LXXIV. THE PERSISTENCE OF VITAMIN A IN PLANT TISSUES.

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It was recognised at a very early stage of research on vitamin A that those plant tissues which have been engaged in the process of carbon assimilation contain relatively large quantities of this factor and that resting tissues such as seeds contain very small quantities, if any, of it. Its absence from etiolated leaves (*i.e.* those which have never been exposed to light) has been shown, and also its presence in all plant tissues so far examined which contain lipochromes and which have also been exposed to the light. Of underground structures, the only ones found to contain more than the merest trace are the carrot and the yellow sweet potato, each of these also containing lipochromes [Steenbock and Gross, 1919; Steenbock, Sell and Boutwell, 1921; Steenbock and Sell, 1922; Coward, 1923, 2]. Comparisons between different green vegetables or salads have sometimes been drawn with regard to the vitamin content, but apparently very little consideration has been paid to the possible differences of vitamin content of leaves of the same plant grown or preserved under different conditions. Hart, Steenbock, Hoppert and Bethke [1922] drew attention to a difference in vitamin content of two different samples of hay but ascribe it to the method of curing rather than to a difference in the hay on cutting. Drummond and his co-workers [1924] had also noticed a very marked difference in the vitamin A content of butters made from the milk of cows fed at the beginning of the experiment on a sample of hay which had been cut when growing vigorously and, later, on one which had been cut when it was beginning to wither. That this difference in the butters could be ascribed largely to a difference in the vitamin content of the two samples of hay was shown in the results of a feeding test of the hays themselves on rats. An isolated observation by Coward and Drummond [1921] that shoots of *Tradescantia* kept in water-culture solution in the dark for some 8 weeks had lost only very little of their vitamin activity, and a further observation by Coward [1923, 1] that sunflower seedlings exposed to the light and then kept in the dark for (*a*) 1, (*b*) 8 days had apparently lost none of their activity, seemed to make it desirable to investigate the question of the persistence of vitamin A in plant tissues more fully. It was decided to use wheat seedlings

for this purpose because they could be grown easily under laboratory conditions and also to compare green and yellowing autumn leaves, for supplies of these growing under natural conditions could easily be obtained.

EXPERIMENTAL.

Wheat seeds (Carter's Red Stand-up) were set in pots on successive days so that seedlings of the same age should be available for feeding on each day of the test. They were kept in the dark until 8 days old, transferred to the light as the experiment demanded and the shoots cut between 9 and 9.30 a.m. each morning. These were carried in a covered box to the animal room and were eaten immediately by the rats. The basal diet was that in general use in these laboratories. It consisted of:

Caseinogen, heated in thin layers 24 hours, 105°	20 g.
Starch (rice)	50 „
Hardened bean seed oil	10 „
Salt mixture	5 „
Yeast extract	5 „
Lemon juice	5 cc.

The daylight to which the shoots were subjected was never very brilliant, the experiment being carried out in July and August of 1924. It did not vary much from day to day, but any variations would be felt equally in the different experiments as they were all run concurrently with this purpose in view. At first the test was carried out on one shoot, then the dose was doubled and finally three shoots were used to obtain continued growth.

A. *Etiolated shoots.*

Eight days after the seeds were set, the shoots had grown to a height of about 3 inches. No evidence of even traces of vitamin A was obtained by giving one, two or three shoots to each test rat per day (Fig. 1 *a*).

B. *Effect of one day's exposure to light.*

Each day a pot containing 8 days old seedlings was exposed to such daylight as there was for 1 day. The shoots turned green and produced a small amount of vitamin A as shown in the curves of Fig. 1 *b*.

C. *Effect of absence of light after exposure to it.*

The pot of seedlings which had been exposed to light for 1 day was brought back into the dark room.

(1) First 4, then 8 and later 12 shoots were cut off close to the root and distributed equally between four small test tubes each containing 1 cc. tap water, the cut ends only standing in the water. After 24 hours the water from each test tube was given to a test rat by a pipette to see whether the vitamin had diffused into the water. The curves in Fig. 1 *c* show that there was apparently no diffusion.

(2) The shoots from the above experiment were given to other rats which only showed really satisfactory growth when the highest dose of three shoots was given (Fig. 1 *d*).

