CCXLIX. THE ESTIMATION OF VITAMIN B₁ IN BLOOD

II. A FURTHER MODIFICATION OF MEIKLEJOHN'S METHOD

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MEIKLEJOHN [1937] described a method, based upon the work of Schopfer, of estimating vitamin B₁ in blood. The method uses the ability of the vitamin to promote the growth of a fungus, *Phycomyces blakesleeanus*. I have already shown that his claim "that the method... provides a quantitative estimate of the true vitamin B₁ content of the blood" is not justified because blood invariably contains substances other than vitamin B₁ that affect the growth of the fungus [Sinclair, 1938]. These substances fall into two groups. First, the method is not specific for vitamin B₁, since other substances can replace the vitamin as a growth factor. Such substances include the degradation products of the vitamin and certain compounds very closely related to these. They may be important since they can be produced by heating the vitamin in a slightly alkaline medium; for instance, the vitamin might be destroyed by cooking, but if a sample of blood was drawn shortly after the cooked food was eaten, the pyrimidine and thiazole components of the vitamin might be present in blood and so give a falsely high estimate by the test. Work using other fungi is in progress to test the importance of this possible error. In the tests that I have done during the last 3 years it has been avoided as far as possible by collecting most of the samples of blood at least 2 hr. after food was eaten. This paper is concerned with the second group of substances, namely those that have an adjuvant or inhibitory effect upon the growth of the fungus under the conditions of the test, but that cannot replace vitamin B₁ as a growth factor. I have shown that the adjuvant action of blood "is probably due to more than one factor; sources of nitrogen and salts in the blood, the buffering power of the blood and the more solid medium produced when blood is added, particularly in large amounts, probably all contribute to it. Under certain conditions, blood may be shown to have an inhibitory action on the growth of the fungus" [Sinclair, 1938]. One simple way of showing the presence of adjuvant substances was to add vitamin B₁ to blood: addition of small amounts of the vitamin usually produced a greater growth than expected and addition of excess vitamin invariably did so.

If one assumes that the growth produced by a given amount of vitamin B₁ in the presence of blood is always in the same ratio to the growth produced by the same amount of vitamin B₁ in the absence of blood, one can correct for the adjuvant action of blood by multiplying the weight of fungus obtained in the presence of blood by the factor:

$$\frac{\text{weight of fungus obtained with excess vitamin B}_1}{\text{weight of fungus obtained with excess vitamin B}_1 + \text{blood}}$$

The crucial test of this assumption is to add known amounts of vitamin B₁ to blood from which the vitamin has been removed; but it has as yet proved

impossible to destroy the vitamin in blood without also affecting the adjuvant factor. It will be shown below that discrepancies obtained with Meiklejohn's method are eliminated when the correction is applied to allow for the adjuvant action of blood, using samples of blood that contain widely different amounts of vitamin B₁ (3–12 $\mu\text{g.}/100\text{ ml.}$), and adding various further amounts of the vitamin (0.025–0.2 $\mu\text{g.}$) to different volumes of blood (0.5–5.0 ml.). These results would appear to justify the assumption.

EXPERIMENTAL

The technique of the experiments has already been described [Sinclair, 1938]. In that paper I showed that samples of 1, 2 and 3 ml. blood usually did not give the same values per unit volume. Determinations upon samples of human blood from 235 different cases were then quoted. The number of relevant samples has now been increased to 425; the 3 ml. value (expressed in $\mu\text{g.}/100\text{ ml.}$ blood) has been at least 1 $\mu\text{g.}$ higher than the 1 ml. value in 61 %, at least 1 $\mu\text{g.}$ lower in 14 %; in 54 samples the growth with 3 ml. blood was greater than that given by excess vitamin. When the values are corrected, the different volumes of blood give approximately the same values per unit volume (Table I). In

Table I. *Comparison of corrected and uncorrected estimates of vitamin B₁ ($\mu\text{g.}/100\text{ ml.}$) obtained with different volumes of blood*

	Vol. of blood ml.	Sample of blood										
		1	2	3	4	5	6	7	8	9	10	11
Uncorrected	1	8	10	7.5	12.5	14.5	17	7.5	12	9	10.5	5
	2	9.5	11	8	—	20	13	16	14	10.5	—	7.5
	3	10.5	—	13.5	20.5	?	?	?	?	18	20	7.5
Corrected	1	7	7.5	3.5	11	7.5	12	7	8	6.5	9	4
	2	7	7	3	—	7.5	10	6.5	9	6.5	—	4.5
	3	8.5	—	3	10	7.5	12	7	8.5	8	10	4.5

* In some cases the growth of fungus was greater than that obtained with excess of added vitamin in absence of blood. In such cases it is not possible to translate the weights of growth into apparent amounts of vitamin B₁ and a question-mark has been placed in the table to indicate this.

