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Avitaminosis. XVIII. Peripheral Nerves in Vitamin B₁ Deficiency as Observed by Polarized Light.*

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Although the literature is full of reports on nerve degeneration in avian polyneuritis and B deficiency in the rat and other mammals, the most recent contributions in this field reveal conflicting evidence.¹ In order to circumvent uncertainties in the interpretation of Marchi preparations and any possible artefacts that may be introduced during fixation and staining, Sutton, Setterfield and Krauss² have recently applied the polarized light technique for the study of degeneration of myelinated nerves in vitamin A deficiency. This technique was found by Setterfield and Baird³ to have advantages over the Marchi method of staining with osmic acid, since it does not depend upon fixation and staining but on the chemical structure of the myelin sheath; moreover, it is more rapid, more sensitive and constant.

The polarized light technique, however, as employed by Sutton and associates requires previous fixation of tissues in formalin, which procedure may introduce artefacts. We have applied the technique of Sutton and co-workers in studies of vitamin B deficiency and have checked the formalin procedure by elimination of all fixatives as well as all staining operations. Small pieces of spinal cord, trigeminal, sciatic and optic nerves from avitaminotic and litter mate controls on the same plane of nutrition, were fixed in 95% alcohol in liquid air, and dehydrated *in vacuo*, at $-32^{\circ}\text{C}.$, by the Altmann-Gersh-freezing-drying technique, as modified by Scott and Williams.⁴ Frozen sections were made and compared under polarized light with those fixed in 10% formalin. Twelve groups of rats were used for this comparative study. The pathological animals of 9 groups were on diets deficient only in vitamin B₁ and had varying degrees of polyneuritis, ranging from slight to marked paralysis. The avitaminotic animals of the rest of the groups

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¹ Prickett, C. O., *Am. J. Physiol.*, 1934, **107**, 459; Zimmerman, H. M., *Arch. Path.*, 1932, **13**, 207; Duncan, D., *Arch. Neurol. and Psychiat.*, 1931, **25**, 327.

² Sutton, T. S., Setterfield, H. E., and Krauss, W. E., *Ohio Agr. Exp. Sta. Bull.*, No. 545, Dec., 1934.

³ Setterfield, H. E., and Baird, T. T., *Stain Technology*, 1936, **11**, 41.

⁴ Scott, G. H., and Williams, P. S., *Anat. Rec.*, 1936, **66**, 475.

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.