

were on a diet deficient only in the vitamin B complex. No demonstrable differences were observed by the 2 methods of study. We are now certain that the formalin fixation, previous to the use of polarized light, is quite a dependable procedure.

In addition to the above, we have studied, to date, 2 polyneuritic and 3 complex groups, involving a total of 32 animals, 16 pathological and an equal number of controls. The most regular and marked myelin degeneration was found in the sciatic and trigeminal nerves. About 50% of the avitaminotic animals showed a slight degeneration of the spinal cord. The optic nerves were normal in all pathological animals suffering from either polyneuritis or a deficiency of the vitamin B complex. The extent of loss of weight had no direct relationship to the severity of nerve degeneration. Nerve degeneration has been observed in animals that did not develop paralysis on the vitamin B complex deficient diet.

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Bisulphite Binding Substances in the Blood in Health and in Disease, Particularly Vitamin B₁ Deficiency.

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Bisulphite binding substances (B.B.S.) in the blood have been reported increased in vitamin B₁ deficiencies both in experimental animals¹ and in clinical "wet beriberi".² The present study of the B.B.S. in the blood was undertaken to determine its value in distinguishing vitamin B₁ deficiencies from other disease states.

Oxalated blood samples were taken fasting and at rest from 30 healthy controls, and from 110 patients. The method of Clift and Cook³ was modified to adapt its use to trichloroacetic acid filtrates of whole blood. Five milliliters of oxalated blood were precipitated with 20 ml. of 10% trichloroacetic acid, allowed to stand 30 minutes and centrifuged. Five milliliter aliquots of the supernatant liquid were adjusted to pH 2 by the addition of 1.5 ml. of normal sodium

¹ Thompson, R. H. S., and Johnson, R. E., *Biochem. J.*, 1935, **29**, 694.

² Platt, B. S., and Lu, G. D., *Quart. J. Med.*, 1936, **5**, 355.

³ Clift, F. P., and Cook, R. P., *Biochem. J.*, 1932, **26**, 1788.

hydroxide and allowed to react with 0.2 ml. of saturated sodium bisulphite solution for 15 minutes. Twenty-five milliliters of distilled water and 2 ml. of fresh starch solution were added and the excess of bisulphite titrated out with normal and N/10 iodine solutions adjusting the end point with N/200 iodine solution and N/100 sodium thiosulphate solution. The bound bisulphite was released by the addition of 2 gm. of solid disodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$) and titrated within 5 minutes with N/200 iodine solution. The values were expressed in milligrams of pyruvic acid per 100 ml. of blood, using the expression: 1 ml. N/200 iodine = 0.22 mg. pyruvic acid. In most cases the non-protein nitrogen, the plasma carbon dioxide capacity and blood sugar were measured on the same samples. In a smaller group the urine was tested for sugar, acetone, acetoacetic acid and pyruvic acid.

Results. Thirty normal subjects had values for B.B.S. in the blood ranging between 3.66 and 5.75 mg. with an average of 4.74 mg. per 100 ml. Values for B.B.S. above 6.0 mg. are accepted as elevated. Normal values were found in the following conditions: arteriosclerosis, compensated heart disease, carcinomatosis, glomerulonephritis, hepatitis with jaundice, severe anemia, toxemia of pregnancy, acute alcoholism, chronic alcoholism without vitamin deficiency, chronic alcoholism with vitamin (B₁) deficiency after treatment. Increase in the B.B.S. was found in the following diseases: decompensated heart disease, unregulated diabetes, vascular nephritis, lobar pneumonia and certain other febrile diseases, chronic alcoholism with vitamin (B₁) deficiency untreated, terminal uremia, pancreatitis, and pernicious vomiting. Among the factors usually associated with an elevation in the B.B.S. were acidosis, ketosis, anoxemia, "toxemia" and uremia. In certain cases of "vitamin B₁ deficiency" and of diabetes, however, the elevation in the B.B.S. could not be related to these factors. In both of these conditions abnormal amounts of pyruvic acid were found in the urine in a few of the cases in which it was studied. Whether or not the elevation of the B.B.S. in these conditions represents an elevation also of the pyruvic acid in the blood awaits further observations.

Conclusions. An elevation of the B.B.S. in the blood is found in a variety of different diseases, including vitamin B₁ deficiencies. Since vitamin B₁ deficiencies rarely occur without the possible complication of one or more of the other diseases in which there may be an elevation of the B.B.S., we have not found the B.B.S. in the blood of aid in diagnosing vitamin B₁ deficiency.