Table I uncorrected values are compared with the corresponding corrected ones; the mean of the uncorrected 3 ml. values is 15 $\mu\text{g.}/100\text{ ml.}$ and the mean of the corresponding 1 ml. values is 8.8; the means of the 3 and 1 ml. corrected values are 7.9 and 7.6 respectively.

In my previous paper I showed that adding small amounts of vitamin B₁ to blood usually produced a greater growth than was expected. When the weights of fungus are corrected this discrepancy disappears. The results of six typical experiments are summarized in Table II, in which the figures in column *a* represent the apparent amount of vitamin B₁ in the blood (expressed in $\mu\text{g.}/100\text{ ml.}$ blood) estimated by subtracting the amount of added vitamin from the estimated total vitamin content; the figures in column *b* represent the corresponding corrected values. It will be seen that whereas the apparent values may vary widely, the corrected values agree within the limits of experimental error. The degree of variation is indicated by the standard deviation of the individual observations in each column. But in the case of the uncorrected values, it must be remembered that some growths of fungus were too large to be translated into apparent amounts of vitamin B₁; in these cases the estimate of the variation is therefore a minimal one.

Table II. *Effect of added vitamin B₁ upon the estimated vitamin content of different samples of blood (column a, uncorrected values; column b corrected values, $\mu\text{g.}/100\text{ ml.}$)*

Blood ml.	Added vitamin B ₁ $\mu\text{g.}$	Sample of blood											
		1		2		3		4		5		6	
		a	b	a	b	a	b	a	b	a	b	a	b
0.5	0	11	9	—	—	—	—	—	—	—	—	—	—
0.5	0.05	9	6	—	—	—	—	—	—	—	—	—	—
0.5	0.1	6	2	—	—	—	—	—	—	—	—	—	—
0.5	0.15	8.5	2	—	—	—	—	—	—	—	—	—	—
0.5	0.2	15	7	—	—	—	—	—	—	—	—	—	—
1.0	0	8	7	10	7.5	7.5	3.5	12.5	11	14.5	7.5	5	4
1.0	0.025	—	—	11	7.5	8	3	14.5	10.5	—	—	—	—
1.0	0.05	10	8	11.5	7	9	3	16	12	—	—	5.5	4
1.0	0.1	8.5	6	16	9	11.5	2	15	12	40	8	8.5	4
1.0	0.15	13	8.5	31	11.5	—	—	—	—	—	—	—	—
1.0	0.2	18	6	?	11	—	—	—	—	—	—	—	—
2.0	0	9.5	7	11	7	8	3	—	—	20	7.5	7.5	4.5
2.0	0.025	—	—	13	8	9	3	—	—	—	—	—	—
2.0	0.05	10.5	7.5	19	8.5	11	3	—	—	—	—	8	6
2.0	0.1	9	6	?	9.5	16	2	—	—	?	8	—	—
2.0	0.15	8.5	5.5	—	—	—	—	—	—	—	—	—	—
2.0	0.2	7	4	?	9	—	—	—	—	—	—	—	—
3.0	0	10.5	8.5	?	—	13.5	3	20.5	10	?	7.5	7.5	4.5
3.0	0.05	9.5	7.5	?	—	—	—	?	9.5	?	7.5	—	—
3.0	0.1	9.5	7	?	—	—	—	?	10	?	8	?	5.5
3.0	0.2	?	5.5	?	—	—	—	—	—	—	—	—	—
4.0	0	?	7.5	?	—	11.5	2.5	—	—	?	9	—	—
4.0	0.05	?	6.5	?	—	—	—	—	—	—	—	—	—
4.0	0.1	?	5.5	?	—	—	—	—	—	?	8	—	—
5.0	0	?	5.5	—	—	—	—	—	—	?	—	—	—
5.0	0.05	?	5.5	—	—	—	—	—	—	—	—	—	—
Mean		10.4	6.3	15.3	8.7	10.5	2.8	15.7	10.7	24.8	7.9	7.0	4.6
σ		2.8	1.8	7.0	1.6	2.7	0.5	3.0	0.9	13.4	0.5	1.4	0.8

