SUMMARY

The action of H_2O_2 and I_2 on fibrinogen has been studied. Fibrinogen in phosphate buffer solution reduces these substances rapidly at 25° and pH 6.6. The reaction with I_2 is associated with the aromatic residues of the protein. The reducing power of fibrinogen is not changed by denaturation, but the reducing power towards H_2O_2 is increased on conversion to fibrin.

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The Vitamin B_1 Content of Potatoes

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The use of the mould *Phycomyces blakesleeanus* as a test organism for estimating vitamin B_1 was first worked out by Schopfer & Jung [1937b]. The fungus responds to such small quantities of the vitamin that the method is the most sensitive known; on this account it was adapted for estimating the vitamin B_1 content of human blood by A. P. Meiklejohn [1937] and Sinclair [1938; 1939]. The method has been used to estimate vitamin B_1 in plant materials by several American workers [Burkholder & McVeigh, 1940; Robbins, 1939; Bonner & Erickson, 1938]. It has the advantage, as compared with other methods, of requiring such small samples of plant material that it can be used to study the distribution of the vitamin in a single plant or part of a plant.

This paper deals with the use of the *Phycomyces* method for estimating the vitamin B_1 content of potato tubers, and with variations in the vitamin content and its distribution in the potato plant.

METHODS

The method employed was an adaptation, for use with plant material, of the method for blood estimations worked out by Meiklejohn and Sinclair. It consisted essentially in the comparison of the growth of *Phycomyces* in cultures containing potato samples with a dose-response curve obtained from a series of control cultures containing known amounts of the vitamin.

Medium. The *Phycomyces* cultures were grown in 50 ml. conical flasks; each flask contained 4 ml. of a mixture of equal parts of the following solutions in distilled water:

Glucose (A.R.)	Equal weights of solid and water
Asparagine (recrystallized)	4 g./100 ml.
KH_2PO_4 (A.R.)	1.5 g./100 ml.
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (A.R.)	0.5 g./100 ml.

The volume of liquid in each flask was made up to 10 ml. by the addition of vitamin B_1 solution and distilled water, or distilled water only.

Control cultures. A range of 12 control cultures was set up in every experiment, containing the following amounts of vitamin B_1 in 10 ml. liquid: none, 0.05, 0.1*, 0.15, 0.2*, 0.25, 0.3* $\mu\text{g.}$, and excess (2.5 or 5 $\mu\text{g.}$) (quantities marked * in duplicate). A solution of vitamin B_1 containing 1 $\mu\text{g./ml.}$ was freshly prepared for each experiment from a stock solution in 75% ethanol (pH 4.5) kept in a refrigerator. The required volume of the dilute solution was added to medium and water in each flask, immediately before the adjustment of the pH .

Potato samples. The size of sample was designed to give a reading in the central part of the dose-response curve. Preliminary experiments showed that, for potato, samples of between 0.1 and 0.2 g. were correct. According to the amount of vitamin expected, samples were taken of 0.1, 0.15, or 0.2 g. (± 10 mg.), in triplicate from each tuber, or part of a tuber, to be tested, by taking out cores with a small cork borer and cutting off the required length. Skin samples were taken by removing the corky skin and cutting a thin slice off the exposed surface, and sprout and leaf samples by cutting off a small piece. All samples were

quickly weighed and immediately blanched by putting them straight into the liquid medium in the culture flasks, which were held at a temperature of 80–100° in a steamer.

Sterilization. All culture flasks were sterilized by steaming for 45 min. on 3 successive days. Just before the second steaming, the dilute vitamin B₁ solution was added to the control flasks; some of the potato cultures received excess vitamin as well, and this was added at the same time, the pH of the liquid in all flasks being brought to 6.4 with N/20 NaOH. The potato samples were broken up with a glass rod.

Inoculation and incubation. Stock cultures of *P. blakesleeanus* (sex –) were maintained on dextrose-peptone-malt agar, and also on the experimental liquid medium, with potato and excess vitamin B₁. Inocula were made, normally from a liquid culture, by removing the aerial mycelium and stirring it in sterile distilled water. 1 ml. of the resulting spore suspension was pipetted into each culture flask. The flasks were incubated 12–16 days in the dark at room temperature.

