Vitamin C in the Walnut (*Juglans regia*)

BY F. WOKES AND R. MELVILLE

Ovaltine Research Laboratories, King's Langley, Herts and the Royal Botanic Gardens, Kew

(Received 30 April 1948)

The remarkably high concentration of vitamin C in unripe walnuts (*Juglans regia*) appears to have been first reported by Gerghelezhii (1937), and has been confirmed by Pyke, Melville & Sarson (1942) who also examined a number of allied species and genera. Melville, Wokes & Organ (1943) showed that unripe walnuts and nuts from allied species may also contain considerable amounts of apparent vitamin C, the provisional term suggested by Wokes, Organ, Duncan & Jacoby (1943) to describe substances occurring in certain foods, and, although not possessing the antiscorbutic potency of vitamin C, not distinguished from it by the indophenol dye titration as usually carried out. Differentiation between true and apparent vitamin C in walnuts can, however, be effected by modifications of Lugg's (1942) formaldehyde method (Mapson, 1943; Wokes, Organ & Jacoby, 1943).

During the last four years we have studied the distribution of true and apparent vitamin C in different tissues of the walnut at intervals throughout the season, estimating them in the minute amounts of material permitted by the high concentration of the vitamin. Our data provide information on the possible functions and phytosynthesis of vitamin C which may be applicable to other plant material containing this vitamin.

METHODS

Collection of material

The majority of the samples were taken from a typical tree of *J. regia* growing at Kew, and the results were checked on a second tree at Kew and others at Berkhamsted and King's Langley. The material was at once put into air-tight boxes and transported as quickly as possible to the laboratory, where it was either examined immediately or stored in the refrigerator. Tests showed that there was no significant loss of vitamin C during transport, and very little change in moisture content except in leaves, where as much as 10% might be lost between collection and dissection. In view, however, of the variable moisture content of the leaves as collected at different times of day, and also of probable changes in weight through varying metabolic activity during the day, we adopted Chibnall's (1923) procedure and expressed our results on the basis of fresh weight. Since our findings were largely comparative we do not think this procedure led to serious errors as far as leaves were concerned. With fruits, stems, buds, catkins and other parts the moisture losses were insignificant.

Preparation of extracts

Nuts. The walnut is a drupe-like fruit in which the outer green husk is derived from two whorls of fused bracts. In this paper the epidermis and a few subjacent cell layers are referred to as epicarp, and the bulk of the green husk belonging to the inner bracts as mesocarp. The white inner portion, which eventually becomes woody, corresponds with endocarp and consists of two carpels. The testa or seed coat is well developed at an early stage. The endosperm remains fluid until the fruit is almost full size, the shell beginning to harden before the endosperm is replaced by the embryo. An attempt was made to separate these tissues from one another and to follow the seasonal changes of vitamin content in them. Shallow cuts were made along the line of union of the two carpels, which were forced apart and the undamaged kernel obtained free from adherent endocarp. The epicarp was then removed in thin slices, followed by the mesocarp, and finally the endocarp was cut in small pieces. Separation of mesocarp from endocarp was ensured by rejecting adjacent parts of these tissues. If a separate sample was required of the liquid contents of the kernel in young nuts, this was obtained by piercing the unbroken testa with a fine-pointed teat pipette and withdrawing the liquid by suction. Any of the liquid coming in contact with endocarp was removed by filter paper. When a sample of the larger whole nut was taken, the first cut along the carpellary junction was followed by a second cut at right angles, each cut being taken right to the centre to include the kernel. The two diagonally opposite quarters were taken for duplicate samples of the whole nut, and the remaining two quarters used for samples of epicarp, mesocarp and endocarp.

Peduncle or fruit stalk. With the object of studying gradients of vitamin C within the plant a rough separation of phloem from xylem and other tissues was effected. In the fruit stalk longitudinal slices of cortex were cut off until the circular zone of pericyclic fibres was reached, as determined by their paler colour. Continuing with longitudinal slices, the tissues from the pericyclic fibres to the cambium were included as phloem, and those inside the cambium as xylem and pith.

Stem. A ring of bud scale scars marked the limit of the previous year's growth. Samples of cortex with phloem of the current year were obtained by peeling off the bark, which came away readily at the cambium. The remaining white cylinder consisted of xylem with pith. In older bark the phloem could be separated from the cortex by careful dissection.