A further experiment upon human blood is recorded in detail in Table III in order to illustrate the method of calculation and the variation between duplicate estimations. Again it will be seen that the corrected values agree extremely well considering the experimental error: for instance, the only bad duplicate estimations are the values of 6 and 12 $\mu\text{g.}/100\text{ ml.}$ obtained with 0.1 $\mu\text{g.}$ vitamin B₁ added to 0.5 ml. blood; but the difference in the corrected weights that produces this large difference in the values is only 6 mg.

A further fact is illustrated in Tables II and III. I have previously shown that "large samples (more than 3 ml.) of blood (to which no vitamin has been added) usually give a growth that is greater than that obtained with excess vitamin B₁" [Sinclair, 1938]. When the weight of this growth is corrected it invariably becomes less than that obtained with excess vitamin, and so the vitamin content of the blood can be estimated. Tables II and III contain examples of this. The largest value for vitamin B₁ in human blood that I have obtained in about 500 estimations was given by a case of myeloid leukaemia. The weights of fungus (mg.) were as follows:

Excess vitamin B ₁ (2.5 $\mu\text{g.}$)	...	...	90.8
1 ml. blood	...	...	122.7
1 ml. blood + excess vitamin B ₁	...	...	160.8
2 ml. blood	...	...	143.3
3 ml. blood	...	...	105.2

Table III. *Effect of added vitamin B₁ upon the estimated vitamin B₁ content of human blood*

Blood ml.	Added vitamin B ₁ μ g.	Wt. of fungus mg.	Vitamin equivalent μ g.	Apparent vitamin content of blood μ g./100 ml.	Corrected wt. of fungus mg.	Vitamin equivalent μ g.	True vitamin content of blood μ g./100 ml.
0	2.5	100.5 99.6	2.5	—	—	—	—
0.5	0	20.0 18.9	0.05 0.045	10 9	18.3 17.3	0.04 0.04	8 8
0.5	0.05	39.3 37.0	0.1 0.095	10 9	36.0 34.0	0.09 0.085	8 7
0.5	0.1	56.3 49.7	0.185 0.15	17 10	51.6 45.6	0.16 0.13	12 6
0.5	2.5	111.1 107.1	> 2.5	—	—	—	—
1.0	0	32.2 29.8	0.08 0.07	8 7	28.4 26.3	0.07 0.065	7 6.5
1.0	0.05	51.4 48.4	0.16 0.14	11 9	45.4 42.7	0.13 0.12	8 7
1.0	0.1	68.7 68.2	0.25 0.25	15 15	60.6 60.3	0.205 0.205	10.5 10.5
1.0	0.2	80.6 80.0	0.345 0.34	14.5 14	71.3 70.7	0.27 0.27	7 7
1.0	2.5	113.6 112.8	> 2.5	—	—	—	—
2.0	0	78.0 78.0	0.32 0.32	16 16	45.6 45.6	0.13 0.13	6.5 6.5
2.0	0.05	98.0 94.8	2.5	?	57.3 55.5	0.19 0.18	7 6.5
2.0	0.1	119.8 116.7	> 2.5	?	70.1 68.4	0.26 0.25	8 7.5
2.0	0.15	124.3	> 2.5	?	72.8	0.28	6.5
2.0	0.2	140.8	> 2.5	?	82.4	0.36	8
2.0	2.5	172.6 169.6	> 2.5	—	—	—	—
				Mean	11.9		Mean
				σ	3.3		σ
							7.7
							1.4

Obviously Meiklejohn's method gives no estimate of the amount of vitamin in the blood, since without added vitamin the samples of blood gave greater growths than that obtained with excess vitamin alone. But the corrected weight for 1 ml. blood is $122.7 \times \frac{90.8}{160.8} = 69.4$ mg., and from the growth-vitamin curve for this experiment, this weight is equivalent to about 0.41 μ g. vitamin B₁. The true amount of total vitamin B₁ in the blood is therefore about 41 μ g./100 ml. Independently the cocarboxylase (vitamin B₁ diphosphate ester) in the sample was estimated by Dr Robert Goodhart; the amount was 42 μ g./100 ml. Since almost all the vitamin in the blood of cases of myeloid leukaemia is in the phosphorylated form [Goodhart & Sinclair, 1939, 2], these values—one obtained by a chemical and the other by a biological method—agree well.

