Distribution of Vitamin B_{12} Between Plasma and Cells

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THE analytical values for vitamin B_{12} are nearly always given for serum or plasma. Since vitamin B_{12} assays may be performed on whole blood without inhibition of the assay micro-organism and without interference by color, we investigated the distribution of vitamin B_{12} between plasma and the cellular elements of the blood. A preliminary report of these findings has been published.[1]

We studied blood from (1) three normal healthy control subjects, (2) four recently diagnosed, still untreated patients with pernicious anemia and (3) a patient with total gastrectomy, who had been given vitamin B_{12} orally, but whose serum level was still very low.

METHOD

The blood was citrated or heparinized, the blood cells were separated by centrifugation and washed six times with acid citrate glucose solution. They were then suspended in a volume of this solution equal to the original volume of whole blood. The microbiologic assay was carried out using Euglena gracilis and Ochromonas malhamensis as described by us on previous occasions.[2] Table I gives the values obtained with euglena, which showed excellent agreement with the ochromonas values.

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The figures given in Table I show the vitamin B_{12} content for 1 ml. of plasma (p) and the vitamin B_{12} content for 1 ml. of cells (c). The content for the whole blood, given in the next column, is thus

$$t = \frac{c.H + p(100 - H)}{100}$$

where H is the hematocrit reading.

RESULTS

The determination on three normal subjects shows that their plasma contained two to four

TABLE I

Vitamin B_{12} Content of Plasma, Red Blood Cells and Whole Blood

Subject	Vitamin B_{12} ($\mu\mu$g./ml.)			Hematocrit (%)
	Plasma	Red Blood Cells	Whole Blood	
Normal Patients				
1	620	170	425	44
2	600	140	410	42
3	320	185	260	47
Patients with Pernicious Anemia				
1	32	115	45	15
2	23	95	35	15
3	76	30	67	20
4	34	55	38	20
Gastrectomized Patient				
1	50	165	75	20
Average of abnormal cases	43	92	52	—

times as much vitamin B_{12} as the cells, volume per volume. In the patients with pernicious anemia (and in the gastrectomized patient) the situation is reversed; the cellular vitamin B_{12} level is, on the average, about 90 $\mu\mu$g. as compared with 165 $\mu\mu$g. in the normal subjects. The plasma level dropped from an average of 500 $\mu\mu$g. in normal subjects to about one-twelfth this value; namely 40 $\mu\mu$g. in the patients with pernicious anemia. With the exception of Case 3 (pernicious anemia) there is actually more vitamin B_{12} in 1 ml. of cells than in 1 ml. of plasma.

In order to learn the ratio between plasma and cells in 1 ml. of whole blood, it is necessary to multiply the figures given in Table I, "c" with the hematocrit reading and "p" with (100 − hematocrit), respectively. Accordingly, in normal serum the cells carry, in general, not more than one-fifth to one-sixth of the total vitamin B_{12}; in pernicious anemia their share is between one-third and almost one-half.

COMMENTS

It is surprising that cells survive at such low vitamin B_{12} levels in the plasma, even when it is considered that their number is substantially reduced. The relatively high proportion of vitamin B_{12} in the cells of the patients with pernicious anemia suggests that vitamin B_{12} used for red cell formation and maturation is efficiently drawn upon, even when the pool of the vitamin is severely depleted. The abnormal cytologic picture in pernicious anemia may be explained by there being enough vitamin B_{12} to initiate cell formation, but not enough for the cells to mature normally. Only plasma well supplied with vitamin B_{12} supports the normal maturation process, in other words the two systems are not independent from each other.

In pernicious anemia, the bone marrow competes with other tissues for the limited store of vitamin B_{12}. When nerve tissue becomes depleted of the vitamin, subacute combined degeneration of the spinal cord ensues. Treatment of pernicious anemia with folic acid may induce both a hematologic remission and subacute combined degeneration of the spinal cord. This suggests that the small store of vitamin B_{12} is mobilized by the action of folic acid towards the bone marrow, alleviating its deficiency, but markedly aggravating vitamin B_{12} depletion in other tissues.

We do not wish at this time to speculate about the mechanism by which this is accomplished; evidently, the cells contain a strong binding factor. Considering this observation from the teleologic point of view, it serves as a reminder of the conservation of protein during starvation in the most vital organs, brain and heart, while muscles and viscera become depleted, or, in a more closely related field the retention of vitamin B_{12} in the fetus in preference to the maternal organism.[3,4]

SUMMARY

Plasma levels reflect closely the vitamin B_{12} supply of the tissues, even though the blood cells from patients with pernicious anemia often contain more than half as much vitamin B_{12} as do normal cells.

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