Leaves. The petiole was freed from its swollen base. The petiolar cortex was separated from the remaining tissues by removing longitudinal slices until the zone of pericyclic

fibres was reached. The green tissue of the leaflets, consisting mainly of mesophyll, was separated by cutting along the midrib and its main branches.

Buds and catkins. The cone-like male catkin buds were readily identified on reproductive shoots but female catkin buds are not differentiated in *J. regia*. Since the nuts always develop from terminal buds, the terminal and lateral buds of both vegetative and reproductive shoots were examined separately.

Roots. These were separated into fine and coarse but were not dissected. In order to minimize losses through enzymic action all pieces cut off were placed immediately in tared weighing bottles containing HPO_3 . Stainless steel knives and safety-razor blades were used.

Estimations

Dye titrations. Visual estimations of 'total' vitamin C were made substantially as described by Harris & Olliver (1942) for true vitamin C, the end point being persistence of the pink colour for 20 sec., and care being taken with the timing when there was sufficient apparent vitamin C present to affect the rate of reaction with the dye. 'Apparent' vitamin C was estimated by the method of Wokes, Organ & Jacoby (1943). The methods of Mapson (1943) and of Snow & Zilva (1944) applied occasionally, when sufficient material was available, showed certain discrepancies because they do not make adequate allowance for the effect of the titration pH on the dye titration value, which has been shown to be very marked in walnut material (Wokes, 1946). 'True' vitamin C was usually determined indirectly by subtracting apparent from total vitamin C. It was occasionally estimated directly by the ascorbic acid-oxidase method, based on the fact that the apparent vitamin C in walnuts acts much more slowly with the enzyme than does true vitamin C. When calculating the concentration of apparent vitamin C the latter was assumed to have the same dye titration value as ascorbic acid. Evidence will be given later in support of this assumption.

Potentiometric estimations were made by the method of Harris, Mapson & Wang (1942) for true vitamin C, with certain modifications advantageous when apparent vitamin C is present (Wokes, Organ & Jacoby, 1943).

Dehydroascorbic acid. Estimations of this substance by the H_2S -reduction method showed that it could only have been a small percentage of the total vitamin C content (e.g. leaves 3-4%, nuts 3-5%, endocarp 5%). Since sufficient material for accurate determination of these minute amounts was often lacking, such estimations were usually omitted.

pH measurements. These were made with a Cambridge portable pH meter, also used in the potentiometric estimations.

RESULTS

Oxidizing enzymes and pH of tissues

Study of these enzymes by the technique previously applied to tomatoes (Wokes & Organ, 1943) showed that the tissues, when examined at pH 6.5, can be placed in the following decreasing order of oxidative activity—epicarp, mesocarp, endocarp, kernel. The natural pH of the endocarp is usually below 5, causing a further decrease in activity. On the other

hand, the natural pH of the epicarp seems always to be above 5 and may reach 7, causing an increase in oxidative activity. The pH of the mesocarp may be above 7 in very small nuts, but gradually falls during growth and may be below 5 in full-grown nuts. Hence, when dissecting walnut fruits for vitamin C assay, particular care should be taken with the epicarp and mesocarp. If these tissues are disintegrated, oxidizing enzymes may destroy half the vitamin and apparent vitamin C within a few minutes. By avoiding prolonged exposure of cut surfaces to air, and by dividing the tissues into not too small pieces, which were dropped immediately into HPO_3 solution, the losses due to oxidizing enzymes are believed to be reduced to a small proportion of the total error. Green tissues outside the nuts, such as fruit stalk, stem, leaves and buds, appear usually to be neutral and to contain considerable amounts of the oxidizing enzymes, and similar precautions are necessary.

