LIVER VITAMIN B₁₂ LEVELS IN CHRONIC LIVER DISEASES

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Liver is apparently the major storage organ of vitamin B₁₂ (B₁₂). Experiments with radioactive B₁₂ have shown that when this substance is administered orally the major part of the absorbed B₁₂ is accumulated in the liver of the human body (1). It has been reported that serum B₁₂ levels in patients with acute or chronic liver diseases are markedly elevated (2—5). In experimental acute hepatic necrosis of rabbits provoked by carbon tetrachloride the marked elevation in serum B₁₂ levels and the decrease in liver B₁₂ levels have been observed (6). B₁₂ levels of the liver tissues from several patients with acute hepatitis have been shown to be low by the studies with biopsy materials (7), and also it has been reported that B₁₂ levels of the liver tissues obtained at autopsy from the patients with cirrhosis of the liver are markedly decreased (8).

The present investigation was carried out to determine the changes in B₁₂ storage of the liver with materials obtained at laparotomy from patients with chronic liver diseases. Preliminary reports of certain phases of this study have been published (9, 10).

MATERIALS AND METHODS

Thirty five patients with chronic liver diseases were studied which consisted of 14 patients with cirrhosis of the liver, 17 patients with Banti's syndrome and 1 patient with obstruction of the hepatic vein and 3 patients with cancer of the liver. Thirteen patients with peptic ulcer of the stomach or the duodenum and 5 patients with gastropotosis were also studied as a control. These were admitted for surgical treatment at the Second Department of Surgery of Nagoya University Hospital from September 1955 to December 1956, and only patients with clear clinical and histopathological evidences of the respective diseases were included. Of the patients with chronic liver diseases there were those to whom B₁₂ had been administered parenterally for therapeutic purposes until 3 days before laparotomy. Doses of B₁₂ injected were 15 or 100 μ g per day. The patients with cirrhosis of the liver or Banti's syndrome underwent the operation of portacaval anastomosis, splenectomy or both. Three patients with cirrhosis of the liver and 4 patients with Banti's syndrome un-

derwent laparotomy twice at varied intervals because of some trouble after the operation or to reexamine portal vein pressure. One patient with Banti's syndrome underwent laparotomy 3 times.

Liver tissues for the B₁₂ assay were resected at laparotomy from the left lobe of the liver by about 500 mg of wet tissues. Immediately upon removal the liver blocks were decapsulated and minced. One hundred mg of the liver pulp was weighed exactly and homogenized in 9.9 ml of ice-cold 0.1 M acetate buffer at pH 4.6 containing 5 mg of potassium cyanide per 100 ml as described by Dawbarn *et al.* (11). The homogenates were heated for 30 minutes in a boiling water bath, cooled and centrifuged. Supernatant obtained was adjusted to pH 6.8 with K₂HPO₄ solution, diluted with distilled water to make 1:100 or 1:200 dilution and used for the assay.

B₁₂ was determined with *Lactbacillus leichmannii* ATCC 4797 by an acidimetric adaptation of U.S.P. method with some modification (12, 6). As the B₁₂ activity in the liver extract was almost entirely destroyed by heating at pH 12 for 30 minutes in a boiling water bath, it was evident that the vitamin activity was due to B₁₂ itself and did not represent any of the nucleic acid derivatives, *i.e.* desoxyribosides. The values obtained were therefore not corrected by subtracting alkali-stable fraction.

RESULTS

In Table I, II and Figure 1, the values for the B₁₂ levels of the liver expressed as μg per g of wet tissues are given for each disease. In cases which had received parenteral B₁₂ administration, doses of B₁₂ are expressed in single and double parentheses as numbers of the injections of 15 and 100 μg of B₁₂ respectively. Since the liver tissues from the patients with peptic ulcer or gastropotosis were histopathologically almost normal, it could be assumed that the values in these patients were the normal values for the B₁₂ levels of the human liver; *i.e.*, range 0.48—1.16, average 0.74 μg per g. Three cases of cancer of the liver examined here were multicentral hepatoma which appeared to have developed from cirrhosis. In cirrhosis and cancer the liver B₁₂ levels

TABLE I
*Liver Vitamin B₁₂ Levels of Patients not Receiving Vitamin
B₁₂ Parenterally*

Kind of disease	No. of cases	Vitamin B ₁₂ levels (μg per g)	
		Range	Average
Cirrhosis of the liver	6	0.02—0.45	0.19
Banti's syndrome	10	0.10—1.12	0.62
Cancer of the liver	3	0.08—0.19	0.13
Peptic ulcer of the stomach or the duodenum	13	0.48—1.16	0.72
Gastropotosis	5	0.65—1.06	0.76

markedly low. In Banti's syndrome, the values were almost within the normal range except one case, the average value being slightly lower than the normal average. Of the cases which had received parenteral B₁₂ administration 2 cases had high levels of the liver B₁₂ but the others were almost within the same range as the cases without parenteral B₁₂ administration, the average values of both cases being almost equal.

