Randomization of the carbon atoms in glucose and fructose during their metabolism in barley seedlings

Incubation of various plant tissues with 1-^{14}C- or 6-^{14}C-glucose or -fructose has shown that during incorporation into sucrose and cellulose partial randomization occurs between carbon atoms 1 and 6 of hexose residues: fructose or glucose isolated from these compounds were found to contain some 10–25% of the total activity of the hexose in the terminal carbon atom remote from the one originally labelled (NEISH[1], EDELMAN, GINSBURG AND HASSID[2], SHAFIZADEH AND WOLFROM[3]). These observations have led to the suggestion that some at least of the hexose molecules are cleaved into triose derivatives which equilibrate before resynthesis to hexose and subsequent polymerization. However, studies by SEEGMILLER and co-workers[4,5], on galacturonic acid residues from strawberry and boysenberry pectin after incorporation of specifically labelled glucose, indicated that there may be an alternative pathway. Furthermore, degradation of cellulose formed by *Acetobacter xylinum* from specifically labelled hexose has indicated that a pentose phosphate system is involved[6].

To elucidate this problem in plant tissue the experiments reported here were undertaken. 1-^{14}C- or 2-^{14}C-glucose or 4:5:6-^{14}C-fructose were used as substrates for sucrose and cellulose synthesis in an attempt to follow all possible interchanges of carbon atoms in the hexose skeleton. Barley was chosen for this work, as aqueous ethanol extracts of whole seedlings incubated with labelled hexose gave chromatograms and radioautographs similar to those of wheat seedlings (EDELMAN *et al.*[2]) and they absorbed tracer amounts of sugar faster than other cereals.

Uniformly-^{14}C-labelled glucose and fructose were prepared by hydrolysis of sucrose isolated from *Canna* leaves which had photosynthesised in an atmosphere containing $^{14}CO_2$. 1-^{14}C-glucose and 2-^{14}C-glucose were purchased from the Radiochemical Centre, Amersham, England. 4:5:6-^{14}C-fructose was synthesised by the method of EDELMAN AND SHIBKO[7]. Degradation of this fructose showed that carbon atoms 4, 5 and 6 were equally labelled and contained 92% of the radioactivity in the molecule; carbons 1, 2 and 3 contained less than 4%.

Seedlings about 3 days old were treated in the way described by EDELMAN *et al.*[2] for wheat seedlings; roots and scutella* were cut from whole seedlings immediately before incubation with tracer amounts of sugar (< 0.2 mg/ml medium). The degradative method for glucose was a modification of that of GUNSALUS AND GIBBS[8] using *Leuconostoc mesenteroides* (see EDELMAN *et al.*[2]).

Excised roots incorporated activity into glucose, fructose, sucrose, hexose phosphate, and other compounds soluble in 80% ethanol, as well as into insoluble substances including cellulose. In contrast, excised scutella accumulated activity mainly into sucrose, with little or none in the monosaccharide fraction (Table I).

TABLE I

DISTRIBUTION OF ACTIVITY IN TISSUE FRACTIONS

Tissue from 30 seedlings was incubated with 5 ml water containing 5 μc uniform-^{14}C-glucose§ (< 0.2 mg/ml) for 4 h at room temperature in darkness.

	Substrate utilized %	*Activity incorporated as % substrate utilized*			
		Free glucose and fructose	*Sucrose*	*Other ethanol-soluble compounds including sugar phosphates*	*Ethanol-insoluble compounds including cellulose*
Roots	90	6.3	8.9	39	20
Scutella	63	1.3	24	15	5.0

§ Similar results were obtained with uniform-^{14}C-fructose.

Experiments using 1-^{14}C-glucose and short time intervals (30 min) confirmed previous findings—not hitherto demonstrated with barley tissue—that carbon atom 6 of incorporated hexose contained about one sixth of the total activity of the molecule; carbon 1 contained almost all the remaining activity. There was little further change on prolonged incubation (10 h). With 2-^{14}C-glucose, a similar proportion of activity appeared in carbon atom 5, and with 4:5:6-^{14}C-fructose a similar proportion in carbons 1, 2 and 3. Results of degradation of free glucose and glucose from sucrose, sugar phosphate and cellulose in typical experiments are shown in Table II. The results of many experiments showed that this randomization within the carbon skeleton of

* The scutellum is the organ through which storage material from the grain is transferred to the seedling.

TABLE II

DISTRIBUTION OF ACTIVITY IN ROOT CONSTITUENTS AFTER INCUBATION WITH SPECIFICALLY LABELLED HEXOSE

Conditions as for Table I, but with 20 μc substrate. Values given as % total activity in molecule.

Carbon atom	*Free glucose*	*Glucose from*		
		Sucrose	*Sugar phosphate*	*Cellulose*
A. 1-^{14}C-glucose				
1	78.0	82 (76.5)*	75.8	75.4
2	1.0	2.7 (0.5)	2.6	4.5
3	0.6	1.3 (2.0)	1.4	1.5
4	0.6	0.8 (2.0)	2.4	1.5
5	0.0	0.2 (0.3)	0.6	0.0
6	16.0	17.8 (17.3)	19.9	18.9
B. 2-^{14}C-glucose				
1	3.5	3.6	6.0	5.0
2	76.5	77.0	72.3	70.0
3	3.5	3.6	6.0	5.0
4	1.2	1.2	1.8	1.6
5	15.0	14.0	14.3	16.2
6	1.2	1.2	2.1	1.6
C. 4:5:6-^{14}C-fructose**				
1	7.0	6.5	8.2	8.4
2	17.6	6.9	9.0	9.1
3		6.4	7.6	7.1
4	81.0	22.5	25.0	27.0
5		22.9	28.6	28.0
6		21.0	27.4	20.0

* Figures in parentheses are for isolated scutella.