Whole fruits (nuts)

Table 1 summarizes our results on nearly 100 *J. regia* nuts, most of which came from the main tree at Kew. They show that the average concentration of vitamin C in very young nuts (0.5-1.0 g.) was 500-1000 mg./100 g. As the nuts grew, this concentration at first rapidly increased, being doubled within a fortnight, and reached a peak of about 2000 mg./100 g. when the nuts weighed 2-3 g. In the Kew samples this peak occurred in 1944 towards the end of June and in 1945 about a fortnight earlier. In 1946 maturation was later than in 1945, the average concentration on 18 June being less than on 30 May in 1945. After the peak concentration was reached the nuts continued to grow rapidly, but the vitamin concentration gradually diminished, being only a half to two thirds of the peak concentration when the nuts were fully grown. Hence the total amount of vitamin C in a given nut increased more and more slowly during the later stages of maturation, and usually reached its maximum before the nuts were fully grown. It then remained fairly constant for several weeks, until formation of the woody shell began, when it fell rapidly. These findings on the main Kew samples were confirmed by results on nuts taken from other trees in 1943-6.

Different tissues in the nuts

These were obtained from nuts belonging to the same samples that provided the data on whole nuts summarized in Table 1. In the smallest nuts it was not possible to separate epicarp completely from mesocarp. The figures for these two tissues in the first two or three samples are, therefore, less accurate than those for later samples in the season (Table 2). In larger nuts, where differentiation between epicarp

Table 1. *Seasonal changes in vitamin C concentration in walnuts*

(Nuts in samples 1, 2, 25 and 45 were too small to be examined individually and therefore were pooled. Weights of nuts taken immediately before examination do not differ by more than 1 or 2% from weights as taken from tree.)

Sample no.	Date gathered	No. of nuts	Average wt. of nuts (g.)	Vitamin C (mg./100 g.)				Vitamin C (mg./nut, mean)
				Individual nuts			Mean	
			(a) From main tree					
1	7 June 1944	6	1	Pooled			—	5
2	13 "	10	1	Pooled			—	10
3	21 "	3	3	2080	1975	1932	1991	60
4	26 "	4	3.6	2060	1940	1920	2000	72
5	4 July	3	10	2065	2045	1915	2010	201
6	12 "	4	20.3	1450	1615	1675	1690	327
7	18 "	4	17.5	1495	1575	1390	1530	262
8	25 "	4	22.6	1563	1335	1405	1563	332
9	1 Aug.	3	24.9	1155	1180	1295	1210	301*
10	9 "	2	32.1	1115	835		975	313*
11	22 "	2	21.7	1150	1280		1215	264
25	17 May 1945	8	0.3	Pooled			928	3
26	30 "	3	0.5	1273	999	931	1068	5
27	6 June	2	1.75	1848	1789		1819	32
28	12 "	3	2.8	1836	2193	1983	2004	56
29	19 "	3	4.1	2074	1799	1677	1517	62
30	26 "	3	14.0	1490	1442	1468	1467	205
31	10 July	3	25.2	1007	1357	1042	1135	286
32	23 "	3	29.7	999	1022	972	998	297
33	8 Aug.	3	32.0	905	1256	882	1014	329
45	18 June 1946	13	0.3	Pooled			699	2
			(b) From other trees					
2k2	29 June 1944	3	10.6	2035	2025	2060	2036	216
2k3	25 June 1945	6	18.5	2037	1770	1732		
				1938	1934	1683	1849	342

* Excluding woody portion.

Table 2. *Seasonal distribution of vitamin C in different tissues of walnut fruits*

(The number of nuts in each sample and date of collection are given in Table 1. The following figures are the sample averages.)

Sample no.	Vitamin C (mg./100 g.) in					
	Fruit stalk	Epicarp	Mesocarp	Endocarp	Testa	Endosperm
1	259	25	—	951	—	—
2	—	360	—	1644	—	—
3	—	791	—	3040	—	—
4	270	940	815	2980	—	—
5	235	985	1000	2855	—	10
6	—	1325	740	2255	65	9
7	—	1000	660	2155	75	20
8	—	1075	740	2280	66	21
9	60	980	750	2120*	65	13
10	—	963	745	2220*	71	22
11	220	1160	1170	3150*	33	21
26	273	342	1290	1532	—	—
27	330	782	1202	2641	—	—
28	189	918	986	2655	—	—
29	272	992	931	3083	—	—
30	246	1002	916	2406	—	—
31	134	921	784	1991	—	—
32	267	913	706	1800		53†
33	316	408	547	1728		31†
34	205	808	867	—	—	—
2k1	—	1170	880	3002	—	—
2k2	—	1195	1085	3140		140†
2k3	—	1410	1203	2678	—	—

* These results are averages on endocarp excluding wood. If wood is included, average results for samples 9 and 10 are 1790 and 1410 respectively.