(3) Shoots which had been left on the plants in the pot during 24 hours in the dark were also tested, and caused resumption of growth in rats, though not such a satisfactory response as was given by the shoots which had been standing in water. It is doubtful whether the difference is great enough to justify a conclusion that the vitamin had travelled down to the living root more readily than it had diffused into the water (Fig. 1 *e*).

Fig. 1 *a, b*. Wheat seedlings.

(4) Other shoots of the same series were tested after the plants had been kept for 16 days in the dark. During this time, they had grown to about three times their original length, a second leaf had grown out of each shoot and the whole had become nearly colourless. A little green colour was left in the oldest leaf, and though the tips of the leaves had become dry and withered, for the most part the leaves were still fairly fresh. That the vitamin had not been destroyed during this time is shown in Fig. 1 *f*.

D. *The vitamin content of the withered leaf.*

A parallel series of seedlings was set and grown in the open air and daylight. The first-formed leaf was tested when the plants were 13 days old and was found to give growth. Similar first-formed leaves were tested when they were 43 days old and quite dried up and withered. They gave no growth (Fig. 2 *a* and *b*).

Hence it appears that the vitamin is not used up in any process carried on in the dark, and is not destroyed until the leaf really dies.

Confirmation of this point was sought in a comparison of the green and yellowing autumn leaves of various trees, *e.g.* lime, plane and poplar, and

also of watercress and cabbage. The tree leaves that were used, both green and yellow, were collected fresh each day from those that had been blown down during the previous day or two. The greenest parts were used for the "green" test, the yellowest (fresh) parts for the "yellow" test. The dead, withered leaves of the lime were also tested. The watercress supply was renewed twice

Grown 8 days in dark, given 1 day in
light, then kept 16 days in dark
(tissue not dry)

Fig. 1 f. Wheat seedlings.

Fig. 2. Wheat seedlings.

* This rat had both legs broken accidentally about this date, which may have accounted for a temporary fall in weight.

a week and a comparison made between the fresh green leaves of the shoots and the yellow ones that had changed colour since coming into the laboratory, during which time the shoots had been freely supplied with fresh tap water. In the case of the cabbage, the comparison was made between the strong green outer leaves and similar leaves that had turned yellow on the plant while its stem was standing in water in the laboratory. It was necessary to renew this supply twice a week in order to ensure the testing of yellowed

leaves that had grown under the same conditions as the green ones. In view of the fact that light is a controlling factor in the formation of vitamin A, the tests were carried out with as nearly as possible equal *areas* of leaf surface, i.e. about 3 square inches daily, rather than equal *weights*.

Two typical results of these feeding tests are seen in Figs. 3 and 4. In each case the growth obtained with the yellow leaves was at least as good as

that obtained with the green; it appears to be even better, but with such small numbers of animals in each test it is difficult to say whether the observed difference has any real significance. The result with the plane leaves, however, appears to be quite definite.

Withered lime leaves which had passed through the clear yellow stage, fallen from the tree and died, gave no growth.

Thus, it would appear that vitamin A is not used up in the yellowing autumn leaf until it actually dies.

One test was carried out on the calyx cups of the winter cherry (*Physalis Alkekengi*). The specimens used had been picked when quite fresh, dried and kept for 12 months. A dose of 0.1 g. per day was sufficient to cause resumption of growth in test animals. A similar test made previously [Coward, 1923, 2] on a different sample immediately after drying had given good growth also on a dosage of 0.1 g. daily.

It is interesting to examine these results in the light of the work of Steenbock and Nelson [1923] on the necessity of the antirachitic factor for growth. No attempt was made to supply the test rats with the antirachitic factor other than whatever may have been present in the shoots, but it may safely be assumed that where continuous growth was obtained (as with the three shoots in many cases), particularly after a long preparatory period (whether this was intentional or not), it must have been the growth-promoting vitamin A which the shoots supplied.

SUMMARY.

1. Vitamin A is not used up in any process carried on by living plant tissue in the dark.
2. It appears to increase when a leaf loses its green colour and becomes yellow.
3. It is completely destroyed when the leaf dries up and dies.
4. It does not diffuse appreciably into water from the cut ends of shoots.

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