CCXL. THE CHEMICAL MEASUREMENT OF VITAMIN B₁ IN FOODSTUFFS AND BIOLOGICAL MATERIAL BY MEANS OF THE THIOCHROME REACTION

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WHEN vitamin B₁ is oxidized in an alkaline medium it is converted into thiochrome [Barger *et al.* 1935]. This substance has a vivid blue fluorescence which can be detected in dilutions of 1 : 2,000,000. Recently Jansen [1936] published a quantitative method for the measurement of vitamin B₁ which was dependent on the conversion of the vitamin into thiochrome and the subsequent measurement of fluorescence by means of a photoelectric cell. When it was attempted in this laboratory to apply the method to the measurement of vitamin B₁ in foodstuffs certain difficulties arose. The following paper indicates the limitations of the method and the means which were devised in order to overcome them.

Since the completion of the work reported below, two papers have appeared in which the thiochrome reaction for the measurement of vitamin B₁ has been used.

Karrer & Kubli [1937] have used Jansen's method with very little modification for preparations of high activity (between 500 and 220,000 i.u. per g.) which had been prepared during the production of crystalline, natural and synthetic vitamin B₁. With substances of this high activity, these workers found it more convenient to compare the intensity of fluorescence by eye rather than use the somewhat delicate fluorimeter of Cohen [1935] in which a photoelectric cell is employed. Where vitamin B₁ in foodstuffs is being measured, however, comparison by direct observation is not sufficiently accurate.

Another recent paper by Goudsmit & Westenbrink [1937] reports the use of a procedure almost identical with that devised by the author for the measurement of vitamin B₁ in urine.

EXPERIMENTAL

(1) *Standardization of the reaction with International Standard adsorbate*

The procedure of Jansen [1936] was carried out with very slight modification. 0.1 ml. of 1% K₃Fe(CN)₆ was required to oxidize each 10 mg. of acid clay. This solution was mixed with 2 ml. redistilled methyl alcohol and 1 ml. 30% NaOH, and the accurately weighed and finely powdered International Standard added. The reagents were shaken for a few moments and made up to 5 ml. with water, and 13 ml. of *isobutyl* alcohol were added. The mixture was again vigorously shaken and finally centrifuged.

The measurement of fluorescence was carried out on the Cohen fluorimeter. The blank reading obtained with 10 ml. of the original *isobutyl* alcohol was subtracted from the galvanometer deflexion given by exactly 10 ml. of *isobutyl* alcohol solution. Compensation for any variation in the intensity of the

fluorimeter lamp was obtained by reading the deflexion given by 10 ml. of a solution containing 0.81 mg. quinine sulphate in 100 ml. $N/10$ H_2SO_4 . This deflexion was always considered to be 100 and readings obtained from solutions of thiochrome were calculated back to this basis.

It can be seen from Fig. 1 that a consistent straight line relationship was obtained with graded amounts of vitamin B₁.

Fig. 1. Relationship between vitamin B₁ and intensity of fluorescence.

Probable error of readings. In a series of determinations of the deflexion given by 20 mg. of International Standard (I.S.) the following results were obtained: 9.0, 9.0, 9.0, 9.0, 9.3, 9.3, 9.5, 9.5, 9.7, 9.8, 9.8, 9.9, 9.9, 10.0, 10.2, 10.5, 10.5, 11.0, 11.0, 12.0, 12.0.

Number of determinations = 21.

Average = 10.0.

Probable error = 0.54, i.e. 5.4%.

Karrer & Kubli [1937] report figures varying between 17 and 22 for 20 mg. of I.S. which compare closely with the figures reported above.

Several precautions are necessary in carrying out the determinations. For example, the solution of ferricyanide is only stable for a day or so, after which free cyanide is given off. It is also necessary to renew the solution of quinine sulphate at adequate intervals since a slow deterioration has been found to occur.

(2) Assay of fuller's earth concentrates of vitamin B₁

A series of concentrates of vitamin B₁ extracted from rice polishings and adsorbed on fuller's earth was assayed. A procedure identical with that adopted for the I.S. substance was followed except that the amount of ferricyanide to be used had to be determined by trial in each case. Usually the amount of $K_3Fe(CN)_6$ required to give the maximum fluorescence was larger than that needed for the I.S.

