An attempt was made to visualize the site of adenosine triphosphatase activity by incubating the diaphragm with and without added ATP in the presence of 0.001 *M* lead acetate (which did not inhibit the reaction). After washing with water and immersing the tissue in 1% sodium sulfide solution (to convert the precipitate of lead phosphate formed at the site of hydrolysis to the readily visible lead sulfide), the control diaphragm showed deposits of lead sulfide only at the cut edges while the diaphragm incubated in the presence of ATP showed marked deposits of lead sulfide conforming mainly to the outline of the muscle bundles at the surface of the tissue.

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Further studies on the formation of ascorbic acid in plants

Tracer studies[1-5] on the urinary L-ascorbic acid recovered from rats administered ^{14}C-labeled D-glucose have led to the following proposed pathway of conversion:

D-glucose → D-glucuronolactone → L-gulonolactone → L-ascorbic acid.

Non-isotopic experiments[6,7] on animals (normal rat) and plants (cress seedling) with various postulated intermediates led to similar conclusions. The latter studies also proposed an analogous pathway for the participation of D-galactose:

D-galactose → D-galacturonate → L-galactonolactone → L-ascorbic acid.

A study[8] of the conversion of D-glucose-1-^{14}C into L-ascorbic acid in the ripening strawberry led to the discovery that unlike the ^{14}C experiments involving the rat[2,4,5], no inversion occurred in the location of label in the ascorbic acid; that is, in the strawberry, carbon-1 of D-glucose became carbon-1 of L-ascorbic acid, in contrast to the path of conversion in the rat where carbon-1 of D-glucose became carbon-6 of L-ascorbic acid.

In view of the observations[7] relating the pathway of ascorbic acid formation in plants and animals, it became a matter of interest to learn whether the strawberry results represented a unique or possibly an alternative pathway, or whether the observation was representative of the normal path of ascorbic acid formation in plants. For this reason, experiments similar to those employing strawberries fed with D-glucose-1-^{14}C were conducted with cress seedlings (*Lepidum sativum*). In a preliminary study, etiolated seedlings, about 44 hours old, were separated from their testas and partially immersed in a 0.1% D-glucose-1-^{14}C solution (6.1 μc) for 33 hours. After an additional 17 hours in distilled water, the etiolated seedlings were ground up in boiling water, cooled, and centrifuged free of insolubles. The ascorbic acid was recovered as previously described[8]. After three crystallizations from glacial acetic acid, the ascorbic acid contained 470 c.p.m./mmole. Carbon-1 (by decarboxylation) contained 300 c.p.m./mmole as ascorbic acid. Apparently, 64% of the counts were in carbon-1.

In order to confirm this observation, a second experiment was performed. Sterile technique was observed as closely as was practical. The seeds (250) were given a preliminary five-minute soak in saturated calcium hypochlorite followed by several rinses with sterile distilled water. The etiolated seedlings were germinated over a period of 66 hours and then treated (without

TABLE I

RADIOACTIVITY OF VARIOUS CARBONS IN L-ASCORBIC ACID FROM D-GLUCOSE-1-^{14}C LABELED CRESS SEEDLINGS

(Solid-sample-counting method)

Carbon	Activity in c.p.m./mmole of ascorbic acid (The values in brackets are the observed c.p.m. above background for each sample)	
	1st Crop	2nd Crop
1	420 (9)*	
1 + 2	500 (48)**	420 (39)**
3	40 (3)	30 (2)
4 + 5	70 (3)	110 (3)
6	120 (6)	120 (7)

* Sample diluted an additional 2.5 times with carrier prior to decarboxylation.

** Counted as calcium oxalate.

TABLE II

RADIOACTIVITY OF VARIOUS CARBONS IN L-ASCORBIC ACID FROM D-GLUCOSE-1-^{14}C LABELED CRESS SEEDLINGS

(Gas-phase-counting method)

Carbon	Activity	
	c.p.m./mg carbon	c.p.m./mmoles of ascorbic acid
1	194	2,330
1 + 2	102	2,450
3	7	80
4 + 5	4	100
6	45	540
		Sum 3,170
1 + 2 + 3 + 4 + 5 + 6*		3,240

* Wet combustion.

disturbing the seedlings) with 5.9 μc of a 0.1% D-glucose-1-^{14}C solution that had been autoclaved for five minutes. After an additional 47 hours at room temperature, the seedlings were harvested free of testas and washed several times until the activity of the wash fell to a low value. The ascorbic acid was separated, diluted with carrier, and crystallized from methanol-ethyl ether and then from glacial acetic acid. Two successive crops of crystals were degraded. The $BaCO_3$ or oxalate precipitate of each fraction was prepared as previously described[8], and counted for a sufficiently long time in a methane end-window proportional counter to distinguish 2 c.p.m. above background with a precision of $\pm$ 1 c.p.m. The results are given in Table I. In addition, the $BaCO_3$ and oxalate samples from the second crop degradation were converted to CO_2 and counted using the more sensitive methane proportional gas-phase-counting method of BERNSTEIN AND BALLENTINE[9]. These results are summarized in Table II.

The results from Table II show that 73% of the total activity of the ascorbic acid from the cress seedlings was located in carbon-1. Another 17% was found in carbon-6. This pattern of labeling is similar to that found in ripening strawberries[8] and is most easily interpreted as evidence that the cress seedling also converts D-glucose-1-^{14}C to L-ascorbic acid without cleavage of the carbon chain and in such a way that the aldehydic carbon of glucose becomes the carboxyl carbon of ascorbic acid.

The present results, together with those previously presented for the strawberry, strongly suggest that ascorbic acid formation in plants differs considerably from the pathway found in the rat. Although it is tempting to speculate upon the nature of the pathway involved in this synthesis, neither the sequence of reactions nor the number of steps involved are known. Further studies concerning these processes are in progress in this Laboratory and will be reported from time to time.

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