

## Vitamin B<sub>12</sub> Content of Cells of the Blood, Bone Marrow and Liver Of the Rat.\* (30961)

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It was reported(1) that Co<sup>60</sup>B<sub>12</sub> injected intraperitoneally into rats can be recovered in the liver, kidneys and also the bone marrow of the limbs, spinal column and skull. In animals rendered anemic through loss of blood there was a fall in radioactive vitamin B<sub>12</sub> in the liver, kidneys and other viscera, and an increased radioactivity could be demonstrated in the bone marrow of the vertebrae and ribs. As an extension of this study, the vit B<sub>12</sub> concentration was determined microbiologically in the cellular elements of the bone marrow, and compared with that of red cells, leukocytes, lymphocytes and liver cells. The results are reported here.

**Material and methods.** Male albino rats of the Hebrew University strain, weighing 145-165 g, fed Purina laboratory chow, were used. The animals were bled from the vena cava under ether anesthesia and killed. The bone marrow cell suspensions were prepared as follows: the tibial bone marrow was removed with the aid of a needle and suspended in cold phosphate-buffered saline (PBS) pH 7.0 and dispersed gently by drawing the fluid up and down in a Pasteur pipette. The bones of the lumbar vertebrae were minced with scissors in a small volume of PBS and homogenized in a loosely fitting glass homogenizer with a teflon pestle. The suspension was then left to settle and the supernatant fluid was removed and centrifuged. The cells were re-suspended in 1-2 ml of PBS, and aliquots of cell suspensions were taken for counting the number of cells and for determination of vitamin B<sub>12</sub>. Cells were counted in a hemocytometer in duplicate. To hemolyze the erythrocytes, the cell suspension was treated with dilute acetic acid and the cell count repeated after treatment. Liver cell suspensions were prepared from livers perfused with ethylenediaminetetra-acetic acid(2). Leukocyte

suspensions were prepared using the method described by Nelken *et al*(3). Lymphocyte suspensions were prepared from mesenteric lymph nodes. Vit B<sub>12</sub> was also estimated in red cell suspensions and in the serum.

Vit B<sub>12</sub> was determined microbiologically with a mutant of *Escherichia coli*(4), with the turbidity measurements carried out in a Beckman DB Spectrophotometer at wavelength 550 m $\mu$ . Release of the bound vit B<sub>12</sub> in the serum and different cell types was achieved by diluting the serum samples or cell suspensions 1:10 with 1 N acetate buffer, pH 4.5, and heating for 30 minutes at 100°C in the presence of cyanide (0.2 mg KCN per 0.5 ml of undiluted serum or cell suspension). The supernatant was assayed.

**Results.** The vit B<sub>12</sub> concentration in the bone marrow of the tibia and vertebrae was 16.04 and 20.94 pg/10<sup>6</sup> nucleated cells, respectively, (Table I). A higher vit B<sub>12</sub> concentration was found in the nucleated cells of the skull, *i.e.*, 39.48 pg/10<sup>6</sup> cells. The average vit B<sub>12</sub> content in the circulating leukocytes was 19.02 pg/10<sup>6</sup> cells (Table II). Somewhat higher vit B<sub>12</sub> concentration was found in the lymphocytes of the mesenteric lymph nodes, *i.e.*, 31.1 pg/10<sup>6</sup> cells (Table III). The vit B<sub>12</sub> content in the nucleated cells of the bone marrow and the circulating leukocytes differed markedly from that found in the red blood cells, the latter being 0.11 pg/10<sup>6</sup> cells (Table II). The highest vit B<sub>12</sub> concentration was present in liver cell suspensions, averaging 560 pg/10<sup>6</sup> cells (Table III).

**Comment.** Few data exist on the vit B<sub>12</sub> content of the bone marrow. According to Halsted(5) vit B<sub>12</sub> concentration in human marrow varies from 7 to 29 ng/g. Using Halsted's results, the ratio of vit B<sub>12</sub> content in the liver to that in the bone marrow is 30:1, which is similar to that observed by us when the ratio was calculated not on the

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TABLE I. Vitamin B<sub>12</sub> Content of Bone Marrow Cells from Different Sites.

Bone marrow site	No. of rats	Erythrocytes (%)	Vit B <sub>12</sub> content		Statistical significance
			pg/10 <sup>6</sup> nucleated cells	mean ± S.E.	
Tibia	13	33	16.04	1.77 (A)	A vs B t = 1.36 P >.1
Vertebrae	13	28	20.94	3.14 (B)	B vs C t = 6.11 P <.01
Skull	13	37	39.48	6.04 (C)	A vs C t = 8.40 P <.01

basis of weight but per million cells. The estimation of vit B<sub>12</sub> content of the rat marrow in a given number of nucleated bone marrow cells has the advantage of enabling a more accurate quantitative evaluation of changes in vit B<sub>12</sub> concentration of bone marrow cells under different experimental conditions.

