SUMMARY

1. The absorption characteristics of ascorbic acid as affected by pH have been determined.
2. A method of assay utilizing the shift of the absorption curve has been suggested.
3. The results of this method when applied to

walnut extracts have been compared with those obtained by the indophenol dye titration.

I wish to thank Dr F. Wokes, the Director of the Laboratories, for affording me the opportunity to publish this work (which, together with that described in the following paper, will form part of the thesis for a Ph.D. degree of the University of London), and Miss N. Baxter for technical help in the preparation of the many extracts used.

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The Occurrence of Ascorbic Acid in the Walnut (*Juglans regia*)

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In an earlier paper (Daglish, 1950*a*) it was shown that hydrojuglone glucoside was more resistant to atmospheric oxidation in walnut extracts than in solutions prepared therefrom by chromatography. The properties of the antioxidant suggested that it was ascorbic acid, high concentrations of which are known to occur in the walnut (Gerghelezhiu, 1937).

Breinlich (1942) postulated that ascorbic acid and juglone formed part of an oxidation-reduction system. If there is a similar interaction between ascorbic acid and hydrojuglone glucoside, then, as the concentration of the latter shows definite seasonal variations in the different tissues of the plant (Daglish, 1950*b*), it would be expected that ascorbic acid would exhibit corresponding variations. In order to determine if this is so, the occurrence of ascorbic acid has been determined throughout the 1949 and 1950 growing periods.

EXPERIMENTAL

Preparation of extracts. In order to avoid variations due to location, age of trees, soil, etc., samples were taken from one mature tree in 1949. The ascorbic acid concentration in the different plant parts exhibited definite seasonal fluctuations. A second tree in a different location was used in 1950 to demonstrate that these variations were characteristic of the walnut as a species and not just a single member.

Within 30 min. of collection, the plant material was extracted into ethanolic HCl (Daglish, 1950*a*), and then assayed for ascorbic acid, dehydroascorbic acid and hydrojuglone glucoside by the spectrophotometric methods previously described (Daglish, 1950*b*, 1951). All results were calculated on a fresh weight basis.

RESULTS AND DISCUSSION

Male catkins. To minimize errors caused by the variation of individual catkins, at least twenty of these were used for each assay. Duplicate assays at any one time differed by not more than 8.5 %, which was less than the variation of successive weekly samples.

When first distinguishable from the vegetative buds, the male catkin buds weighed 30–50 mg. each, and contained approximately 30 mg./100 g. of ascorbic acid. This level was maintained throughout the winter. In the spring there was an initial gradual increase in weight to 0.1–0.15 g. and with this, the concentration of ascorbic acid increased to 160 and 386 mg./100 g. in 1950 and 1949, respectively. This was followed by a rapid decrease in concentration which was not due to a loss of ascorbic acid, but due solely to the 20-fold increase in weight which had taken place. In fact, the amount of ascorbic acid present showed an increase as indicated

by the average amount per catkin. This rose from less than 0.25 mg. in the winter resting state to 1.0–1.5 mg. at 50% full growth, then remained constant or fell slightly. The possible sources of this extra ascorbic acid were, at first, the cortex and phloem of the adjoining stem, and later, by translocation from the unfolded leaves or by photosynthesis in the green catkins themselves. Some slight evidence for this was obtained both from ringing experiments and by growing catkins on isolated branches in water.

Rings of bark were removed from stems of trees before any signs of development in the catkins were visible. Catkins which developed above the rings were very stunted in growth, averaging 20–25% the weight of normal catkins, were brown rather than green in colour, and showed no ascorbic acid on assay. In contrast catkins, grown on isolated branches in water, developed to maturity and formed and shed pollen. The vegetative buds on the same stems, however, remained dormant. The weight of the catkins was again only 25% that of controls and no ascorbic acid could be detected.

It would thus appear that ascorbic acid was necessary for full and rapid growth, and that in the catkins this was obtained, first of all from the adjoining stem, then later from the leaves by translocation.

Vegetative buds. As with male catkin buds, precautions were taken to minimize sampling errors and those due to individual variations in vegetative buds. These were first detected in late June when they weighed about 30 mg. each. With the increase in weight to 50–60 mg. in the same season, the concentration of ascorbic acid increased from 100 to 450 mg./100 g. As this level was approximately the same as that in the leaf stalk, it suggested that the ascorbic acid was received from the mesophyll. After reaching full size the concentration fell steadily, until in the winter dormant state it was difficult to detect any ascorbic acid.

With the development of the buds in the spring the concentration rose rapidly to 300 mg./100 g. and this rise was continued in each of the tissues derived from the buds. As with all the other tissues of the plant investigated, the increase in concentration of ascorbic acid occurred just before the increase in growth.

Mesophyll, midribs and stalks. The seasonal variations in the concentration of ascorbic acid in these tissues are shown in Fig. 1, together with the growth curve of the leaflets. The rapid growth in the early part of the summer was preceded by a similar increase in the concentration, which reached in the mesophyll 750 and 850 mg./100 g. in 1949 and 1950, respectively. The midribs and stalks showed somewhat lower levels, and except at the very beginning of growth, there was a concentration gradient from

the mesophyll into the stalks which was maintained throughout the growing season. The initial reverse flow suggested that the bud residues were the source of ascorbic acid required by the unfolding leaves until they were able to synthesize this.

Fig. 1. The seasonal variation in concentration of ascorbic acid during 1950. —, mesophyll; ---, midribs; - · - · -, stalks; ○—○, weight (mg.) of average leaflet.

Fig. 2. Seasonal variation in concentration of dehydroascorbic acid during 1950. —, mesophyll; ---, midribs; - · - · -, stalks.

