122. VITAMIN METHODS

2. A NOTE ON VITAMIN B₁ IN URINE AS DETERMINED CHEMICALLY AND BIOLOGICALLY

BY LESLIE J. HARRIS AND Y. L. WANG

*From the Dunn Nutritional Laboratory, University of Cambridge
and Medical Research Council*

(Received 31 July 1941)

WANG & HARRIS [1939] described a method for estimating vitamin B₁ in urine by a modification of the thiochrome reaction and found that their results agreed well with those determined biologically. Hills [1939] claims to have improved the procedure, yet states that the results by his method are 30 % lower on the average than those determined by him according to our method. This apparent discrepancy has prompted us to carry out yet another series of biological comparisons on urine. The new determinations increase our confidence in the reliability of our method.

Two urines from normal subjects have been tested, one having a relatively high and the other a low content of vitamin B₁. For the biological determination it was necessary to adsorb the vitamin before dosing. For this purpose the urines were diluted 3–4 times with water, adjusted to pH 4 and treated with 'clarit' clay, 10 g. per 1600 ml. of diluted urine. The urines were analysed by the thiochrome method before and after adsorption, the percentage of the vitamin adsorbed being found by difference. In one instance 86 % was shown to be adsorbed and in the other 82 %. In the biological tests the adsorbates were administered to the rats directly. As the biological and chemical values both refer to the adsorbate, the small amount lost on adsorption does not enter into the comparison. Normally it would be included in the chemical test, which we carry out on the whole urine without prior adsorption [cf. however, Hills, 1939; Jowett, 1940].

The bradycardia method was used for the biological tests and the results are summarized in Table 1. To make the check as accurate as possible a fairly large number of duplicate doses were tested at each level. To equalize conditions for the rat, the solution of vitamin B₁ used as the standard was not administered as such but was first poured on to specimens of 'clarit' clay identical with those used for the urine adsorbate. The dose-response curve used for calculating the results is given in Fig. 1.

The technique for the thiochrome test was the same as that previously described.

Occasionally, especially with stale specimens, the final matching of fluorescence is found to be difficult. Sometimes, in such instances, a preliminary treatment with permanganate may help to improve the end point by removing the blank fluorescence without significant destruction of the vitamin B₁. This procedure can only be used with safety, however, with urines which are not too strongly reducing, otherwise appreciable destruction of the vitamin does occur. The working directions are as follows: before washing with water-saturated *isobutanol*, add 2 drops of conc. H₂SO₄ to 5 ml. of the urine. Then add a 1 % solution of KMnO₄ drop by drop with continual stirring, allowing complete decoloration before the next drop is added. Discontinue the addition when a faint pink colour appears which persists for about 1 min. Add one drop of 0.5 % H₂O₂ solution and continue as in the usual procedure.

Table 1. *Biological tests*

Sample	Vit. B ₁ content of urine, i.u./ml. by thio- chrome test	No. of i.u. administered according to thio- chrome test	Days cured		Activity as read from reference curve		Biological value found as % of chemical value
			Individual animals	Av.	Per dose given	Per 1 i.u. given	
Urine 6, M. L. W.	0.154	1	2.25, 0, 1.5, 1.25, 1.25, 2	1.38	1.25	1.25	110
		2	1.25, 1.75, 2.25, 3.25, 2.25, 2	2.17	2.09	1.05	
		3	5.75, 2.5, 3, 1.25, 2.5	3.00	3.00	1.00	
					Av. 1.10		
Urine 7, W. Y. L.	0.40	1	2.25, 0, 1.5, 1.25, 1.25, 1.5	1.29	1.15	1.15	101
		2	2.5, 2.25, 2.25, 1.5, 2, 1.5, 2	2.00	1.90	0.95	
		3	3.75, 2.5, 3, 2.75, 2.5, 2.25	2.79	2.75	0.92	
					Av. 1.01		
Inter- national standard	—	1	0, 1.25, 0, 2, 1, 1.25, 2, 1.25	1.09			
		2	2.25, 3, 1.25, 2, 2, 4, 3.75, 1.25, 1.5	2.33			
		3	2.75, 3, 2.25, 3, 3, 1.25, 2.5, 3.75, 4.5, 3.25, 1.5	2.82

Fig. 1. Dose-response curve. ○ = Standard. × = Urine No. 6. + = Urine No. 7.

Agreement between the chemical and biological findings (Table 1) seems very good. For one urine the biological value is 110% of the chemical and for the other it is 101%. In the earlier tests [Wang & Harris, 1939], on 5 specimens of urine, the biological values were 100, 121, 100, 89 and 128% of the chemical values, making an average of 108%, or an average of 107% for all 7 specimens in the two investigations.

That slightly lower results are given by the thiochrome test than by the biological test accords with our general experience of other animal and plant materials and of foodstuffs [Harris & Wang, 1941]. Methods like those of Hills which give still lower chemical results may therefore be expected to be all the more in error—viz. to the extent of more than 30 %, to judge from the differences between the results given by the two methods as published by Hills.

Jowett, who criticized the uncertainties in the method of Hills, diminished the error by substituting synthetic zeolite for activated earth. But even with this improvement, various possible sources of error remain—including incomplete adsorption and elution—and as Jowett himself concedes, his procedure, although convenient and simple, will not estimate low excretions accurately.

As an additional check we have carried out further recovery tests in which known amounts of vitamin B₁ were added to urine. The recovery has always been virtually complete (Table 2).

Table 2. *Recovery of vitamin B₁ added to normal urines*

Specimen	Vitamin B ₁ present μg. per ml.	Additional vitamin B ₁ added μg. per ml.	Additional vitamin B ₁ found μg. per ml.	Recovery %
Urine 1	0.118	0.16	0.15	94
	0.118	0.16	0.15	94
Urine 2	0.472	0.32	0.36	112
Urine 3	0.378	0.32	0.31	97
	0.408	0.32	0.287	90
Urine 4	0.0812	0.32	0.291	91

Since this note was written, a study by Slater [1941] has also appeared. His findings accord excellently with our own. He concludes: 'the thiochrome method of Wang & Harris can be satisfactorily applied to the determination of thiamin in milk and cereal products [and urine]... Criticisms by Hills [1939] of the Wang & Harris method of determining thiamin in urine appear to be unjustified... The recovery of added thiamin is quantitative in all cases.'

SUMMARY

A further comparison has been made of the results obtained by the improved thiochrome method of Wang & Harris with those obtained by biological tests (bradycardia method). The agreement is good. On one specimen of urine the biological value was 110 % of the chemical and on another 101 %; in earlier tests the values were 100, 121, 100, 89, 128 %; average for both series is 107 %. Recovery tests confirm the reliability of the method.

REFERENCES

- Harris & Wang (1941). *Biochem. J.* **35**, 1050.
 Hills (1939). *Biochem. J.* **33**, 1966.
 Jowett (1940). *Biochem. J.* **34**, 1348.
 Slater (1941). *Aust. J. exp. Biol. med. Sci.* **19**, 29.
 Wang & Harris (1939). *Biochem. J.* **33**, 1356.