

the extreme hypotonicity of heart-lung-kidney urine, and its low chloride content, have been difficult to interpret. The urine in this preparation differs in chloride content so markedly from that in the intact animal that the reasons for the difference must be elucidated before the isolated perfused kidney results can be applied to normal renal physiology. The findings reported here indicate that the hypotonic urine may be due, in part, to a relatively low glomerular filtration rate caused by greater constriction in afferent than in efferent arterioles.

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**A Comparison of Some Methods for the Extraction of Vitamin B<sub>1</sub> from International Standard Acid Clay.**

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Williams and co-workers<sup>1</sup> in their description of an improved method for obtaining crystalline vitamin B<sub>1</sub> from rice polish have stated that by using barium hydroxide to extract the vitamin from activated fullers' earth no more than a 50% recovery could be obtained. For this reason they chose to use quinine sulfate to displace the vitamin from fullers' earth. Recently Kinnersley and Peters<sup>2</sup> have reported that by the use of a baryta extraction method, vitamin B<sub>1</sub> can be quantitatively removed from the acid clay used as the international standard. However, they made no comparison of the baryta method of extraction with the quinine extraction method proposed by Williams. In this communication we present the results of our comparison of the Williams' extraction method with other proposed methods together with some data on the potency of crystalline vitamin B<sub>1</sub> in terms of international units.

The method of assay used throughout this work was the Ammerman and Waterman<sup>3</sup> modification of the Smith curative procedure. In most cases the test substance was injected subcutaneously since as reported by Kinnersley and Peters and confirmed by us there is no appreciable difference in the response of the animal to oral or

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<sup>1</sup> Williams, Waterman and Keresztesy, *J. Am. Chem. Soc.*, 1934, **56**, 1187.

<sup>2</sup> Kinnersley and Peters, *Biochem. J.*, 1936, **30**, 985.

<sup>3</sup> Ammerman and Waterman, *J. Nutrition*, 1935, **10**, 25.

subcutaneous administration. We considered as the curative dose that amount of substance which would produce complete relief of severe symptoms in 60-80% of the test animals and keep these animals free from convulsions for 4 or more days.

Extractions of the acid clay were made using the Williams method, the Kinnersley and Peters method and an alkaline methyl alcohol method suggested by Smith and Seidell.<sup>4</sup> These extracts together with the untreated acid clay and crystalline vitamin B<sub>1</sub> were administered to polyneuritic rats. The results are given in Table I.

TABLE I.  
Curative Dose of International Standard, Extracted and Unextracted, and of Crystalline Vitamin B<sub>1</sub>.

Substance	Form administered	Curative dose	No. rats used	No. rats cured	% cured
		mg.			
Int. St'd for B <sub>1</sub>	Untreated	20	25	17	68
" " " "	Extracted—M. I. Smith technic	20	9	6	67
" " " "	" Kinnersley & Peters	18	10	6	60
" " " "	" Williams <i>et al.</i>	10	30	19	63
Crystalline B <sub>1</sub>	Aqueous solution	0.005	55	40	73
" "	Quinine sulfate solution	0.005	20	15	75

It is apparent that according to our criterion the curative dose of the international standard is 20 mg. Likewise the curative doses of the extracts prepared either by the baryta method of Kinnersley and Peters or by the alkaline methyl alcohol method of Smith are approximately the equivalent of 20 mg. of the acid clay. These results are in close agreement with the published findings of these investigators. However, when an extract prepared by the Williams quinine sulfate procedure is administered to polyneuritic rats the curative dose is equivalent to 10 mg. of acid clay. Thus it is evident that quinine sulfate liberates from the acid clay approximately twice as much vitamin as is extracted by the other procedures. This difference is not due to a potentiating influence of quinine for we have found that the quantity of crystalline vitamin B<sub>1</sub> necessary to cure polyneuritis in rats is practically the same whether administered in aqueous solution or in a solution to which 0.5% of quinine sulfate has been added.

Within the past few months there have appeared several papers reporting on the potency of crystalline vitamin B<sub>1</sub> hydrochloride in terms of international units. Waterman and Ammerman,<sup>5</sup> using a rat growth assay method, found 200 international units per milli-

<sup>4</sup> Smith and Seidell, *U. S. Public Health Reports*, 1936, **51**, 685.

<sup>5</sup> Waterman and Ammerman, *J. Nutrition*, 1935, **10**, 35.

gram; Moll,<sup>6</sup> using both the pigeon day dose method and a rat curative method, reports 500 international units per milligram; Smith, by his rat curative method, finds 333 international units per milligram; B. C. Jansen,<sup>7</sup> using a modified Smith curative method, finds approximately 300 international units per milligram; and Kinnersley and Peters, by their pigeon curative method, interpreted in terms of their catatorulin test report 500 international units per milligram.

Our own findings approximate either 400 international units per milligram or 200 international units per milligram, depending upon whether the untreated international standard clay or its quinine sulfate extract are used for comparison. The larger figure of 400 international units per milligram falls generally within the range reported by others using a curative technic, whereas, the lower figure of 200 international units per milligram is in close agreement with the findings obtained by employing a growth method of assay. This may be interpreted as indicating that only 50% of the vitamin present in the standard adsorbate product is available to the severely depleted polyneuritic rats, whereas the total vitamin effect is obtained on the less severely depleted animals used in a growth test.

The fact that the quinine sulfate extract of the international standard is apparently twice as potent as either the untreated clay or its alkaline extracts indicates that the form in which this substance is administered will largely determine the response. It would seem, therefore, that any estimation of vitamin activity in terms of the present international unit must be weighted in terms of the method of assay employed; before such a comparison is made it should be determined that the vitamin present in both standard and test substance is available to the test animal to the same extent.

*Summary.* A comparison of different methods of removing vitamin B<sub>1</sub> from the international standard clay shows that the quinine sulfate method yields approximately twice the amount of vitamin that is obtained by alkaline extraction methods.

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<sup>6</sup> Moll, *E. Merck Jahresbericht*, 1935, **49**, 56.

<sup>7</sup> Jansen, *Z. für Vitaminforschung*, 1936, **5**, 254.