A similar gradient from the mesophyll was established for dehydroascorbic acid (Fig. 2). This showed a maximum concentration at the same time as the reduced form, after which there was a steady fall in both until the leaf fall.

Stem. The concentration of ascorbic acid in the stem was only 150–200 mg./100 g. at its maximum. These figures were obtained on the outer cortex and phloem. The xylem and pith gave still lower values. As these concentrations were lower than that in the stalks, it would appear that the stem provided a path of conduction of ascorbic acid from the photosynthetic centres of the mesophyll to the rest of the plant.

In contrast, the concentration of dehydroascorbic acid remained fairly constant at 250 mg./100 g. throughout the year, and it is possible that it forms a source of ascorbic acid for the developing plant parts in the following year.

Fig. 3. The effect of growth upon the concentration (mg./100 g.) and amount (mg.) per fruit of ascorbic acid in whole fruits. $\bigcirc$ — $\bigcirc$, growth; —, concentration; - - -, amount per fruit.

Fruits. Fig. 3 shows the relationship between the increase in weight and the amount and concentration of ascorbic acid in whole fruits for 1950. An almost identical figure was obtained for 1949, although the concentration was somewhat higher (2000 mg./100 g. as compared with 1500 mg./100 g. for 1950). The concentration reached its peak when the fruits weighed 1–2 g., whereas the average amount per fruit was a maximum when they were fully grown.

Analysis of the separate tissues showed both the epicarp and mesocarp to contain 800–900 mg./100 g. throughout the entire growing period (Fig. 4), whilst the endocarp gave much higher values (3400 and 2500 mg./100 g. in 1949 and 1950). These figures

were obtained on the endocarp immediately prior to lignification. As this process proceeded, the concentration in the tissue as a whole showed a steady

Fig. 4. Seasonal variation in concentration of ascorbic acid in the tissues of the fruit. —, epicarp; - - -, mesocarp; - · - · -, endocarp; x—x, seed; ·····, shell.

Fig. 5. The effect of lignification on the concentration of ascorbic acid in the endocarp of the fruit. —, weight of soft tissue; - - -, weight of total tissue; - · - · -, concentration of ascorbic acid in the soft tissue; ·····, concentration of ascorbic acid in the total tissue; x—x, dehydroascorbic acid in the soft tissue.

decline to the fall of the fruit (Fig. 5). However, if the soft part of the tissue alone were considered, the concentration remained reasonably constant until the rapid decrease when maturation was complete.

This suggested that the high level was maintained only by virtue of the diminishing weight of the tissue.

Although surrounded by these extremely high concentrations, the seed (Fig. 4) showed very low levels, and the woody shell showed a steadily decreasing amount of ascorbic acid.

If the fruit is considered in conjunction with the rest of the plant, it is possible to follow the concentration gradient of ascorbic acid from the mesophyll of the leaves to the fruit stalk and possibly into the mesocarp. As the epicarp contains chlorophyll, it can supply further quantities of ascorbic acid by photosynthesis. These facts, however, do not explain the high levels in the endocarp which can be maintained by preformed ascorbic acid only if it is translocated against a steep concentration gradient, which is improbable (Wokes & Melville, 1948).

An alternative suggestion is that the ascorbic acid is synthesized in the endocarp. The intermediates in this proposed synthesis are first, precursors which are translocated from other parts of the plant, secondly, glucose (which is found in very high concentrations in the fruit), and thirdly, dehydroascorbic acid.

Zilva, Kidd & West (1938) showed that there was a change in the relative proportions of ascorbic and dehydroascorbic acids in Bramley's Seedling apples as the fruit developed, with the equilibrium shifting in favour of ascorbic acid. In the walnut, when the fruits are small, a concentration gradient of the

oxidized form exists both from the outer tissues of the fruit and from the stem, into the endocarp.

It will be shown in a future paper, that in the presence of ascorbic acid, the epicarp and mesocarp are able to synthesize hydrojuglone glucoside. Examination of extracts failed to reveal sufficient dehydroascorbic acid to account for the high concentration of the glucoside in these tissues. It is presumed, therefore, that the dehydroascorbic acid is rapidly removed and utilized by the endocarp.

SUMMARY

1. A spectrophotometric assay for ascorbic acid and dehydroascorbic acid has been applied to extracts prepared from various parts of the walnut (*Juglans regia*).

2. Definite seasonal variations have been found for the concentration and amount per unit of these substances.

3. In order to explain the high concentration of ascorbic acid in the endocarp, which appears to preclude its translocation as such from the other parts of the plant, it is suggested that dehydroascorbic acid may be utilized by this tissue.

I wish to thank Dr F. Wokes, the Director of the Laboratories, for affording me the opportunity to publish this work, and Miss N. Baxter for technical help in the preparation of the many extracts used.

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Non-protein Nitrogenous Constituents of Rye Grass: Ionophoretic Fractionation and Isolation of a 'Bound Amino-acid' Fraction

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About one-sixth part of the organically combined nitrogen of freshly picked green leaves is in the form of compounds of low molecular weight, in the main free amino-acids. Since these are probably the most reactive nitrogenous compounds when leaves are eaten by the ruminant animal, or made into silage and so forth, it is important to have better knowledge of their nature, quite apart from the importance of this knowledge for plant physiology.

During the latter part of the nineteenth century, studies of plant juices, especially those by Schulze, were very fruitful for biochemistry, and particularly the biochemistry of nitrogenous substances. Subsequently, however, such studies were rather neglected. Plant physiologists indeed made extensive series of measurements of the concentrations of individual compounds or groups of compounds, and their variation with changing conditions, but