Drying and weighing of fungus. At the end of incubation the mats of *Phycomyces* mycelium were removed from the culture flasks, washed 3 times in tap and once in distilled water, dried for 2–3 hr. at 100° and weighed to 0.1 mg.

Calculation of results

A dose-response curve was constructed from the 12 control cultures in every experiment by plotting the dry weight of mycelium in mg. (or the average of duplicates) against the logarithm of the amount of vitamin B₁ (μg.) in each culture. The curves were always S-shaped, and the middle part, corresponding to 0.1–0.25 μg. vitamin B₁, was very nearly a straight line [cf. Sinclair, 1939]. The amount of vitamin in the potato samples was calculated by reading from the curve the figure corresponding to the dry weight of fungus from each sample flask. The results are expressed as μg. vitamin B₁/100 g. fresh weight of potato.

RESULTS

A. Comparison with thiochrome method. The *Phycomyces* method is open to one serious theoretical

objection; Schopfer & Jung [1937a] showed that the fungus responds to the pyrimidine and thiazole halves of vitamin B₁, if they are supplied together, as well as to the intact vitamin. It was therefore thought advisable to check the fungus method by comparing it with the thiochrome method as described by Pyke [1939]. The results of 3 comparisons on tubers of the variety Majestic are given in Table 1; the figures obtained by the 2 methods are of the same order in each case. This makes it reasonably certain that vitamin B₁ itself, and not its decomposition products, was being estimated by the fungus method.

B. Variations during growing season. In both 1941 and 1942, at intervals during the growing season, between July and late September, tubers were dug from plants in the same field and assayed for vitamin B₁. In 1941 the material was obtained from Spalding, Lincolnshire, by Mr P. H. Gregory; the two varieties Majestic and King Edward, growing in adjacent fields, were sampled by taking 1 tuber from each of 4 plants of each variety for each determination. In 1942 the material was obtained from a plot on the Rothamsted farm planted with Scottish seed Majestic; 2 tubers were taken from each of 2 plants at each sampling. Three cores were taken from the middle of each tuber.

From Table 2 it will be seen, first, that the samples are reasonably constant in vitamin B₁ content; the standard errors of the mean values for each sample are small enough to permit a valid comparison between samples.

Secondly, the vitamin B₁ content of the tubers of both varieties increases continuously during the growing season. The figures for Majestic show that the increase continues up to the time when main-crop potatoes are usually lifted, and also show a very good agreement between parallel samples in

Table 1. Comparison of methods for estimation of vitamin B₁

Date	Nature of material	Comparison	Vitamin B ₁ (μg./100 g.)	
			Fungus method	Thiochrome
April 1942	Stored tubers	Different tubers of same batch	76	61
			76	72
May 1942	Seed tubers (sprouted)	Different tubers of same batch	57	54
			46	—
			78	—
July 1942	Freshly dug young tuber	Both tests on same tuber	51	67

Table 2. Variations in vitamin B₁ content of tubers dug in summer

Majestic 1941		King Edward 1941		Majestic 1942	
Date of digging	(μg./100 g.)	Date of digging	(μg./100 g.)	Date of digging	(μg./100 g.)
18. vii. 41	61 ± 1.6	20. vii. 42	63.1 ± 4.0	20. vii. 42	55.6 ± 4.7
9. viii. 41	74.8 ± 3.9	12. viii. 42	67 ± 2.1	12. viii. 42	75.6 ± 5.1
8. ix. 41	91.3 ± 4.6	15. ix. 42	77.3 ± 7.4	15. ix. 42	135.7 ± 10.2
25. ix. 42	141.3 ± 8.7				

Each figure is the mean of 12 determinations: 3 cores each from 4 tubers.