These results support the claim that a correction can be applied to allow for the adjuvant action of blood, the correction being based, as described above, upon the increase in weight of fungus produced by blood in presence of excess vitamin B₁. I believe that if the precautions described elsewhere are borne in

mind [Sinclair, 1938], the results obtained by Meiklejohn's method can be corrected to give an approximately true estimate of the total vitamin B₁ in blood.

The volume of blood tested

To the clinical procedure of estimating the degree of saturation of the body with vitamin B₁ by assaying the vitamin in blood, certain other facts are relevant. Meiklejohn [1937] recommended that duplicate estimations of the vitamin should be done on 1, 2 and 3 ml. samples of blood, and on a 2 ml. sample to which 0.1 μ g. vitamin B₁ was added. This requires 16 ml. blood. I have criticized the adding of 0.1 μ g. vitamin B₁ to 2 ml. blood because the values obtained (about 0.3 μ g. with normal human blood) are too large to be read accurately from the growth-vitamin curve [Sinclair, 1938]. The 3 ml. sample should always be omitted; it gives values that tend to be higher (expressed per unit volume) than those given by the smaller samples and therefore if it is omitted in a particular case because 16 ml. blood cannot be obtained the estimate of the vitamin content of the blood will probably be falsely low when compared with average values obtained with 1, 2 and 3 ml. samples. Further, the medium that contains 3 ml. blood usually becomes solid after tyndallization at pH 6.5, and it is then possible that all the vitamin is not available for the fungus. Other things being equal, the most accurate estimate is obtained with amounts of blood producing the largest growths of fungus that fall on the steep part of the growth-vitamin curve. The corresponding amount of vitamin is about 0.1 μ g. The best method of translating weights of fungus into amounts of vitamin B₁ is to plot the results obtained with the control series of flasks on a logarithmic scale. If this is done it is found that the logarithm of the weight of fungus is directly proportional to the logarithm of the amount of vitamin B₁ present between 0.05 and 0.25 μ g. vitamin B₁; the mid-point of the straight line corresponds to 0.12 μ g. This happens to be approximately the mean apparent value for vitamin in 1 ml. normal human blood. Therefore the apparent values for normal blood can, other things being equal, be estimated with greatest accuracy in 1 ml. blood. In the case of the corrected values, the mean ($\pm \sigma$) for vitamin B₁ in normal human blood is $7.4 \pm 1.4 \mu\text{g./100 ml.}$ Apparent values of 7 or less and true values of 4.5 or less may be regarded as being subnormal (see below). These values are the critical ones in estimations that are designed to show whether a given sample of blood contains a normal or subnormal amount of the vitamin. In terms of 2 ml. blood, these values become 0.14 and 0.09 μ g. respectively, and these figures fall on the steep part of the curve. It seems, therefore, that the most satisfactory test, in estimations that are designed to show whether a sample of blood contains an abnormally small amount of vitamin, is obtained with 2 ml. samples, with and without excess vitamin B₁ added; for duplicate estimations this requires a minimum of 8 ml. blood. In addition, a useful check is provided by doing similar determinations upon 1 ml. blood, without addition of vitamin, with 0.1 μ g. vitamin and with excess. Since the true values (expressed per unit volume of blood) are approximately the same with 1, 2 and 3 ml. samples of blood, it is legitimate to compare average values obtained on different samples of blood, even if the average values are not obtained with samples of the same volume. This is not true of the uncorrected values; with these, average values obtained with samples of the same volume must be compared. In the estimations that I have done during the last 3 years, the 3 ml. sample has sometimes, and the 2 ml. sample occasionally, been omitted. I shall therefore quote in this paper and papers now in preparation only values obtained with 1 ml. samples of blood.