** Roots from 50 seedlings incubated for 5 h with 10 μc substrate.

glucose always occurred; comparison of the distribution of ^{14}C (Table II) showed that even the free glucose in roots was derived from the same metabolic pool as the polymers and not by passive diffusion.

The three substrates 1-, 2-, and 4:5:6-^{14}C-hexose were chosen to enable all possible interchanges among carbon atoms in the hexose molecule to be detected within reasonable limits, and their use demonstrated that such interchange occurred almost entirely within the pairs of carbon atoms 1 and 6, 2 and 5, 3 and 4, the degree of randomization being similar in each case; these data can be explained by the hypothesis that a large proportion of the hexose molecule is randomized by cleavage into 3-carbon fragments followed by equilibration and resynthesis to hexose (the labelling of carbons 1 and 3 in the 2-^{14}C glucose experiment indicates that the hexose monophosphate shunt had also occurred to a small extent).

It should be noted, however, that sugar alcohols play a role in carbohydrate metabolism in some animal tissues[9,10] and micro-organisms[11], and the possibility of conversion of hexose to a compound such as D-mannitol whose symmetry allows carbons 1, 2 and 3 to become 6, 5 and 4 respectively on reconversion to glucose cannot be ruled out.

As, by both these hypotheses, activity appearing in the originally unlabelled half of glucose is essentially due to a proportion of the molecules becoming labelled equally in both halves, the appearance of 15–25% activity in the formerly unlabelled half means that at least 30–50% of the molecules have undergone randomization. If equilibration of the triose phosphates is not instantaneous, an even greater proportion than this may have been involved. The fact that such extensive randomization occurs during such short time intervals and in such diverse tissues under differing conditions may indicate that it is a necessary step in either the absorption of hexose or its further polymerization.

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Intracellular distribution of vitamin E and vitamin A in chicken liver

Since tocopherol has been found to be an activator or cofactor in the reduction of cytochrome *c* by DPNH[1] and may also be implicated in oxidative phosphorylation, it seemed of interest to examine the intracellular distribution of this vitamin in liver, and, in particular, its concentration in the mitochondria. Investigations of the vitamin K content have already been made in this laboratory[2], and other workers[3] have investigated the distribution of tocopherol in heart muscle preparations. In the present study vitamin A determinations were also carried out.

22 newly hatched chicks were reared on a "normal" ration[4] for 6 days and then divided into two groups. Group 1692, consisting of 12 chicks, received for 39 days a 20% casein, "fat-free" diet (No. 3 in Table I of ref.[5]). Group 1694, consisting of 10 chicks, received a corresponding 15% casein, "fat-free" diet for 42 days. The diets were supplemented with 100 mg *d,l*-α-tocopherol acetate ("Ephynal", Roche) per kg.

TABLE I

AMOUNT OF NITROGEN, α-TOCOPHEROL AND VITAMIN A IN FRACTIONS OF 100 g FRESH CHICKEN LIVER

Group No.	*1692**				*1694***			
% casein in the diet	*20*				*15*			
Liver fraction	*"N"*	*"M"*	*"P + S"*	*Total*	*"N"*	*"M"*	*"P + S"*	*Total*
g N/100 g fresh liver	0.85	0.37	1.15	2.37	0.84	0.23	1.20	2.27
N content as % of total N	36	16	48	100	37	10	53	100
μg α-tocopherol/100 g liver	415	343	792	1550	446	283	1140	1869
α-tocopherol as % of total tocopherol	27	22	51	100	24	15	61	100
μg α-tocopherol/g N	490	930	690		530	1230	950	
μmoles α-tocopherol/g N	1.14	2.16	1.60		1.23	2.86	2.20	
μg vitamin A/100 g liver	480	167	1650	2297				
vitamin A as % of total vitamin A	21	7	72	100				
μg vitamin A/g N	564	450	1440					
μmoles vitamin A/g N	1.97	1.57	5.03					

* 107.2 g liver from 12 chicks were used for the preparation of the fractions.
** 74.5 g liver from 10 chicks were used for the preparation of the fractions.

The animals were killed by decapitation. The livers were removed, weighed immediately and homogenized in 9 vol. 0.25*M* sucrose using a Teflon homogenizer. The fractionation was carried out as described by SCHNEIDER AND HOGEBOOM[6], except that after separation of the mitochondria the remaining supernatant (containing the submicroscopic particles (P) and the soluble fraction (S)) was not fractionated. The three fractions—nuclei (N), mitochondria (M), and supernatant (P + S)—were freeze-dried in a Stokes "Freeze-dryer apparatus type 103-LPM".

The tocopherol content of the fractions was determined in the following way: Weighed amounts of the fractions were saponified by heating under reflux—in an atmosphere of nitrogen