Table 3. *Effect of storage and sprouting on vitamin B₁ content of potatoes*

Date	Where stored	Variety	No. of tubers	Vitamin B ₁ (µg./100 g.)	Variety	No. of tubers	Vitamin B ₁ (µg./100 g.)
25. ix. 41	Crop as lifted	Majestic	4	141.3 ± 8.7	Doon Star	4	163 ± 12.5
2. i. 42	Rothamsted	"	3	162.2 ± 13.0	—	—	—
	Sutton	"	3	138.2 ± 12.3	—	—	—
	Bonington	"	3	138.2 ± 12.3	—	—	—
5. ii. 42	Rothamsted	Majestic (sprouted)	4	112.4 ± 22.2	King Edward (sprouted)	4	46.7 ± 7.8
8. iv. 42	Sutton	"	4	99.9 ± 8.5	"	4	73.7 ± 4.4
	Bonington	"	4	99.9 ± 8.5	"	4	73.7 ± 4.4
7. v. 42	Scottish seed fully sprouted	"	3	61.8 ± 7.0	—	—	—

1941 and 1942. King Edward tubers would appear to have a lower vitamin B₁ content than Majestic tubers.

C. *Effect of storage.* During the winter and spring of 1941–2, samples were taken from tubers stored in two places: at Rothamsted, where they were kept in paper bags on the laboratory floor, and at the Midland Agricultural College, Sutton Bonington, on trays in a well-ventilated storehouse. The cores for vitamin determinations (3 or 4) were again taken from the centre of each tuber (Table 3).

There certainly is a loss of vitamin B₁ from the centre of the tuber during storage, and apparently the greatest loss takes place in spring, after the tubers have begun to sprout. It may be conjectured that the greater part of the loss is due to sprouting, and not to storage *per se*. This conjecture is supported by the gradient in sprouting tubers (see Section D). Unfortunately there are not many results from stored material, and the material in some cases varied considerably in vitamin B₁ content. This is particularly so in the case of the Rothamsted-stored Majestic, as is shown by the high standard error.

D. *Distribution in the plants. Gradients.* It was thought possible that there might be a movement of vitamin B₁ into the rose end of a sprouting tuber, which would result in a gradient in vitamin content within the tuber. At different times of year, therefore, tubers were cut into 3 parts: the rose end, a

middle slice (which is the part usually taken for sampling), and the heel end. Cores were taken from the 3 parts separately for determinations (Table 4).

There is consistently less vitamin in the heel end than in the rest of the tuber, but the evidence for a gradient cannot be regarded as satisfactory. There is, however, a very much higher content in sprouts than in the middle of the tuber, as is shown in Table 5. (These figures were obtained from white sprouts only, as green sprouts showed a curious anomaly, which is discussed in a later section.)

Table 5. *Sprouts compared with centre of tuber*

Date	Variety	Vitamin B ₁ in sprouts (µg./100 g.)	Vitamin B ₁ in tuber (µg./100 g.)
Jan.	Majestic	174	128
Feb.	Majestic	149	20
Feb.	King Edward	250	53

There was always less vitamin in the layer immediately under the corky skin than in the centre of the tuber; comparisons on different single tubers are given in Table 6. The skin layer, however, was always found to show adjuvant action; this is further discussed in a later section of this paper.

Table 6. *Skin compared with centre of tuber*

Date	Variety	Vitamin B ₁ in centre (µg./100 g.)	Vitamin B ₁ in skin layer (µg./100 g.)
Jan.	Majestic	179	22
Feb.	Majestic	206	9
		76	10
Feb.	King Edward	24	21
		49	18
May	Majestic	78	33

There is a very marked gradient of vitamin content in growing potato plants; in June 1942 two seed tubers of Majestic which had been planted a month previously were dug up and assayed together

Table 4. *Distribution of vitamin B₁ within single tubers*

Date	Variety	Condition of tuber	Position	Vitamin B ₁ (µg./100 g.)
May	Majestic	Fully sprouted seed	Heel	38.4
			Middle	77.8
			Rose	67.7
Oct.	Majestic	Freshly dug, no sprouts	Heel	97.9
			Middle	101.7
			Rose	120.1
Oct.	Majestic	Freshly dug, no sprouts	Heel	117.8
			Middle	209.9
			Rose	165.7

with the small plants growing from them, which were then about 4 in. above the ground. Samples were taken from the heel, middle and rose ends of the seed tubers, the white stem below ground, and from the small leaves at the top of the stem. Sampling from 2 whole plants was repeated in July, when the tops were about 18 in. high. In this case samples were taken from the middle of 2 newly developed tubers, from an internode half-way up the stem, from the blades of a lower leaf, and from the terminal bud and the small leaves round it. The averages of 3 determinations at each point are given in Table 7.

