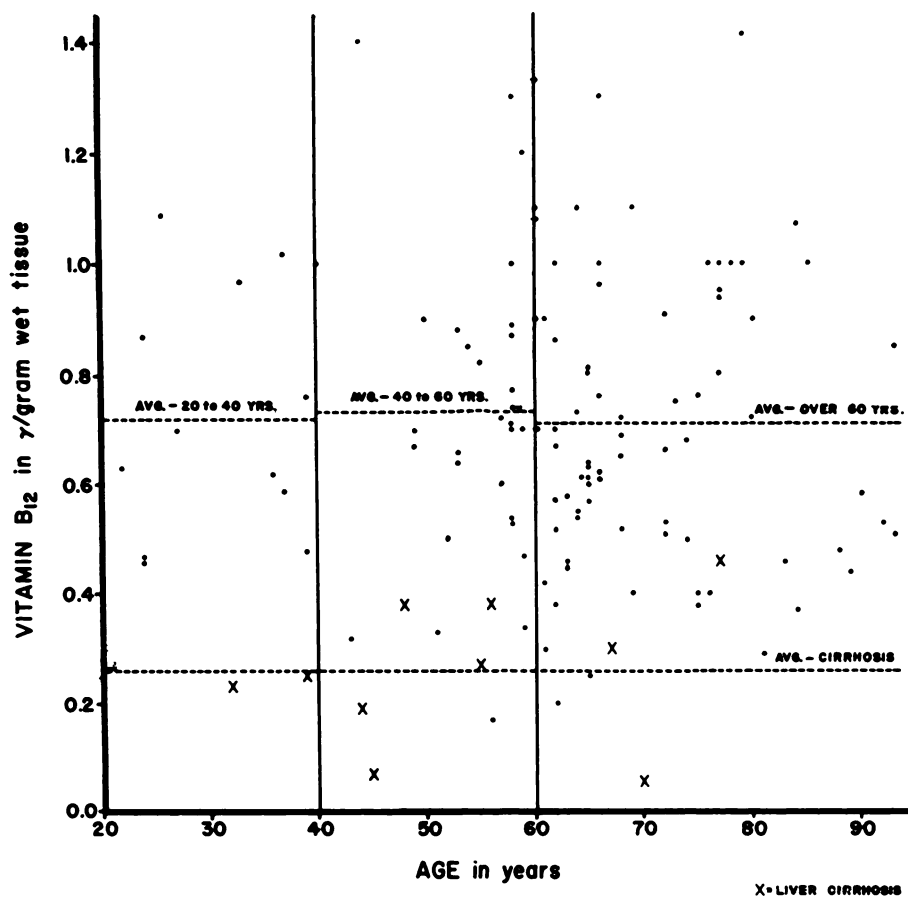


FIG. 1.—The vitamin B<sub>12</sub> content of human liver; 132 cases arranged according to age and including ten cases of cirrhosis.

#### DISCUSSION

A considerable range of values was obtained by the actual measurement of vitamin B<sub>12</sub> in liver tissue in this survey study. However, the figures do not indicate that any widespread difference exists in vitamin B<sub>12</sub> content of liver among the various age groups and they do not support suggestions that a deficiency of this vitamin is prevalent in old age.

Girdwood<sup>14</sup> has reported that liver tissue obtained from pernicious anemia patients was low in vitamin B<sub>12</sub> content. In this study, biopsy liver tissue from two patients with untreated pernicious anemia was assayed for vitamin B<sub>12</sub> and found to contain less than 0.10 γ per Gm.

It was indicated that in this investigation, the cause and duration of illness might influence the vitamin B<sub>12</sub> liver stores. In the survey there were 32 cases of neoplastic disease with an average of 0.69 γ of vitamin B<sub>12</sub> per Gm. of liver, a value not significantly different from the overall average. The only disease associated with a greatly reduced level of the vitamin was cirrhosis. Apparently

the damaged liver is unable to store vitamin B<sub>12</sub>. It would appear that the macrocytic anemia of cirrhosis might result from a vitamin B<sub>12</sub> deficiency, as has been suggested by Wintrobe.<sup>15</sup> However, in the present study, no correlation could be made between the degree of anemia prior to death and the vitamin B<sub>12</sub> content of the liver. That factors other than a vitamin B<sub>12</sub> deficiency are involved in the production of anemia is indicated from clinical results.<sup>16</sup> Only occasionally does the anemia of cirrhosis respond to vitamin B<sub>12</sub> therapy.

Assuming that the maintenance dose for the patient with pernicious anemia of 1  $\gamma$  vitamin B<sub>12</sub> (parenteral) per day represents an adequate daily requirement,<sup>17</sup> some calculations can be made regarding the vitamin B<sub>12</sub> liver stores. If the average total weight of liver tissue is taken as 1500 Gm., then on the basis of the average value of approximately 0.70  $\gamma$  per Gm., enough vitamin B<sub>12</sub> would be present in the liver to last for a three year period. The highest values of 1.4  $\gamma$  per Gm. would be sufficient for six years if all were available to body cells. The measurement of vitamin B<sub>12</sub> in the liver corroborates the clinical evidence that there is a considerable storage of this vitamin in terms of nutritional requirement. It would appear therefore, that a decrease in the absorption of vitamin B<sub>12</sub> from the gastro-intestinal tract would not result in the immediate development of a deficiency state.

