Quantitative Measurement of Vitamin B₁ by the Thiochrome Reaction

IN 1936, Jansen¹ first suggested that vitamin B₁ could be measured quantitatively by conversion into thiochrome with alkaline ferricyanide and measurement of the fluorescence of the thiochrome. Since that time, information has been collected on the use of this method for the assay of vitamin B₁ in urine^{2,3,4} and in other materials including foodstuffs⁵. Evidence, however, is now accumulating that, although results which compare closely with the biological assay can be obtained with crystalline vitamin B₁ and acid-clay adsorbates of the vitamin when the precautions described elsewhere⁵ are exercised, the assessment by the chemical method on certain foodstuffs is significantly lower than the biological results.

Biological assay (Int. units per gm.)	Chemical assay (Int. units per gm.)	Chemical/Biological
9.5 10.0 10.0 10.0 10.5 Avg. = 10.0	6.8 6.3 7.9 8.1 7.6 Avg. = 7.3	73 per cent
11.0 11.0 11.0 11.2 11.2 11.2 11.5 11.5 11.5 Avg. = 11.2	6.8 7.2 8.1 6.7 6.9 8.6 6.2 6.4 6.4 Avg. = 7.0	
12.0 12.0 12.0 12.5 12.5 12.5 12.5 12.5 Avg. = 12.3	5.1 6.5 7.6 6.7 7.2 7.3 7.5 8.6 Avg. = 7.1	58 "
14.0 15.0 15.0 16.0 16.2 20.0 Avg. = 16.0	6.8 6.7 6.2 6.6 5.9 8.1 Avg. = 6.7	

The accompanying table gives the comparative figures for vitamin B₁ in twenty-eight samples of wheat embryo. The biological assays were carried out by Dr. A. Z. Baker of this laboratory by the bradycardia method⁶. A minimum of six rats was used in each case.

Other foodstuffs appear to give similar results. Certain proprietary yeast preparations, for example, contain substances with a considerable blue fluorescence, and frequently no additional intensity of fluorescence develops after ferricyanide oxidation. The chemical assay therefore appears to be zero, although the usual activity of 6–10 international units per gram of vitamin B₁ is known to be present.

Vitamin B₁-pyrophosphate, which appears to be of fundamental biological significance, is insoluble in isobutyl alcohol⁷ and is not therefore included in the chemical assay. It has also recently been suggested⁸ that vitamin B₁ exists combined with protein, in which form it might be expected to be difficult to extract chemically from tissues. Therefore the quantitative recovery of crystalline vitamin B₁ added to a substance under analysis cannot be taken as evidence that the results obtained for that substance by the thiochrome method are a complete measure of the vitamin activity of the material.

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¹ Jansen, B. C. P., *Rec. Trav. chim.*, **55**, 1046 (1936).

² Goudsmit, J., and Westenbrink, H. G. K., *NATURE*, **139**, 1108 (1937).

³ Westenbrink, H. G. K., and Goudsmit, J., *Rec. Trav. chim.*, **56**, 803 (1937).

⁴ Karrer, W., *Helv. chim. Acta*, **20**, 1147 (1937).

⁵ Pyke, M. A., *Biochem. J.*, **31**, 1958 (1937).

⁶ Birch, T. W., and Harris, L. J., *Biochem. J.*, **28**, 602 (1934).

⁷ Kinnersley, H. W., and Peters, R. A., *Biochem. J.*, **32**, 697 (1938).

⁸ Sinclair, H. M., *Chem. and Ind.*, **57**, 471 (1938).

Scotopic Luminosity Curve and the Absorption Spectrum of Visual Purple

MANY attempts have been made to explain the discrepancy between the scotopic luminosity curve and the absorption spectrum of visual purple. Recently, Dartnall and Goodeve¹ have applied the Stark-Einstein law in order to express the luminosity curve in terms of quanta and have shown that the resulting correction was insufficient.

A consideration of various additional possible factors, such as Kundt's rule and the retinal grey, which might explain the discrepancy, convinced me that the scotopic luminosity curve should be corrected for the absorption spectrum of the ocular media. It is known that this spectrum varies considerably with the genus of animal, and for man has not hitherto been determined through the visible spectrum. Recently, however, in collaboration with E. F. McCarthy, I completed measurements of the absorption spectrum in the wave-length range 400–820 mμ for the refractive media of the human eye. Preliminary reports² of this work have already been made and a paper giving the results will appear shortly³.

It will suffice here to give the results obtained by applying the Stark-Einstein law and data on the young normal human eye to transform a scotopic ocular luminosity curve into a very different curve, namely, a scotopic retinal luminosity curve. This permits the comparison of the absorption spectrum of rhodopsin with the retinal luminosity curve instead of with the ocular luminosity curve.

The accompanying graph shows the agreement of the transformed data of Hecht and Williams⁴ with Lythgoe's data⁵ on the absorption spectrum of visual purple. The conclusions drawn from this curve are: (1) The retinal sensitivity for blue is much greater than has been previously assumed. (2) It is not necessary to invoke Kundt's rule, as did Hecht and Williams⁴, to explain the discrepancy between the