Sample	Chemical assay units per g.	Biological assay* units per g.
1/2	58	80
2/4	83	110
12/36	90	100
1/37	140	140
45	140	160
T	170	200
47 ii	194	205
42	200	185
47 i	200	215
44	230	180

* All the biological assays were carried out by Dr A. Z. Baker of this Laboratory.

In this case the average difference between the chemical and the biological assays amounted to 15.3 %. Since, however, the biological method of assay is only accurate to within 20 % [Leong & Harris, 1937] these results appear to be satisfactory.

(3) *Measurement of a solution of pure vitamin*

The procedure again was identical with that described above except that in this case the amount of $K_3Fe(CN)_6$ required was very much less. A satisfactory straight-line graph was obtained with increasing amounts of vitamin, and the assay of the sample of vitamin employed gave the figure of 404 I.U. per mg. This compares with the published figures of Leong & Harris [1937] of 330 units and confirms very closely the determination of Karrer & Kubli of 440 units per mg.

(4) *Measurement of vitamin B₁ in foodstuffs*

It was considered essential for the success of any chemical method of assay that the manipulation should be as simple as possible in order to avoid the loss of vitamin probable in a series of precipitations and filtrations.

The first substance taken was wheat germ. At the outset attempts were made to assay this foodstuff by mixing the powdered germ with a mixture of methyl alcohol, alkali and ferricyanide and extracting with *isobutyl* alcohol. Although reasonable deflexions were given for 200 mg. weights of germ, proportionately larger readings could not be obtained for larger amounts. Another disturbing discovery was that germ heated for 24 hr. at 120° or material autoclaved for 4 hr. at one atmosphere pressure produced a strong fluorescence equivalent to 26 and 70 units of vitamin B₁ per g. respectively. In order to overcome this non-specific fluorescence the device was adopted of running a blank by treating the sample with all the reagents except the ferricyanide. This device is identical with the procedure now published by Goudsmit & Westenbrink for the assay of urine. By following this procedure, reasonable results were obtained for small amounts but again complete extraction could not be obtained with larger quantities. However, when a simple aqueous extract was employed instead of the solid material good results were at once achieved. The method finally adopted was as follows.

Method

The material is finely ground or minced and placed in a solution of 1 % HCl. The mixture is vigorously stirred and just brought to the boil. It is then allowed to cool and made up to a convenient volume.

Four 3 ml. aliquots of the filtered extract are then added to a mixture of 2 ml. methyl alcohol, 1 ml. 30 % NaOH and 0, 0.8, 1.0 and 1.2 ml. 1 % $K_3Fe(CN)_6$ respectively. The solutions are well mixed and then shaken with 13 ml. *isobutyl* alcohol and centrifuged. 10 ml. of the *isobutyl* alcohol layers are taken for the measurement of fluorescence. The fluorescence given by the

solution to which no K₃Fe(CN)₆ was added is subtracted from the fluorescence of the others and the difference multiplied by 100 is divided by the deflexion recorded for the standard quinine solution. If the three replicates containing ferricyanide do not agree closely, a different range of ferricyanide must be tried until maximum fluorescence is obtained.

The reliability of the method was tested on the following substances.

(a) *Assay of milk.* 5 ml. aliquots of milk were treated directly with the reagents as detailed above. To a second 25 ml. of milk a solution containing 10 units of pure vitamin B₁ was added:

Milk alone = 0.25 unit per ml.
Milk + vitamin B₁ = 0.66 unit per ml.
Recovery of added B₁ = 102 %

Published analysis [Baker & Wright, 1935] = 0.23 unit per ml.

(b) *Assay of ham.* 24 g. of ham brought to the boil in 80 ml. of 1 % acid and aliquots tested. To a second 22 g. of ham 33 units of pure vitamin B₁ were added:

Ham alone = 2.4 units per g.
Ham + vitamin B₁ = 3.9 units per g.
Recovery of added B₁ = 100 %

Published biological analysis = 2.2 units per g.