The serum B₁₂ levels of the patients which had not received parenteral B₁₂ administration were determined with *L. leichmannii* ATCC 4797 using the procedures described previously (12). The results are given in Table III.

TABLE III
Serum Vitamin B₁₂ Levels of the Patients Described in Table I.

Kind of disease	No. of cases	Vitamin B ₁₂ levels ($\mu\mu\text{g per ml}$)			
		Free form		Bound form	
		Range	Average	Range	Average
Cirrhosis of the liver	6	20— 66	43	520—2360	1150
Banti's syndrome	10	0— 97	36	200— 850	500
Cancer of the liver	3	96—160	119	590—2040	1090
Peptic ulcer and gastropstosis	18	0— 91	30	100— 730	363

The values of normal human subjects determined by the same procedures were reported (12). The total B₁₂ levels ranged from 160 to 812 $\mu\mu\text{g per ml}$ with a mean of 415 $\mu\mu\text{g per ml}$ and the range of the free form was from 0 to 83 $\mu\mu\text{g per ml}$ (12). In comparison with these values the values in peptic ulcer and gastropstosis were quite normal, and the values in Banti's syndrome were on slightly higher levels within the normal range. But in cirrhosis and cancer the total B₁₂ levels were elevated, mainly due to the increase of the bound form. The close quantitative relationship between the B₁₂ levels of the serum and of the liver in individual cases was not found.

With 3 cases of cirrhosis and 5 cases of Banti's syndrome which underwent laparotomy again after the operation of portacaval anastomosis B₁₂ determinations of the liver tissues were conducted at each laparotomy with the results shown in Fig. 2. Intervals of laparotomies varied from 2 to 11 weeks, and in most cases various doses of B₁₂ were administered parenterally for therapeutic purposes after the first operation until two or three days before the next operation. As will be seen, the liver B₁₂ levels were decreased following the operation of portacaval anastomosis in all cases. The most remarkable decrease was observed in a case, whose value fell in 2 weeks to 22 per cent of the pre-operation value. The case underwent the third laparotomy 11 weeks after the second operation; the fall of the liver B₁₂ levels was slight. Another characteristic in the decline of the liver B₁₂ levels following the operation of portacaval anastomosis was that in the cases having considerably high B₁₂ levels of the liver before the operation the decrease was

Numbers in single and double parentheses are doses of vitamin B₁₂ injected, expressed as numbers of injections of 15 and 100 μ g, respectively.

FIG. 2 Changes in the Liver Vitamin B₁₂ Levels of Patients with Cirrhosis of the Liver and Banti's Syndrome Following the Operation of Portacaval Anastomosis.

more marked than in those having low B₁₂ levels. For example, in cases with more than 0.45 μ g B₁₂ per g of the liver the decrease was more than 50 per cent except one case and in cases with less than 0.30 μ g the decrease was slight.

DISCUSSION

It was shown that the liver B₁₂ levels was remarkably lowered both in cirrhosis of the liver and in cancer of the organ possibly developed from cirrhosis, and that even in cases to which B₁₂ had been administered parenterally the liver B₁₂ levels did not elevate significantly with a few exceptions. Swendseid *et al.* (8) have estimated the B₁₂ levels of the liver tissues obtained at autopsy from 132 subjects, and have found that the average value for all age groups was approximately 0.70 μ g per g of wet tissues and in cirrhosis the average value was 0.26 μ g per g. Jansen (7) has found that the B₁₂ level of the liver tissue obtained by biopsy from a patient with cirrhosis of the liver was less than 0.05 μ g per g of wet tissues, and it was very low in comparison with the value 0.50 to 1.80 μ g per g which were found in normal livers by Heinrich *et al.* (13). Low levels of the liver B₁₂ in cirrhosis may be attributed to the damaged capacity to store the vitamin. The disease associated with a markedly reduced level of the liver B₁₂ is pernicious anemia. Wolff *et al.* (14, 15) have reported that B₁₂ levels of the liver tissues obtained by biopsy ranged from 0.02 to 0.14 μ g per g in 6 patients with untreated pernicious anemia, while 0.47 to 1.14 μ g were found in normal subjects. Girdwood (16) has reported very low B₁₂ levels of the liver tissues obtained from pernicious

anemia patients who died without treatment. Swendseid *et al.* (8) also have found that biopsy liver tissues from 2 patients with untreated pernicious anemia were found to contain less than 0.10 μg per g. The liver B₁₂ levels of some patients with cirrhosis in the present study were as low as these values in untreated pernicious anemia patients. However, the serum B₁₂ levels of patients with cirrhosis were elevated quite differently from those of pernicious anemia patients whose levels are extremely low. High levels of the serum B₁₂ in patients with cirrhosis of the liver have been noted by several workers (2–5).