The amount of vitamin B₁ in normal human blood

Few figures have been published for the amount of vitamin B₁ in normal human blood. To illustrate a particular point, Meiklejohn [1937] included in his paper results obtained with different volumes of human blood from six selected cases. The values ranged from 6.5 to 14.0 $\mu\text{g.}/100\text{ ml.}$ These figures, of course, were not intended to indicate the amount of vitamin in normal human blood; the lowest figure was obtained with blood from a case of malignant cachexia [Meiklejohn, personal communication]. Laurent & Sinclair [1938] published the results of two analyses of the apparent vitamin B₁ in blood, made by a modification of Meiklejohn's method, upon a patient with peripheral neuritis associated with pyloric stenosis; they stated that "a value below 7 $\mu\text{g.}$ per 100 ml. is regarded as being abnormal, and, out of about 200 determinations made by one of us (H. M. S.) on normal and pathological samples of blood, the first (4 $\mu\text{g.}$ per 100 ml.) is the lowest value that has been encountered".

Later, Rowlands & Wilkinson [1938] published a paper on "The clinical significance and estimation of blood vitamin B₁." Their paper has been misinterpreted, since subsequent writers have concluded, for instance, that Rowlands & Wilkinson were led by Schopfer's discovery "to devise a technique for the estimation of the vitamin in human blood", whereas in fact they appear to have used Meiklejohn's method without modification. They stated: "We have employed Schopfer's technique and applied it to the estimation of the vitamin B₁ content of human blood; our method is very similar to that since described by Meiklejohn [1937], details of which were kindly made available to us by Prof. R. A. Peters. The test we used was as follows." There follows a description of Meiklejohn's method without modification or further acknowledgement, and a list of experimental evidence in support of the method nearly all of which was originally put forward by Meiklejohn (whose name is mentioned in connexion with one item only—tests on deficient pigeons). This paper by Rowlands & Wilkinson is not considered in any detail here because their results, as they subsequently admitted, are statistically worthless (*Brit. med. J.* 1938, 2, 1060, 1110), but it may be noticed that in one part of their paper they regarded a figure of 5.5 $\mu\text{g.}/100\text{ ml.}$ as "gross deficiency" and elsewhere a figure below this value was considered a "normal value".

Recently Lehmann & Nielsen [1939] have applied Meiklejohn's method without modification, but state that a detailed account and criticism of his method will appear in another paper. They found that the values for 36 normal men ranged from 7 to 13 $\mu\text{g.}/100\text{ ml.}$, and for 10 normal women from 8 to 12; the average for each group was 9 $\mu\text{g.}/100\text{ ml.}$, but no statistical analysis is given. Morell [1938], in a paper entitled "Ein neuer biologischer Vitamin B₁-Test", comments at length on the paper of Rowlands & Wilkinson and describes what is in fact Meiklejohn's test without any reference to him; nothing new is added by this paper. Guhr [1939] includes Meiklejohn's paper in his bibliography but does not refer to it in his text; when he states that the work of Schopfer & Jung has been extended by English investigations, he mentions only "Rowland und Wilkinson" and proceeds to describe what is in fact Meiklejohn's method. Guhr's results are, as he admits, surprising. The amount of vitamin B₁ in the blood of ten normal subjects varied from 8 to 10 $\mu\text{g.}/100\text{ ml.}$; but six normal hospital sisters, who had no signs or symptoms of deficiency of vitamin B₁, gave very low values: one gave 0 $\mu\text{g.}/100\text{ ml.}$ on one occasion and "traces" on another; four others gave "traces" and the sixth sister gave a value of 5 $\mu\text{g.}/100\text{ ml.}$ Five patients, none of whom might be expected to be deficient, gave values

between 3.8 and 10.8 $\mu\text{g.}/100\text{ ml.}$ (the mean $\pm\sigma$ may be calculated to be 7.3 ± 2.6); 19 pregnant or puerperal women gave values that ranged from 0 (4 cases) or "traces" (6 cases) up to 18 $\mu\text{g.}/100\text{ ml.}$ Guhr concludes that the physiological variation of vitamin B₁ in the blood can be very wide, and normal people may have no vitamin B₁ in their blood—a remarkable discovery.