† These results were obtained on whole kernel, including both testa and endosperm.

and mesocarp was more complete, the concentration of vitamin C in the epicarp was sometimes but not always higher than in the mesocarp. The peak concentration was generally reached at a later date in the epicarp than in the mesocarp or endocarp. The concentration in the endocarp was always much higher than in other tissues, but fell rapidly when lignification of the shell began. It seemed possible that, during the dissection of the epicarp and mesocarp, a loss of vitamin C occurred which might explain why the concentration of this vitamin in these two tissues was so much lower than in the endocarp. We, therefore, compared the sum of the results on the different tissues in a number of dissected nuts with those on whole nuts from the same samples (Table 3). Allowing for the weights of the different tissues, the results on the dissected nuts were very similar to those on the corresponding whole nuts, ranging from 84 to 119 and averaging 99 % of the latter in 15 nuts from five samples. We, therefore, felt satisfied that our findings were not being affected seriously by loss of vitamin C during dissection. The kernels always contained very little vitamin C, but the concentration in the testa was always higher than in the endosperm, when separation of these two tissues was effected. These findings on the main Kew samples in 1944 and 1945, confirmed by results on other samples in 1943-6, made it difficult to assume that the large amount of vitamin C in the endocarp had come entirely, or even mainly, from the epicarp and mesocarp where the concentration was always much lower. We, therefore, considered as another possibility translocation of the vitamin from the leaves.

Table 3. *Loss of vitamin C during dissection of walnuts*

	Vitamin C		
	Wt. of tissue (g.)	Concn. (mg./100 g.)	Content (mg.)
Epicarp	2.23	921	20.5
Mesocarp	16.87	784	132.2
Endocarp	7.10	1991	141.4
Kernel	2.64	0.50	1.3
Total	28.84		295.4

Average vitamin C concentration = 1007 mg./100 g.

(b) Vitamin C concentration (mg./100 g.)

In whole nut 31/1	1357
In whole nut 31/2	1042
Mean	1200
In dissected nut 31/3	1007
In dissected as percentage of whole nuts	84
Percentage assumed loss during dissection which scarcely exceeds sampling error	16

Leaves

In whole leaflets from the main Kew tree the average concentration of vitamin C was found to be highest in the earlier part of the season (May and June) and to fall from a peak of about 1000 mg./100 g. to less than half this concentration as the season progressed (Table 4). These leaflets were on the same shoots as, and therefore adjoined, the nuts of the corresponding samples in Tables 1 and 2. Being nearest to the nuts, they formed the most likely

Table 4. *Vitamin C in walnut leaves and stems*

Sample no.	Date gathered	Vitamin C (mg./100 g.) in					Main stem: cortex and phloem
		Whole leaflets	Mesophyll	Vascular tissue	Rachis	Petiole	
4	26 June 1944	856	—	—	—	—	—
9	1 Aug.	440	—	—	—	60	—
11	22 "	—	700	240	—	120	—
25	17 May 1945	876	930	663	458	308	353
26	30 "	908	1039	557	391	170	153
27	6 June	838	970	530	447	264	192
28	12 "	1037	1137	756	383	339	305
29	19 "	755	758	477	293	151	64
30	26 "	698	869	287	239	90	102
31	10 July	717	878	483	275	176	112
32	23 "	553	668	260	202	104	635
33	8 Aug.	530	715	231	171	105	239
34	11 Sept.	503	520	150	171	40	708
35	2 Oct.	319	—	—	165	46	335
36	8 "	741	621	340	322	255	217
37	15 "	388	308	179	38	60	275
38	22 "	371	278	70	124	81	272
43	15 Apr. 1946	227	—	—	—	—	83
44	29 "	230	—	—	530	438	122
45	18 June	814	853	750	475	445	235
46	1 July	1048	1161	804	450	350	245