Table 7. *Gradients in vitamin B₁ content in growing plants*

Date	Part of plant	Vitamin B ₁ (µg./100 g.)	
		Plant A	Plant B
8 June	Planted seed tuber: heel	25	27
	middle	39	24
	rose	47	40
	White stem below ground	103	93
	Foliage—small leaves	>1200	>1200
20 July		Plant C	Plant D
	Young tubers—middle	53	51
		78	41
	Green stem—internode	9	9
	Lower leaf—blade	129	153
	Terminal bud and leaves round it	>285	>115

In June the planted seed tubers have their highest vitamin B₁ content in the rose end; the stem has a rather larger content, and the value for the tops is enormously high. The amount of mycelial growth in the cultures on foliage was so large as to be right off the curve; the true vitamin content can only be guessed at. In July stems contain very little vitamin and leaves a good deal; but even the terminal buds do not approach the enormously high figure for June foliage.

E. *The presence in potato of adjuvant factors for the growth of Phycomyces.* *Phycomyces* responds to other growth factors than vitamin B₁; Schopfer & Jung [1937b] said that it requires 'une constellation de facteurs auxogènes.' Sinclair [1938] described the 'adjuvant action' of blood; blood and vitamin B₁ supplied to the fungus together produced more growth than the sum of the two separately. He attributes this action to a combination of causes, notably the buffering action exerted by blood, and its nitrogenous constituents.

In every experiment reported in this paper some cultures were grown on potato and excess vitamin, to test for the presence of adjuvant action; such action, shown by excess growth over the sum of potato and vitamin cultures separately, was exhibited by tuber material only in the cases shown in Table 8.

Table 8. *Potato tubers showing adjuvant action in vitamin B₁ test*

Date	Variety	Condition of tuber
1941		
June	Majestic	Fully sprouted seed
July	Majestic	Freshly dug, newly formed
Aug.	Majestic	Freshly dug, newly formed
1942		
Apr.	King Edward	From store, sprouted
June	Majestic	Seed, 1 month planted
July	Majestic	Freshly dug, newly formed
Aug.	Majestic	Freshly dug, newly formed

Mature tubers, from September to April, never displayed any adjuvant action, nor did seed potatoes at the time of planting in 1942. Samples of skin layer (see Table 6) always showed a marked action, but the factor was not present in sprouts. Stem from growing plants (Table 7) showed adjuvant action in 2 cases, but leaves never did.

The adjuvant factor in potato apparently operated only in presence of excess vitamin B₁, as is shown by the experiment reported in Table 9, in which parallel sets of cultures were set up, 1 with a range of concentrations of vitamin B₁ alone (column A), a 2nd with the same range of vitamin concentrations plus potato samples taken from a single tuber (column B), and a 3rd set containing samples from the same tuber without added vitamin (column C). The increased growth due to the addition of potato (B—A) is no larger than that produced by potato alone, except in the presence of excess vitamin (2.5 mg.).

Table 9. *Growth of fungus with potato alone and on potato plus vitamin B₁*

Vita- min B ₁ supplied (µg.)	Fungus growth with vitamin B ₁ only (mg.) A	Fungus growth with vitamin B ₁ + potato (mg.) B	Diff. (B—A)	Growth with potato alone (mg.) C	Adju- vant action
0.05	18.2	51.7	33.5	36.8	None
0.1	31.2	72.2	41.0	42.0	None
0.2	57.2	87.8	30.6	39.2	None
0.3	69.2	103.2	34.0	36.8	None
2.5	89.2	155.6	66.4	—	+

As the adjuvant factor only appears to operate after the need of the fungus for vitamin B₁ has been satisfied, it was not allowed for in calculating the results [cf. Sinclair, 1939], as it was assumed that the vitamin supply was acting as a limiting factor under the conditions of the experiment, so that the growth of the fungus was in fact a measure of the vitamin present.

