

The Assay of Vitamin B₁₂ in Blood Serum and Urine

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Of the microorganisms at present in use for vitamin B₁₂-assay, the following have been tested:

1. *Euglena gracilis* var. *bacillaris*. The method described by Ross¹ has been somewhat modified. The inoculum is washed and the chlorophyll determined photometrically after 10 days incubation.

2. *Escherichia coli* 113-3. The medium described by Burkholder² has been used. The glucose is autoclaved and added separately and the incubation time is 24 hours.

3. *Ochromonas malhamensis*. The method described by Ford³ has been used with an incubation time of 5 days.

Specificity. In the presence of methionine, *E. coli* does not require vitamin B₁₂ as a growth factor. Only exceptionally the serum-concentration of methionine is so high as to interfere with the vitamin B₁₂-assay. Some of the vitamin B₁₂-like compounds, obtained from Dr. Ford, have been tested for growth activity. The results, which are given in Table 1, show the high specificity of *Ochromonas malhamensis* compared to the others.

Table 1.

	<i>Eugl. grac.</i> var. <i>bacill.</i>	<i>E. coli</i> 113-3	<i>Ochrom.</i> <i>malham.</i>
Cryst. vitamin B ₁₂ (cyanocobalamin)	100	100	100
Factor A	77	50	0
Factor B	13	26	0
Pseudovitamin B ₁₂	27	5	0
Vitamin B ₁₂ III (Bernhauer)	91	79	32

Sensitivity. A vitamin B₁₂-conc. of 0.5 μg/ml is sufficient for stimulating growth of *Euglena gracilis*. The corresponding values for *E. coli* and *Ochromonas malhamensis* are 5 and 2.5 μg/ml, respectively. Because of the need of a method of maximum sensitivity for the assay of vitamin B₁₂ in serum and urine, *Euglena gracilis* has been used in this investigation.

Results. The normal range changes with age from relatively high values at birth to lower conc. during the first years. During youth high values are found and in senescence relatively low conc. compared to the serum-conc. of vitamin B₁₂ in middle age. Very low conc. of vitamin B₁₂ in serum and urine have been found in pernicious anemia in relapse. Extremely high values have been demonstrated in chronic myelocytic leucemia. Normal to moderately increased values have been found in cases of polycythemia, osteosclerotic anemia, Hodgkin's disease, cancer metastases of the bone-marrow, hepatic cirrhosis and diabetes mellitus. In acute leucemia, chronic lymphocytic leucemia and myeloma normal values have been found. The excretion in the urine of vitamin B₁₂ is normal in all cases except pernicious anemia. The vitamin B₁₂-assay has been shown to be of great value in establishing the diagnosis of pernicious anemia in relapse at a very early stage and especially of subacute combined degeneration of the cord without characteristic findings in the blood and bone-marrow.

1. Ross, G. I. M. *J. Clin. Pathol.* 5 (1952) 250.
2. Burkholder, P. R. *Science* 114 (1951) 459.
3. Ford, J. E. *Brit. J. Nutrition* 7 (1953) 299.

Hemoglobin Formation in the Rat Liver Nucleus

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The nucleus of the liver cell contains a large part of the iron content of the cell. A few hours after intraperitoneal injection of ⁵⁹Fe a considerable part of this iron is found in the liver cell nucleus. Part of this iron is due to the presence of hemoglobin iron.

The presence of hemoglobin iron in the nucleus is not due to contamination with hemoglobin of the circulating blood as the specific activity of the latter is appreciably lower than that of the hemoglobin present in the nucleus.