

waves of increased amplitude. In shock irreversible to transfusion there occurs a progressive decrease in cortical electrical activity which is shown on the electroencephalogram as a continued reduction in the frequency and voltage (amplitude) of the wave pattern. The effects on the electroencephalogram of blood

transfusion in reversible and irreversible hemorrhagic shock are discussed. It is tentatively suggested that the electroencephalographic response to blood transfusions in shock may be used as a means of differentiating the reversible and irreversible forms.

Received November 8, 1949. P.S.E.B.M., 1949, v72.

Vitamin B_{12b}: Some Properties and its Therapeutic Use.* (17528)

H. LICHTMAN, J. WATSON, V. GINSBERG, J. V. PIERCE, E. L. R. STOKSTAD, AND
T. H. JUKES

From the Department of Medicine, Kings County Hospital, Brooklyn, N. Y., and Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

The preparation of two red fractions from liver extract,(1) both clinically active in pernicious anemia, was reported by Smith who suggested that one fraction might arise from the other by proteolysis. In later publications(2,3) it was reported that paper chromatography enabled 2 red factors to be recognized in liver extract. Both of these were

active for *L. lactis* Dorner. In our investigations it was found that concentrated liver extract yielded a pink fraction by chromatography with silicic acid. Upon purification, this fraction yielded a red pigment with an absorption spectrum differing from that of vitamin B₁₂. An apparently similar fraction was obtained from cultures of *Streptomyces aureo-*



FIG. 1.
Crystals of vitamin B_{12b} $\times 425$.

* Paper presented at the Eastern Section of the American Federation for Clinical Research at Boston, Mass., on Dec. 3, 1940.

The expenses of this investigation were defrayed by a Lederle grant.

1. Smith, E. L., *Nature*, 1948, v161, 638.
2. Smith, E. L., and Parker, L. F. J., *Proc. Biochem. Soc.*, May 29, 1948, p. viii.
3. Cuthbertson, W. F. J., and Smith, E. L., *Proc. Biochem. Soc.*, January 22, 1948.

TABLE I.
Vitamin B_{12b}: Hematological Response in Patients with Addisonian Pernicious Anemia.

Case No.	1	2	3	4	5
Vit. B _{12b} , γ , I.M. daily	1	1	1	1.5	2
Initial RBC, mil/cmm	1.90	2.60	1.74	1.75	1.47
Day of reticulocyte peak	8	8	9	7	9
Max. reticulocytes, %	11.4	14.0	18.0	8.3	15.1
Expected max. retic., %	18	5	18	18	24
RBC, mil/cmm, at 3 wk	3.06*	4.36	2.84	3.17	2.95
Expected RBC, at 3 wk	3.11*	3.43	3.19	3.29	3.17

* At 2½ wk.

faciens and red crystals of a biologically active compound "vitamin B_{12b}"(4) were obtained from this fraction. The crystals of vitamin B_{12b} are shown in Fig. 1. In the present investigation crystalline vitamin B_{12b} was prepared from cultures of *S. aureofaciens* by absorption on charcoal, precipitation with ammonium sulfate, chromatography upon silicic acid columns and crystallization from acetone-water mixtures. The vitamin was dissolved in 0.9% sodium chloride solution at pH 7.0. The solution was sterilized by filtration through a Seitz filter. The final concentration of vitamin B_{12b} was adjusted to 10 μ g per ml.

Clinical Observations. The solution of crystalline B_{12b} containing 10 μ g per ml was used for assay in 5 patients with Addisonian pernicious anemia. All patients had megaloblastic bone marrows and histamine-fast achlorhydria. The age range was 55 to 75 years. Vitamin B_{12b} was administered by intramuscular injection daily for 21 days in the dosage schedule given in Table I. Hematologic response seemed to be optimal whether the amount given was 1, 1.5, or 2 micrograms. One patient had a higher reticulocyte peak than expected, one the same and three somewhat lower. The relative unreliability of the reticulocyte count as a quantitative index of the formation of red blood cells has been recently emphasized by several observers.(5,6) In contrast to the fluctuation of the reticulocyte peak in relation to that of the expected

standard, the erythrocyte regeneration was within maximal limits in all 5 patients at the end of 21 days of therapy. It thus seems that 1 to 2 μ g of vitamin B_{12b} is hemopoietically equivalent to 1 U.S.P. unit of liver extract and is then comparable to the potency of vitamin B₁₂.(6,7)

The detailed hematologic data of Case 2 who showed the most dramatic response are presented in Fig. 1. Therapy resulted in the usual reversal of the megaloblastic marrows to normal normoblastic cellularity. The white blood cell counts rose from leukopenic to normal figures with a loss of hypersegmentation of the neutrophils. In the only patient (Case 3) who has been maintained longer than 3 weeks on vitamin B_{12b}, the red cell count has risen to a nearly normal level at the end of 7 weeks.