The thiochrome method of Jansen has been applied to whole blood or plasma [Jansen, 1938]. Various modifications of the method have been introduced. According to Schneider & Burger [1938] the average amount of vitamin B₁ in normal serum is 6.4 $\mu\text{g.}/100\text{ ml.}$; their values on 12 subjects ranged from 3.8 to 10.0; from their figures the mean ($\pm\sigma$) may be calculated to be 6.4 ± 1.9 . Widenbauer *et al.* [1939] obtained a mean value of 7 $\mu\text{g.}/100\text{ ml.}$ on serum from 20 normal subjects of various ages; their values ranged from 2 to 11, but as the individual figures are not given σ cannot be calculated. Ritsert [1939] obtained a range of 3–15 $\mu\text{g.}/100\text{ ml.}$ blood from 12 normal persons; the mean ($\pm\sigma$) may be calculated to be 7.9 ± 3.9 . Therefore from his figures the range within which 95 out of 100 observations might be expected to fall is 0.1–15.7 $\mu\text{g.}/100\text{ ml.}$, or if the values are expressed to the nearest 0.5 $\mu\text{g.}$, a value of zero cannot be considered abnormally low; if the number of observations (12) is taken into consideration when interpreting the statistics, a value of zero is certainly not significantly low. Ritsert was unable to find cocarboxylase in blood. Hennessy & Cerecedo [1939] have published a modification of the thiochrome method that will estimate either free vitamin B₁ or cocarboxylase. They quote no figures for the latter in blood, but give the range 9–12 $\mu\text{g.}/100\text{ ml.}$ for the former; the number of observations is not recorded. Benacchio [1939] obtained values of 3.6 and 3.2 $\mu\text{g.}/100\text{ ml.}$ serum. Gaehtgens [1939] has also published values obtained by the thiochrome method, but I have been unable to obtain his book.

Recently Heyns [1939] has described a yeast-test which is applicable to blood; no actual values for human blood are given, but two tests with this showed practically no total vitamin B₁. Goodhart & Sinclair [1939, 1, 2] have described a method for the estimation of cocarboxylase in blood, based upon the method of Ochoa & Peters [1938]. On 26 healthy adults they obtained a mean value ($\pm\sigma$) of $7.0\pm2.1\text{ }\mu\text{g.}/100\text{ ml.}$ Because most of the vitamin in blood is phosphorylated and associated with the corpuscles the blood cell count is of importance in considering the amount of vitamin B₁ in blood [Sinclair, 1939, 1], and values for plasma or serum tend to be unreliable because they are greatly affected by slight haemolysis of the blood [Jansen, 1938]. By a combination of the method described by Goodhart & Sinclair and of the modification of Meiklejohn's method described in the present paper, both total vitamin B₁ and cocarboxylase may be estimated on 10 ml. blood; by these methods the metabolism of the vitamin has been partly elucidated [Sinclair, 1939, 2]. Some of the discrepancies between the results mentioned above are probably due to failure to recognize that most of the vitamin in blood is phosphorylated and bound to protein [Sinclair, 1939, 2].

Using the modification of Meiklejohn's method already described [Sinclair, 1938] (which estimates both free vitamin B₁ and cocarboxylase, whether bound or not) the following results have been obtained upon healthy adults during the last 3 years. Forty-four observations upon different samples of my own blood taken throughout this period and under various conditions (such as after fasting for 24 hr. or after being on a diet low in vitamin B₁ for a week) have given apparent values that range between 8.0 and 13.5, with a mean ($\pm\sigma$) of $9.5\pm1.5\text{ }\mu\text{g.}/100\text{ ml.}$ In 15 cases the corresponding true values have been calculated: they range from 5.0 to 9.0 with a mean of $6.2\pm1.1\text{ }\mu\text{g.}/100\text{ ml.}$ A

group of 73 "normal" adults has included 47 males and 26 females. They consisted of healthy medical students, members of the staff of this Department and of a local hospital, and surgical patients (for instance, cases of fractures). Their ages ranged from 17 to 78 years. A statistical analysis of the figures in the male and in the female groups has shown that there is no significant difference between the means of the two groups. Including all the 73 figures together, the apparent values for vitamin B₁ range from 8.0 to 15.0, with a mean of 10.6 ± 1.7 $\mu\text{g./100 ml.}$ Since 95 out of 100 observations may be expected to fall within the range of $\pm 2\sigma$, the range of apparent values in normal adults may be considered to be 7.2–14.0 $\mu\text{g./100 ml.}$, and a value of 7.0 or less can be considered to be abnormally low. The corresponding true values, obtained on 45 of the subjects, ranged from 5.5 to 10.5 with a mean of 7.4 ± 1.4 . The range of true values for vitamin B₁ in normal adults may therefore be considered to be 4.6–10.2 $\mu\text{g./100 ml.}$, and a value of 4.5 or less can be considered to be abnormally low. These results are shown graphically in Fig. 1. It is clear from this figure, and even more obvious

Fig. 1. Vitamin B₁ in the blood of healthy adults. (Circles, apparent values; dots, true values. The lines are drawn at the level mean $- 2\sigma$ calculated from the 73 apparent and 45 true values.)

if the frequency-distribution curve is plotted, that the individual observations are not normally distributed about the arithmetic mean. But in calculating the standard deviation on the assumption of a normal distribution, a greater scatter amongst the low values has been allowed for than in fact occurs, and therefore the lower limit, mean $- 2\sigma$, becomes a more rigid test of the normality of an individual observation.