Following the operation of portacaval anastomosis the liver B₁₂ levels of the patients with cirrhosis or Banti's syndrome were generally decreased even during parenteral administrations of the vitamin. This may agree with the fact that liver functions and histopathological changes of the liver tissues were deteriorated following the operation (10). In the report of Jones *et al.* (5) a patient was included who underwent the operation of portacaval anastomosis and whose serum B₁₂ levels were remarkably elevated following the operation. The decrease of B₁₂ storage of the liver following the operation is apparently due to the release of the vitamin into blood stream. It has been found that the serum B₁₂ levels of rabbits receiving carbon tetrachloride were rapidly and markedly increased and concomitantly the liver B₁₂ levels were decreased, with close time and quantitative relationships to each other and to the development of acute hepatic necrosis provoked by carbon tetrachloride (6). It may be suggested therefore that when the liver cell damages are in progress, the stored B₁₂ in the liver is released into blood stream. According to Yamamoto *et al.* (17) the rise of the serum B₁₂ levels after carbon tetrachloride administration was not observed in rats receiving a low B₁₂ diet. They had low B₁₂ levels of the liver unlike the rats fed a sufficient diet. Similar observation was also noted in the present report that the fall of the liver B₁₂ levels following portacaval anastomosis was not marked in cases whose pre-operation levels were low.

SUMMARY

Range and average values were determined for the vitamin B₁₂ levels of the liver tissues obtained at laparotomy from 35 patients with chronic liver diseases and from 18 patients with peptic ulcer or gastropotosis. In cirrhosis the average value was 0.19 μg per g, in cancer of the liver possibly developed from cirrhosis 0.13 μg per g, in Banti's syndrome 0.62 μg per g, and in peptic ulcer and gastropotosis 0.74 μg per g. In cirrhosis and Banti's syndrome the liver B₁₂ levels of the cases receiving parenterally B₁₂ did not rise significantly with a few exceptions, the average values remaining on the similar levels described above.

Following the operation of portacaval anastomosis the liver B₁₂ levels of the patients with cirrhosis or Banti's syndrome fell even during parenteral administration of the vitamin. The fall following the operation was not remarkable in cases whose pre-operation levels of the liver B₁₂ were low.

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REFERENCES

1. Glass, G. B. J., Boyd, L. J., Gellin, G. A., Stephenson, L., *Arch. Biochem. Biophys.* **51**, 251 (1954).
2. Rachmilewitz, M., Aronovitch, J. Grossowicz, N., *J. Lab. Clin. Med.* **48**, 339 (1956).
3. Lear, A. A., Harris, J. W., Castle, W. B., Fleming, E. M., *ibid.* **44**, 715 (1954).
4. Jandl, J. H., *J. Clin. Invest.* **34**, 390 (1955).
5. Jones, P. N., Mills, E. H., Capps, R. B., *J. Lab. Clin. Med.* **49**, 910 (1957).
6. Kato, N., Murakami, S., *Vitamins* **13**, 549 (1957); *J. Lab. Clin. Med.* (in press).
7. Jansen, L., Vitamin B₁₂ and Intrinsic Factor edited by Heinrich, H. C., Ferdinand Enke, Stuttgart, (1957). p. 541.
8. Swendseid, M. E., Hvolboll, E., Schick, G., Halsted, J. A., *Blood* **12**, 24 (1957).
9. Narita, Y., Kato, N., *Vitamins* **14**, 147 (1958).
10. Imanaga, H., Isobe, Y., *Jap. J. Surgery* (Japanese). **57**, 1014 (1956).
11. Dawbarn, M. C., Hine, D. C., Austral. *J. Exptl. Biol. Med. Sci.* **32**, 1 (1954).
12. Kato, N., *Vitamins* **13**, 112 (1957); *J. Vitaminol.* **5**, 24 (1959).
13. Heinrich, H. C., Lahann, H., *Z. Vit. Horm. Fermentforschg.* **6**, 126 (1954).
14. Wolff, R., Drouet, P. L., Karlin-Weissman, R., *Compt. rend. Acad.* **232**, 568 (1951).
15. Drouet, P. L., Wolff, R., Karlin-Weissman, R., Rauber, G., *Bull. Soc. Méd. Hôp. Paris* **67**, 281 (1951).
16. Girdwood, R. H., *Biochem. J.* **52**, 58 (1952).
17. Yamamoto, R. S., Okuda, K., Chow, B. F., *Proc. Soc. Exptl. Med. Biol.* **94**, 497 (1957).