Clinical improvement was also very satisfactory, beginning on the 3rd, 4th or 5th day of treatment, and was characterized by improved appetite and a sense of well-being. Severe glossitis in one patient completely subsided within 10 days. Neurological symptoms such as paresthesias disappeared in all cases. A patient who had advanced combined system disease had striking improvement within 3 weeks, including the reversal of a strongly positive Romberg's sign.

Oral administration of 5 μ g of vitamin B_{12b} daily for 10 days was undertaken with a 6th patient with Addisonian pernicious anemia, having an initial red cell count of 2,090,000 per cmm. There was no response, the reticulocytes varying from 0.4% to 1.4%. The patient was then given the same therapy with

4. Pierce, J., Page, A. C., Jr., Stokstad, E. L. R., and Jukes, T. H., *J. Am. Chem. Soc.*, 1949, v71, 2952.

5. Clark, G. W., *Am. J. Med. Sci.*, 1948, v216, 71.

6. Jones, E., Darby, W. J., and Totter, J. R., *Blood*, 1949, v4, 827.

7. West, R., and Reisner, E. H., *Am. J. Med.*, 1949, v6, 643.

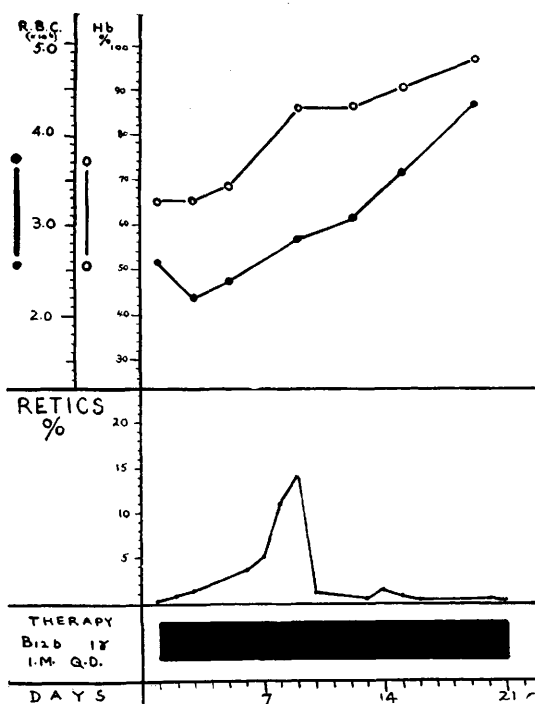


FIG. 2.

Hematological response of a patient with Addisonian pernicious anemia to vitamin B_{12b}. (Case 2).

the simultaneous addition of 150 ml of neutralized gastric juice daily for 12 days. The reticulocytes began to rise slowly on the 8th day to reach a peak of 3.4% on the 11th day. There was a concomitant red blood cell rise of 240,000 per cmm during this time, and slight but definite clinical improvement occurred. This is similar to the results obtained by Berk *et al.*(8) using vitamin B₁₂.

We observed one patient with megaloblastic anemia of pregnancy who had failed to respond to 3 weeks of therapy with 150 U.S.P. units of refined liver extract intramuscularly, and also showed no hematologic response to 1 µg daily of vitamin B_{12b} given intramuscularly in a 10 day period. The severe glossitis, unaffected by liver therapy, cleared completely on B_{12b}, and later there was a maximal hematologic response to pteroylglutamic acid. Several similar vitamin B₁₂ refractory cases have been reported. The problem of different types of deficiencies occurring in megaloblastic anemias other than Addisonian pernicious anemia has been recently reviewed by Mueller *et al.*(9)

Summary. A pink fraction obtained from liver and from *Streptomyces aureofaciens* has been separated from vitamin B₁₂ by silicic acid chromatography and has been shown to have different ultraviolet and visible absorption spectra. This new substance, vitamin B_{12b}, has been prepared in crystalline form and has been found to be effective parenterally in the treatment of patients with Addisonian pernicious anemia in amounts of 1 to 2 µg daily.

We are indebted to Mrs. Helen Jakubowski for performing the blood examination.

8. Berk, L., Castle, W. B., Welch, A. D., Heinle, R. W., Anker, R., and Epstein, M., *New England J. Med.*, 1948, v239, 911.

9. Mueller, J. F., Hawkins, V. R., and Vilter, R. W., *Blood*, 1949, v4, 1117.

Received November 9, 1949. P.S.E.B.M., 1949, v72.

Sterols and Fatty Acids in the Nutrition of Entozoic Amoebae in Cultures.* (17529)

A. M. GRIFFIN AND W. G. MCCARTEN

From the Department of Bacteriology, Hygiene and Preventive Medicine, George Washington University, Washington, D. C.

The many excellent media which have been

* Part of a project supported by a grant from the Division of Research Grants and Fellowships of the U. S. Public Health Service.

suggested for culture of entozoic amoebae suffer from one serious deficiency when used for quantitative studies. Statistically satisfactory reproduction of results is the exception rather