The adjuvant action of potato does not appear to be due to the same causes as that of blood; it is

not due to buffering; cultures with and without potato both reach a pH value of 4.0 at the end of incubation. Neither is it due to nitrogenous compounds; in the absence of asparagine, potato and excess vitamin give very little growth as compared with blood and excess vitamin. This is seen in Table 10, which also shows the additive effect of potato and blood when supplied together; this is further evidence that the factors in potato and blood are distinct.

Table 10. *Growth of mycelium on blood and on potato*

Vitamin B ₁ (μg.)	Additions to medium			Growth of mycelium	
	Potato (g.)	Blood (ml.)	Asparagine (mg.)	(mg.)	Excess growth in presence of both factors (mg.)
5	—	—	40	77	—
—	0.15	—	40	38	—
—	—	1	40	29	—
5	0.15	—	40	121	6
5	—	1	40	136	30
5	0.15	1	40	188	44
5	0.15	—	—	12	—
5	—	1	—	115	—

It is possible that the adjuvant action of potato observed in these experiments is due to the 'factor Z' described by Robbins [1939]. It is interesting in this connexion that royal jelly from a queen bee cell, which has been reported to be rich in pantothenic acid [Pearson & Burgin, 1941] had no stimulating action whatever upon *P. blakesleeanus*, either in the presence or absence of excess vitamin B₁.

F. *The anomalous action of green sprouts.* White sprouts from potatoes kept in the dark (Table 5) were found to have a high vitamin content. All attempts to repeat this work on green sprouts, however, failed completely. In presence of green sprouts the fungus did not grow at all; there was no visible mycelium, and the pH of the medium was unchanged. A second inoculation of fungus spores into the same flask failed like the first. It seems likely, to judge from the high vitamin B₁ content of white sprouts and young leaves, that green sprouts also have a high content, but this cannot be proved, because of their toxic action on the fungus.

It was thought that the toxic action might be due to the solanine in the sprouts; accordingly solanine and solanidine were supplied to the fungus under a variety of conditions, but with very little effect. 2 mg. solanine produced a slight diminution of growth, but smaller quantities no effect at all; the same was true of 4 mg. and smaller quantities

of solanidine. There was only one exception: in a single case complete inhibition of growth occurred in a culture containing white sprouts and 2 mg. solanine. It cannot be pretended that the toxic action of green sprouts is explained, but it may be noted that the thiochrome method also failed on green sprouts; the pepsin digest, which was coffee-coloured, gave no thiochrome reaction at all.

DISCUSSION

The vitamin B₁ content of potato tubers is important from the point of view of nutrition, as sources of this vitamin are somewhat restricted in a war-time diet. It appears from this work that mature tubers of the variety Majestic have a vitamin B₁ content, in autumn, of about 140 μg./100 g. On this basis a daily ration of 12 oz. of potatoes, if properly cooked in their skins, would supply 470 μg. of vitamin, or nearly half the usual estimate of the daily requirement of the vitamin (1 mg.). Potatoes, which are eaten by the great majority of people, are at any rate not a negligible source of the vitamin, particularly in the autumn and winter months.

The vitamin B₁ content of the young tubers increases continuously during the growing season; from this fact, and from the very high vitamin content of potato leaves, it can be presumed that the vitamin is synthesized in the leaves in summer and transferred to the young tubers as they grow. In winter the vitamin apparently remains in the tuber till sprouting begins, when it moves out of the centre of the tuber into the sprouts; but further evidence is needed on this point.

It is interesting to note that, in contrast to ascorbic acid, which is usually concentrated in the skin and adjacent layers of fruits and vegetables, vitamin B₁ in potatoes is concentrated in the centre of the tuber rather than in the skin.