source of any vitamin C passing into the latter from the leaves. The weight of each leaflet increased rapidly during the first few weeks after unfolding of the bud, but had become fairly constant by the beginning of June. The amount of vitamin C in each leaflet reached a maximum of about 10 mg. in June or July and then fell gradually. The total amount of vitamin C in the leaves examined after this date was perhaps 5-10 % of the amount in the nuts borne on the corresponding shoots. Examination of different leaflets in a leaf showed no marked variation in vitamin C concentration, the lowest concentration being usually not more than 10 % below the highest concentration. In our dissection of leaflets differentiation of mesophyll from vascular tissue, though necessarily incomplete, was sufficiently efficient to reveal marked differences between the vitamin C concentrations in the two tissues; that in the mesophyll always being much higher. The peak concentration in the mesophyll coincided with that in the whole leaflet. To test for any loss of vitamin C during dissection of the leaflets we compared in a number of leaflets the sum of the results obtained on the dissected mesophyll and vascular tissue of the leaflet with the concentration of vitamin C found in the corresponding whole leaflet on the opposite side of the leaf. Allowing for the weights of the dissected tissues, there was good agreement between the dissected and whole leaflets throughout the period when nuts were on the trees, the weighted mean concentration in the dissected leaflets ranging from 87 to 110, and averaging 97 % of the concentration in the corresponding whole leaflets in 12 samples collected from May to September. In three samples of older leaves collected on 9, 15 and 22 October the percentages fell to 76, 71 and 59 respectively, probably because the oxidizing enzymes were liberated more readily from the senescent cells. However, for the period when nuts were on the tree we felt satisfied that our findings were not being seriously affected by loss of vitamin C during dissection.

When studying the possible translocation of vitamin C from the mesophyll of the leaf to other parts of the walnut tree we determined its concentration in the rachis and petiole through which it would have to pass *en route* to the stem. Data from 17 samples summarized in Table 4 show that there is always a fall in concentration in passing from the vascular tissue of the leaflets through the rachis to the petiole. Separation of the cortex and phloem of the petiole by dissection from the xylem and pith showed that the concentration of vitamin C in the outer tissues was three to four times that in the inner, whereas the proportions by weight were approximately equal. The concentration in the outer tissues was, therefore, at least twice that in the rachis or petiole. This distribution suggests that the vitamin is translocated mainly, if not entirely, in the phloem.

It was not possible to separate the phloem completely from other tissues for a vitamin C determination, but an estimate of the amount of phloem present from transverse sections suggests that the concentration in the phloem is probably not less than three times that reported for the whole rachis and petiole in Table 4.

It should be stated that invariably our results on the mesophyll and vascular tissue of the leaf, the rachis, petiole and the cortex and phloem of the stem were obtained on samples taken from adjoining parts of the same shoot.

Stem

The outer tissues, cortex and phloem were usually separated from the xylem and pith. The concentration of vitamin C in the former was found to be three to four times that in the xylem and pith and the results from 18 samples are included in Table 4. These were all obtained from stems of the current year's growth (c.y.g.) to which the leaves analyzed were attached. In order, however, to obtain a more complete picture of the distribution of vitamin C in different parts of the stem, we extended our observations to other parts of the stem, including the previous or last year's growth (l.y.g.) and still older parts originating as far back as 1920. Stems were also examined in the winter when leaves were absent. The data on 34 samples, collected between August 1944 and October 1946, are summarized in Table 5. The wide variations from one sample to another are due probably to varying amounts of brittle cortex adhering to the phloem. Examination of the separated phloem in a number of samples low in vitamin C (e.g. nos. 21, 22, 26, 27) showed that about twice as much vitamin C was present in the phloem alone as in the combined tissues. The dissection of cortex from phloem was a tedious procedure needing 2 or 3 hr. to obtain a sufficient quantity for an assay, and time was not always available to prepare replicates from all the samples. However, our data suggest that the concentration of vitamin C in the phloem of stems of the current year is usually above 200 mg./100 g. and may reach a peak between 600 and 1000 mg./100 g. The concentration in the phloem of older stems is usually lower than in that of the current year.

Buds and catkins

The difference in vitamin C concentration between the lateral and terminal buds of vegetative shoots in any one sample was found to be smaller than that between either lateral or terminal buds of successive samples. During the autumn and winter when the buds were very small we therefore economized material by pooling lateral and terminal buds, the results being quoted as for vegetative buds in Table 5. There was likewise no significant difference

Table 5. *Seasonal distribution of vitamin C in buds, catkins and stem*

(Older stem sample corresponded to growth in previous years back to 1920. 'Reproductive buds' were terminal buds on reproductive shoots, which might produce female flowers. C.Y.G. = current year's growth; L.Y.G. = last year's growth.)