#### SUMMARY

Range and average values are reported for the vitamin B<sub>12</sub> content of liver tissue obtained at autopsy from 132 individuals. The average value for all age groups was approximately 0.70  $\gamma$  per Gm. of wet tissues. In cirrhosis the average value was 0.26  $\gamma$  per Gm. It was concluded that there are no apparent differences in vitamin B<sub>12</sub> stores in the various age groups. Also, the amount of vitamin stored is relatively large in terms of requirement and is probably sufficient to last for a period of several years.

#### SUMMARIO IN INTERLINGUA

Es reportate le valores maximo-minimal e medie del contento de vitamina B<sub>12</sub> in histos hepatic obtenite al necropsia de 132 individuos. Le valor medie pro omne gruppos de etate esseva approximativement 0,70  $\mu\text{g}$  per g de histo humide. In casos de cirrhosis, le valor medie esseva 0,26  $\mu\text{g}$  per g. Le conclusion esseva que il existe nulle apparente differentias del reservas de vitamina B<sub>12</sub> in le varie gruppos de etate. In plus, il pare que le quantitate del vitamina accumulate es relativemente grande in comparation con su requirimentos. Illo es probabilemento sufficiente pro un periodo de plure annos.

#### REFERENCES

- <sup>1</sup> HEINLE, R., WELCH, A. D., SCHARF, V., MEACHAM, G. C., AND PRUSOFF, N. H.: Studies of excretion (and absorption) of Co<sup>60</sup>-labeled vitamin B<sub>12</sub> in pernicious anemia. *Tr. A. Am. Physicians* 65: 214, 1952.
- <sup>2</sup> SCHILLING, R. F.: Intrinsic factor studies. II. The effect of gastric juice on urinary excretion of radioactivity after oral administration of radioactive vitamin B<sub>12</sub>. *J. Lab. & Clin. Med.* 42: 860, 1953.
- <sup>3</sup> GLASS, G. B. J., BOYD, L. J., AND GELLIN, G. A.: Surface scintillation measurements in humans of the uptake of parenterally administered radioactive vitamin B<sub>12</sub>. *Blood* 10: 95, 1955.

- <sup>4</sup> SWENDSEID, M. E., HALSTED, J. A., AND LIBBY, R. L.: Excretion of Co<sup>60</sup>-labeled vitamin B<sub>12</sub> after total gastrectomy. *Proc. Soc. Exper. Biol. & Med.* **83**: 226, 1953.
- <sup>5</sup> REISNER, E. H., JR., ROSENBLUM, C., AND MORGAN, M. D.: Urinary excretion of orally administered Co<sup>60</sup>-labeled vitamin B<sub>12</sub> in normal subjects and patients with pernicious anemia and sprue. *Clin. Res. Proc.* **11**: 56, 1954.
- <sup>6</sup> HALSTED, J. A., SWENDSEID, M. E., GASSTER, M., AND LEWIS, P. M.: Absorption of radioactive vitamin B<sub>12</sub> in disease of the small intestine: Relation to macrocytic anemia. *Trans. Clin. & Climat. Assn.* **65**: 1954.
- <sup>7</sup> GLASS, G. B. J., BOYD, L. J., GOLDBLOOM, A. A., AND ROSEN, S.: Effect of intrinsic factor on intestinal absorption of B<sub>12</sub> in aged people. *Fed. Proc.* **14**: 57, 1955.
- <sup>8</sup> SCHWARTZ, S. O. AND LEGERE, H.: Relapses in pernicious anemia. *J. A. M. A.* **124**: 637, 1944.
- <sup>9</sup> MACDONALD, R. M., INGELFINGER, F. J., AND BELDING, H. V.: Late effects of total gastrectomy in man. *New Eng. J. Med.* **237**: 887, 1947.
- <sup>10</sup> CHOW, B. F.: Vitamin B<sub>12</sub> and Aging. *Nutrition Symposium Series 9*: 59, 1954.
- <sup>11</sup> SWENDSEID, M. E., GASSTER, M., AND HALSTED, J. A.: Limits of Absorption of orally administered vitamin B<sub>12</sub>. Effect of intrinsic factor sources. *Proc. Soc. Exper. Biol. & Med.* **86**: 834, 1954.
- <sup>12</sup> GLASS, G. B. J., BOYD, L. J., STEPHANSON, L., AND JONES, E. L.: Metabolic interrelations between intrinsic factor and vitamin B<sub>12</sub>. *Proc. Soc. Exper. Biol. & Med.* **88**: 1, 1955.
- <sup>13</sup> WRIGHT, L. D., SKEGGS, N. R., AND HUFF, J. W.: The ability of thymidine to replace vitamin B<sub>12</sub> as a growth factor for certain Lactobacilli. *J. Biol. Chem.* **175**: 475, 1948.
- <sup>14</sup> GIRDWOOD, R. H.: The occurrence of growth factors for Lactobacillus leichmannii, Streptococcus faecalis and Leuconostoc citrovorum in the tissues of pernicious anemia patients and controls. *Biochem. J.* **52**: 58, 1952.
- <sup>15</sup> WINTROBE, M. M.: Relation of disease of the liver to anemia. *Arch. Int. Med.* **57**: 289, 1936.
- <sup>16</sup> UNGLEY, C. C.: The chemotherapeutic action of vitamin B<sub>12</sub>. *Vit. and Hor.* **13**: 137, 1955.
- <sup>17</sup> BETHELL, F. H.: Vitamin B<sub>12</sub> requirements of human beings. *The Vitamins*, Academic Press Inc., p. 523, 1954.