(c) *Assay of wheat germ.* 5 g. of germ were finely ground and brought to the boil in 100 ml. of 1 % HCl. 3 ml. aliquots of the filtered solution were tested. To a second 5 g. of germ 45 units of pure vitamin B₁ were added.

Wheat germ alone = 10.7 units per g.
Germ + vitamin B₁ = 20.0 units per g.
Recovery of added B₁ = 103 %

Published biological analysis [Baker & Wright, 1937] = 6–20 units per g.

(5) *Analysis of other foodstuffs*

Material	Chemical determination units per g.	Published biological assay [Baker & Wright, 1935] units per g.
Dried brewer's yeast	13	6–23
Egg white	Nil	Trace
Egg yolk	1.6	1.4
Rice bran	5.4	5.6–7.4
Wheat germ A	5.6	5.9–18.7
Wheat germ B	8.0	

(6) *Vitamin B₁ in animal tissues*

Material	Chemical assay units per g.	Published biological assay [Leong, 1937] units per g.
Rat liver	0.8	1.5
Rat kidney	0.2	0.2
Rat muscle	0.6	0.4

(7) *Measurement of vitamin B₁ in urine*

The amount of vitamin B₁ in urine is too small for it to be possible to measure it directly on the sample, and concentration by adsorption on fuller's earth was adopted. The following experiments were carried out to check the validity of the analysis.

(a) 1 ml. of a solution of pure vitamin B₁ containing 11 units was diluted to 100 ml. and made acid to Congo red with HCl. 100 mg. of fuller's earth were added

and shaken for 10 min. The adsorbate was dried with alcohol and ether and 20 mg. amounts were submitted to the chemical procedure.

$$\begin{aligned}\text{Vitamin B}_1 \text{ recovered} &= 9.3; 12.3 \text{ units} \\ &= 10.8 \text{ (average)} \\ &= 98\% \text{ recovery}\end{aligned}$$

(b) 100 ml. of urine were brought to pH 4.5 with HCl and treated with 1 g. of fuller's earth. 20 mg. aliquots of the dried adsorbate were assayed. A second 100 ml. of urine to which 23 units of vitamin B₁ had been added was similarly treated. The adsorbates from each solution were dried and 20 mg. portions tested.

	Activity of fuller's earth units per g.
Urine alone	10
Urine + 23 units vit. B ₁	35
Recovery	110%

(8) *Variation in the concentration of vitamin B₁ in different samples of milk*

The chemical determination of vitamin B₁ in milk may be simply carried out as was detailed above. Since milk is a foodstuff of great nutritional importance the analysis of several samples was carried out.

Source	Nature	Vitamin B ₁ units per ml.
Sherborne, Dorset	Raw	0.11
Mevagissey, Cornwall	"	0.06
London, W. 6	Pasteurized	0.17
London, S.E. 16	"	0.12
London, W. 6	"	0.12
Deptford	"	0.12
London, S.W. 10	"	0.11
London combine (a)	"	0.18
London combine (b)	"	0.14
London, S.E. 1	Sterilized	0.04

A point which may not be without significance is the fact that the sample used previously to verify the method was found to contain 0.25 unit of vitamin B₁ per ml. This milk was examined in May whereas the samples given above were produced at the end of July and August after an exceptionally dry summer.

DISCUSSION

From the above results it appears that the chemical method for determining vitamin B₁ by its conversion into thiochrome and the measurement of the fluorescence of the latter substance is suitable for application to a wide range of materials. While the probable error of the method is low compared with other methods of vitamin assay and while in most cases the recovery of added vitamin is good, a great deal depends on the satisfactory behaviour of the fluorimeter. In the early stages of this work progress was slow because the magnitude of the galvanometer deflections was too small for reliance to be placed on the readings obtained. Vibrations in the building and rapid fluctuations in the intensity of the light source must also be guarded against. If, however, care is taken in the use of the instrument, and if a blank determination is run without ferricyanide on each unknown material, the thiochrome method of Jansen promises to be of great importance in the investigation of vitamin B₁.

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