Sample no.	Date gathered	Vitamin C (mg./100 g.) in					
		Stem (cortex and phloem)			Buds		Male catkins
		C.Y.G.	L.Y.G.	Older	Veg.	Reprod.	
12	29 Aug. 1944	135	—	—	—	—	270
13	4 Sept.	—	—	—	169	20	40
14	11 "	—	—	—	35	106	—
15	10 Oct.	160	—	—	174	109	65
16	14 Nov.	215	—	—	270	200	130
17	20 "	160	—	—	210	—	80
18	27 "	170	—	—	90	100	180
19	8 Jan. 1945	499	147	—	163	—	460
20	21 "	222	177	178, 98	401	266	603
21	28 Feb.	113	298	328	199	511	169
22	13 Mar.	185	130	170	61	155	505
23	4 Apr.	158	—	—	315	232	133
24	13 "	—	—	—	—	—	61
25	17 May	353	379	—	500	946*	—
26	30 "	153	188	—	617	1068*	—
27	6 June	192	163	112, 93	389	—	—
28	12 "	305	119	108	507	—	—
29	19 "	64	51	59	293	—	—
30	26 "	102	63	—	194	—	—
31	10 July	112	93	—	231	—	—
32	23 "	635	267	—	203	—	—
33	8 Aug.	239	173	—	287	—	—
34	11 Sept.	708	84	80, 53	99	—	357
35	2 Oct.	335	260	378, 226 70, 141	63	54	873
36	9 "	217	240	175	197	—	1001
37	15 "	275	217	—	406	—	825
38	22 "	272	278	—	492	—	207
39	31 "	260	216	—	396	—	403
40	—	327	184	—	226	—	525
41	15 Jan. 1946	202	160	—	150	—	220
B	21 Mar.	240	—	—	—	—	364
42	2 Apr.	120	70	—	236	214	115
43	15 "	83	59	—	159	—	100
44	29 "	122	40	—	—	—	64
45	18 June	235	199	—	—	—	—
46	1 July	245	354	—	1042	—	—
47	23 Sept.	—	—	—	—	—	542
48	24 Oct.	85	139	192	338	—	420

* Results obtained on female catkins.

between the lateral and terminal buds of reproductive shoots during the autumn and winter, when the concentrations were similar to those in the buds from the vegetative shoots, i.e. 225 and 211 mg./100 g. respectively. In the spring there were marked increases in vitamin C concentration in both types of bud shortly before they opened. After this the concentration fell to an average of about 200 mg./100 g. in the unopened vegetative buds. In the autumn when reproductive buds were again available the vitamin C concentration in both kinds of buds had fallen to a still lower level.

Male catkin buds, on account of their shape, were much easier to distinguish than female reproductive buds. Data on them were obtained for 12 samples

gathered at regular intervals between 11 September 1945 and 29 April 1946 (Table 5), and show rather wide fluctuations in vitamin C concentration. However, the vitamin C content per catkin was found to be more uniform. From August until the end of the year the weight of the catkins remained fairly constant at 50–70 mg. and the vitamin C content was usually less than 0.1 mg. Early in the following year the catkin buds grew rapidly until by March or April they weighed 0.5–1.0 g. and their vitamin C content increased up to 1.0 mg. Soon afterwards the catkins shed their pollen and fell off. When catkin buds next appeared in the autumn, their content of vitamin C was at the initial low level of about 0.1 g.