DISCUSSION

The uselessness of most of the work done so far upon the amount of vitamin B₁ in blood or serum is distressing. The arbitrary way in which Rowlands & Wilkinson [1938] call a figure of 5.5 $\mu\text{g./100 ml.}$ "gross deficiency" when a

figure below this is called a "normal value" has already been mentioned. Apart from workers from this Department, none of the authors concerned have treated their figures statistically, although some of them will doubtless do so when they report their results in greater detail. In the hands of Guhr [1939] Meiklejohn's method seems useless for clinical purposes because a value of 0 for total vitamin B₁ can be obtained with the blood of healthy adults. But I am encouraged to believe that my results are more trustworthy than Guhr's by the fact that Lehmann & Nielsen [1939] have obtained results very similar to mine. The latter authors, who used Meiklejohn's method, obtained a range of 7-14 $\mu\text{g.}/100\text{ ml.}$ upon 46 normal subjects; as already mentioned I obtained a range of 8-15 upon 73 normal subjects. I look forward to the promised publication of a critical analysis of Meiklejohn's method by these authors.

Turning to the thiochrome method, this promises to be useful for whole blood, and even for serum or plasma if great care is taken to avoid haemolysis or contamination with blood cells and if the vitamin is freed from protein by warming or digestion with pepsin. Although the results obtained by Ritsert [1939] for unphosphorylated vitamin B₁ in blood are not encouraging since a value of zero is not significantly low, the modification of Hennessy & Cerecedo [1939] may prove valuable. It is, however, surprising that the latter authors obtained such high values for unphosphorylated vitamin B₁ in blood (9-12 $\mu\text{g.}/100\text{ ml.}$), and still more surprising that Ritsert found no cocarboxylase in blood; in this Department we have found most of the vitamin in blood to be in the phosphorylated form [Goodhart & Sinclair, 1939, 2], and Heyns [1939] has also stated that by far the largest amount of vitamin B₁ occurs in blood in the form of cocarboxylase, although he is probably referring to the blood of animals other than man since his two tests with human blood yielded practically no result. It is also surprising that Schneider & Burger [1938] and Widenbauer *et al.* [1939] obtained average values of between 6 and 7 $\mu\text{g.}$ in 100 ml. serum, using the thiochrome method; Meiklejohn [1937], using a concentration of oxalate that may be haemolytic, found that about 20% of the vitamin B₁ in blood was present in plasma, and I obtained an average value of 1.1 $\mu\text{g.}/100\text{ ml.}$ (i.e. about 14% of the figure for blood) for normal plasma and serum, taking care to avoid haemolysis.

Considering all the above facts, it seems that the total vitamin B₁ in blood or in serum can be most accurately estimated by the modification of Meiklejohn's method that I have described in this paper, and that a value of 4.5 $\mu\text{g.}/100\text{ ml.}$ or less for human blood is significantly low.

SUMMARY

1. A modification of Meiklejohn's method for the estimation of total vitamin B₁ in blood is described, whereby a correction can be made for the adjuvant action of blood upon the growth of the fungus, and the apparent values obtained by the original method can be translated into true values. The method estimates both free vitamin B₁ and cocarboxylase.

2. Samples of 2 ml. blood are the most satisfactory, and the test can be performed in duplicate with a total of 8 ml.

3. Various workers have attempted to assay vitamin B₁ in blood or serum. Their results are mentioned and briefly criticized.

4. The true values for total vitamin B₁ in the blood of healthy adults of both sexes ranged from 5.5 to 10.5 $\mu\text{g.}/100\text{ ml.}$ with a mean ($\pm\sigma$) of 7.4 ± 1.4 . A value of 4.5 or less can be considered to be abnormally low.

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