Vit B<sub>12</sub> concentration of the nucleated bone marrow cells is similar in the tibia and vertebrae. A significantly higher vit B<sub>12</sub> concentration was found in the nucleated cells of the skull. Although the cellularity of the skull was much less than that of the vertebrae and tibia, the cell composition did not differ essentially in the various marrow sites to explain the difference in vit B<sub>12</sub> content. Vit B<sub>12</sub> content of the circulating leukocytes was found to be similar to that of nucleated cells

TABLE II. Vitamin B<sub>12</sub> Content of Leukocytes, Erythrocytes and Serum.

Exp No.	Vit B <sub>12</sub> content		
	Leukocytes,* pg/10 <sup>6</sup> cells	Erythrocytes, pg/10 <sup>6</sup> cells	Serum, pg/ml
1	23.4	.10	510
2	21.4	.12	1179
3	17.9	.15	960
4	15.8	.11	790

\* In each experiment the leukocytes were separated from blood derived from 3-5 rats.

TABLE III. Vitamin B<sub>12</sub> Content of Liver Cells and Lymph Node Cells.

Exp No.	Vit B <sub>12</sub> content		
	Liver cells, pg/10 <sup>6</sup> cells	Lymphocytes, pg/10 <sup>6</sup> cells	Serum, pg/ml
1	593	27.0	740
2	629	30.5	780
3	726	36.5	1280
4	794	—	—
5	364	—	1040

of the vertebrae and tibia. Kidd and Thomas (6) found lower concentrations in human leukocytes (3.84 pg/10<sup>6</sup> cells). Our findings of vit B<sub>12</sub> concentrations in human leukocytes of 5 normal subjects did not differ significantly from those obtained in rat leukocytes. The reason for the discrepancy between our findings and those of Kidd and Thomas is not clear; it may be due to differences in technique and bioassays employed.

In striking contrast to the high concentration of vit B<sub>12</sub> in the leukocytes and bone marrow cells is the very low concentration found in erythrocytes. It appears that all the cellular elements containing nuclei have a high vit B<sub>12</sub> content. It is not surprising that the highest concentration of vit B<sub>12</sub> was found in the liver cells which contain about 20 times more vit B<sub>12</sub> than the leukocytes, since the liver represents the main storage organ for vit B<sub>12</sub>.

*Summary.* Vit B<sub>12</sub> content was determined microbiologically in different cellular elements of the rat. The highest concentration was found in liver cells. The vitamin B<sub>12</sub> content of nucleated bone marrow cells and circulating leukocytes was of the same magnitude, except for the nucleated cells of the skull which had a higher concentration than the other marrow sites. The lowest concentration of vit B<sub>12</sub> was found in the red cells.

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## Renal Arteriovenous Reduction in Hemoglobin Concentration in the Anesthetized Dog.\* (30962)

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A renal arteriovenous reduction in hemoglobin concentration has been observed in the dog in terms of oxygen capacity(1,2) and in the rat in terms of oxyhemoglobin(3). Since this finding would not necessarily have been expected from what was known of the physiology of hemoglobin and the physiology of the kidney, it was considered of interest to investigate the matter further.

*Procedure.* The hemoglobin concentration in arterial blood was compared with that drawn from a renal vein at the same time. Adult mongrel dogs were anesthetized with 20 mg/kg of sodium pentobarbital given intravenously, supplemented with additional doses of 5 mg/kg as needed. Two groups of experiments were performed. In the first, samples of about 1 ml were taken from the right femoral artery and left renal vein by puncturing the vessels with hypodermic needles just before they were to be drawn. The vessels were exposed by dissection. The renal vein was approached through a ventral laparotomy and the viscera were usually retracted out of the way just before the samples were to be taken. A total of 109 pairs of blood samples were taken from 13 different dogs.

In the second group of experiments samples of about 1 ml were drawn from the aorta opposite the left renal artery and from the left renal vein through polyethylene catheters that had been implanted some time before. The animals were heparinized to avoid clot-

ting in the catheters. Two to 5 ml of blood were drawn through each catheter and discarded just before the samples were taken to clear the catheters of the blood that had remained in them. A total of 89 pairs of blood samples were taken from 15 different dogs.

In both groups of experiments duplicate subsamples of each sample of blood were analyzed for hemoglobin concentration in terms of both carbon monoxide hemoglobin and acid hematin and in the second group also in terms of cyanmethemoglobin. The sample, which was discharged from the syringe or catheter into a small paraffin cup, was agitated at the time the subsamples were taken to avoid any possibility of settling of the red cells. In the first group of experiments subsamples of 0.1 ml were each measured into 25 ml of distilled water. Two tenths ml of concentrated ammonium hydroxide was added to clear this somewhat cloudy solution. Aliquots of the resulting clear solution were then analyzed for carbon monoxide hemoglobin and acid hematin. In the second group of experiments 0.2 ml subsamples were each measured into 20 ml of M/60 phosphate buffer (pH 6.6). This buffered solution was cleared by addition of 0.05 ml of concentrated ammonium hydroxide. Aliquots of it were then analyzed for carbon monoxide hemoglobin, acid hematin, and cyanmethemoglobin.

*Results* are summarized in Tables I and II. The results of the first group of experiments demonstrate that a renal arteriovenous reduction in hemoglobin, as reported by others, may be observed. Reductions of as much as 3.58 g % were found. In 27 instances, the

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