DISCUSSION

When unripe walnuts were found to contain remarkably high concentrations of vitamin C, its origin and functions in the plant became of interest. In view of much evidence that in other fruits the vitamin C content is influenced by the degree of exposure to sunlight, it seemed likely that the source of the vitamin might be green tissues, such as the epicarp of the fruit or the leaf mesophyll where photosynthesis is most active. If the vitamin is synthesized in the epicarp, and translocated to the endocarp it would have to move against a steep concentration gradient. Such a movement could not take place by simple diffusion, but is not impossible where activities of living cells are involved (Stiles, 1936). In order to establish a gradient favourable to direct diffusion from epicarp to endocarp it would be necessary to show that the vitamin was confined to particular cells or regions of the epicarp which in some instances would have to contribute less than a quarter of the total weight of the tissue. Although no chemical evidence was obtained on this point, a segregation of morphologically similar cells into a tissue meshwork was observed in sections in which certain cells became brown on exposure, probably by the oxidation of juglone derivatives, while adjacent cells remained unstained for some time. Such a segregation of materials in the outer tissues of the fruit is rendered less unlikely by the existence of considerable differences in vitamin C concentration in the mesocarp and endocarp, the limiting cell layers of which must be extremely effective barriers to the reverse movement of the vitamin in order that the concentration may be maintained in the endocarp. This may have an evolutionary background in that the green husk of the walnut is not a true mesocarp, but arose by the fusion of several bracts with the true fruit represented by the endocarp and its contents. The presence of such boundary layers renders it probable that translocation from epicarp and mesocarp takes place through their vascular tissues to the base of the fruit and thence up into the endocarp.

As it was not possible to establish the existence within the fruit of a gradient of vitamin C favourable to the translocation and accumulation of the vitamin in the endocarp, other parts of the plant were considered. Concentrations of over 1000 mg./100 g. were found in the leaf mesophyll in May and June, while the concentration in the midrib and lateral nerves was generally about 40% of that in the mesophyll. As the mesophyll contained a meshwork of small nerves it is probable that the actual concentration in the green assimilatory cells was well over 1000 mg./100 g. throughout the period from May to August, but may have fallen below this level in September and October. There was always a clear gradient from the mesophyll to midrib and through the rachis as far

as the base of the petiole. Beyond this point vitamin C in course of translocation from the leaf would have to pass through a zone of stem with a higher concentration in the cortex and phloem, and then through a zone usually of a still higher concentration in the peduncle of the fruit, before it could reach the endocarp. The low concentration in the xylem and pith of the stem renders it unlikely that translocation takes place through the wood, and indicates the phloem as the only possible tissue for this function. The dissection of the fruit stalk into cortex, phloem and xylem plus pith enabled us to show that the highest concentration of vitamin C was in the cortex, where it was about twice that in the fruit stalk as a whole, probably on account of the photosynthetic activity of the green parts. The concentration in the phloem as dissected was about equal to that in the whole stalk. The possibility of greater concentrations in the sieve tubes and companion cells of the phloem was considered, since movement of the translocate may be confined to them. Estimates of the area of true phloem consisting of sieve tubes, companion cells and phloem parenchyma were made from sections of the rachis, petiole, stem and fruit peduncle. Assuming the bulk of the vitamin C to be concentrated in these cells, and applying a factor to the observed data the irregularities in the gradient from leaf to endocarp were somewhat reduced. Only if companion cells alone are considered, and the assumption made that the bulk of the vitamin present in the phloem is confined in these slender cells, would the anatomical evidence be favourable to a positive translocation gradient from the leaf mesophyll to the endocarp of the fruit. In the absence of a method for isolating the companion cells it was not possible to confirm this theory. Thus with the available data we are unable to demonstrate that the vitamin C in the endocarp comes mainly either from the epicarp or the mesophyll.

An alternative hypothesis, that vitamin C is translocated as a precursor or other form of compound which can yield ascorbic acid after it reaches the endocarp, will be considered in a later paper. In this connexion it is interesting to note that, in contrast with many other fruits, the pH of many walnut tissues, both from the fruit and from other parts of the plant, is slightly alkaline. Only in the endocarp is the usual acidity sufficient to improve the stability of vitamin C, whilst the concentration of ascorbic acid oxidase is much lower than in other walnut tissues. Thus any such precursor of vitamin C may be expected to differ from ascorbic acid in being resistant to the action of ascorbic acid oxidase and being stable in neutral solution (pH 6-8).