The adjuvant action, which is due to some factor or combination of factors not fully explained, appears in tubers only in certain definite physiological states, e.g. in old fully sprouted, and young developing, tubers, between April and August. Adjuvant factors in potato have previously been described [Robbins, 1939], but information is lacking as to the age or physiological state of the tubers from which they were extracted. It can definitely be said that the adjuvant action of potato is due to a different cause from that of blood, but the factor responsible cannot be identified with any previously described.

SUMMARY

1. The *Phycomyces* method of estimation is valid for determining the amount of vitamin B₁ in potatoes.

2. The vitamin B₁ content of Majestic tubers at the time of lifting (September) is about 140 µg./100 g.

3. During the growing season the vitamin B₁ content of tubers increases continuously up to the time of lifting of maincrop potatoes.

4. The vitamin B₁ content of stored tubers falls during the winter and spring; the greater part of the loss takes place in spring, and is apparently due rather to sprouting than to storage, especially as white sprouts have a higher vitamin B₁ content than the tuber which produces them.

5. The vitamin B₁ content of young potato leaves is very high. •

6. An adjuvant factor of undetermined nature is present in the centres of tubers between April and August, and always present in the skin layer.

7. Green sprouts completely inhibit the growth of the fungus.

I should like to express my thanks to the following: to F. C. Bawden, under whose auspices the work was carried out, and N. W. Pirie, for advice; to P. H. Gregory for potato samples and help in planning the experiments; to H. M. Sinclair for a culture of *Phycomyces blakesleeanus*; to my brother, A. P. Meiklejohn, for calling my attention to the possibilities of the method, and to E. M. Crook and D. J. Higgins for supplies of solanine and solanidine.

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The Total Nitrogen Content of Egg Albumin and Other Proteins

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The question of amino-acid analysis became one of increasing importance in this laboratory during 1933, when the isolation of proteins from various forage grasses had been successfully accomplished and evidence for their content of the dietetically essential amino-acids was clearly desirable.

Work has continued without interruption since then, but at times progress has been slow on account of the laborious nature of many of the incidental investigations that we have felt called upon to undertake. During the first two years some preliminary studies of the better-known methods for base and dicarboxylic acid analyses were made by Miller [1935; 1936]; these served to emphasize the large amount of humin formation that attended the hydrolysis of our relatively impure leaf products, and little further progress was made until the origin of this humin had been satisfactorily established by Bailey [1937] and Lugg [1939]. The observation that cystine and, to a lesser extent, tyrosine can condense, like tryptophan, with the furfural derived from pentosan-like impurities during acid hydrolysis to form humin led Lugg, in continuation of work started in Adelaide, to investigate the reliability and limitations of the better-known indirect methods for estimating these three amino-acids, and also methionine [cf. Lugg, 1939]. Meanwhile, Tristram [1939] had explored the applicability to these impure leaf proteins of the Block small-

scale gravimetric method of base analysis; progress had been made in an attempt to improve the Foreman method of dicarboxylic acid analysis; and the separation of the monoamino-acids by the Dakin-Fischer methods had been explored. With the experience thus gained it was possible to carry out partial amino-acid analyses of a series of protein preparations from forage crops [cf. Chibnall, 1939].

At this stage in our work we were not specifically interested in obtaining strictly quantitative values for the dicarboxylic acids and bases, for such a refinement was considered unnecessary in view of the somewhat impure nature of our leaf products; all that we had set out to attain was an analysis for each product comparable, from a nutritive point of view, with those already in existence for many seed and animal proteins. During 1936-8 however Bergmann & Niemann reported partial analyses of several proteins, and on the results of these they formulated certain stoichiometric laws governing protein structure. Two of the proteins they had selected for consideration, egg albumin and haemoglobin, had already been submitted to dicarboxylic acid analysis in this laboratory, and our results, which we were fairly certain were still several per cent too low, were higher than theirs and definitely not in keeping with the general conclusions they had drawn. Moreover, in agreement we believe with many others, we were not satisfied with the values they had assigned to the molecular weight of the average amino-acid residue.