The function of vitamin C in plants is often stated to be concerned with growth. Its occurrence in higher concentration in young actively growing

shoots supports this hypothesis. Our data have shown that the vitamin C concentration in the vegetative buds increased markedly just before opening, the peak occurring in very young leaves. In the reproductive buds and catkins there was a similar increase just before rapid growth began. In the phloem of the stem, adjoining the cambium, there was a peak concentration at the time of active growth. Schopfer (1943) has suggested that vitamin C may also be connected with photosynthesis and the production of sugars. Our data support this suggestion in so far as high concentrations of the vitamin were found in the mesophyll and in other green tissues (e.g. cortex of green stems) where photosynthesis was most marked. However, photosynthesis cannot very well explain the function of the vitamin C in the endocarp, where the highest concentrations occur. Since these concentrations fall rapidly when the woody shell is developing, and since in a given nut the concentration of vitamin C in the woody part is much lower than in the non-woody part, we suggest that the vitamin may be utilized in the formation of the lignin. This hypothesis may also explain the function of the vitamin C in the stem. The concentration gradient of vitamin C in the stem declines towards the roots. In the large trees yielding our main sample we could only follow this gradient 2 or 3 ft. down the shoots into the main stem. By examining a sapling about 4 ft. high we

were able to establish the complete gradient from the buds down to the finest roots (Table 6).

Table 6. *Concentration gradient of vitamin C in walnut sapling examined March 1945*

(C.Y.G. denotes current year's growth; L.Y.G., last year's growth.)		Vitamin C (mg./100 g.)
Buds		258
Stem cortex and phloem:	C.Y.G.	252
	L.Y.G.	183
Roots		140
Fine roots		94

SUMMARY

1. Studies on samples of different tissues collected during three seasons from *Juglans regia* trees growing in several districts round London showed, in the endocarp, remarkably high concentrations of vitamin C, which may be used in lignification. This vitamin C probably originates mainly in photosynthetic centres in the leaves.

2. Examination of the intervening tissues showed that physiological conditions (acidity, concentration of enzymes and of vitamin C) militate against translocation of vitamin C as such from leaf to nut. The experimental findings favour an alternative hypothesis, which will be considered later.

REFERENCES

- Chibnall, A. C. (1923). *Ann. Bot., Lond.*, **37**, 511.
 Gerghelzhiu, H. K. (1937). *Bull. appl. Bot. Genet. Plant Breeding, U.S.S.R. Suppl.* **84**, **2**, 206.
 Harris, L., Mapson, L. W. & Wang, Y. L. (1942). *Biochem. J.* **36**, 183.
 Harris, L. & Olliver, M. (1942). *Biochem. J.* **36**, 155.
 Lugg, J. W. H. (1942). *Aust. J. exp. Biol. med. Sci.* **20**, 273.
 Mapson, L. W. (1943). *J. Soc. chem. Ind., Lond.*, **62**, 223.
 Melville, R., Wokes, F. & Organ, J. G. (1943). *Nature, Lond.*, **152**, 447.
 Pyke, M., Melville, R. & Sarson, H. (1942). *Nature, Lond.*, **150**, 267.
 Schopfer, W. H. (1943). *Plants and Vitamins*. Waltham, Mass: Chronica Botanica Co.
 Snow, G. A. & Zilva, S. S. (1944). *Biochem. J.* **38**, 458.
 Stiles, W. (1936). *An Introduction to the Principles of Plant Physiology*. London: Methuen.
 Wokes, F. (1946). *Nature, Lond.*, **158**, 133.
 Wokes, F. & Organ, J. G. (1943). *Biochem. J.* **37**, 259.
 Wokes, F., Organ, J. G., Duncan, J. & Jacoby, F. C. (1943). *Biochem. J.* **37**, 695.
 Wokes, F., Organ, J. G. & Jacoby, F. C. (1943). *J. Soc. chem. Ind., Lond.*, **62**, 232.

The Accuracy of the Svedberg Oil-turbine Ultracentrifuge

By R. CECIL AND A. G. OGSTON, *Department of Biochemistry, University of Oxford*

(Received 30 April 1948)

1. THE SEDIMENTATION CONSTANT

The values of sedimentation constants which appear in the literature show more variation than one would expect to obtain from measurements made on well defined substances by an accurate physical method. Measurements made in Oxford on several proteins which are believed to be definite and homogeneous have always given values lower than those obtained

elsewhere (Table 1); correction of these values for errors described in the present work would further increase these discrepancies. The large difference between the values for the sedimentation constant of insulin of Gutfreund & Ogston (1946) and of Miller & Andersson (1942) finally led us to investigate the errors of our Svedberg ultracentrifuge.