walk-in refrigerator at 0°. The buffer utilized was: K_2HPO_4, 0.067 *M*; KH_2PO_4, 0.042 *M*; $MgCl_2$, 0.06 *M*; nicotinamide 0.03 *M*, *p*H 7.[5] Surviving tissue slices were prepared in the usual way with a Stadie slicer. Cell-free homogenates were prepared from tissue minces following rapid homogenization for 20 seconds in a loose fitting Potter–Elvehjem glass homogenizer. Two and a half volumes of buffer were employed. Cell debris, cells and nuclei were removed by centrifugation at 2000 r.p.m. for 7 minutes. The remaining particulate fraction was subjected to centrifugation at 20,000 r.p.m. for 30 minutes (40,000 *g*) and the resulting mitochondria were washed with cold buffer solution and resedimented (fraction M). The mitocnondria were extracted by the method of deDuve.[6] The microsomal fraction was obtained after centrifugation at 36,000 r.p.m. for 30 minutes (100,000 g.). A Spinco preparative ultracentrifuge was utilized for all the separations and the speeds indicated refer to dial readings. Flask additions were 1 milligram each of uridine triphosphate, diphosphopyridine nucleotide, adenosine monophosphate and C^{14}-glucurone (1 mc./mmole); except when specified. The time interval between preparation of the glucurone solution and the incubation of the solution is given for each experiment. The evolved CO_2 was absorbed by a saturated $Ba(OH)_2$ solution layered with toluene. Measurements of radioactivity were performed in a flow counter; the results were corrected to infinite thinness.

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Biosynthesis[1] of C^{14}-Labeled Cotton Cellulose from D-Glucose-1-C^{14} and D-Glucose-6-C^{14}

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To study the mechanism of controlled ignition of cellulose nitrate which results in the formation of a variety of decomposition products,[2] we were interested in the production of specifically labeled cellulose-C^{14}. The biosynthesis of labeled cellulose has been investigated in the cultures of *Acetobacter xylinum*[3] and *Acetobacter acetigenum*,[4] in a maturing cotton boll,[5] and in growing wheat seedlings.[6,7] Greathouse and associates have reported that the biosynthesis in a maturing cotton boll required the introduction of D-glucose-1-C^{14} at the time of maximum sugar translocation (21 days after fertilization of the flower) and the product cotton cellulose-C^{14} gave on hydrolysis a 44% radiochemical yield of D-glucose-1-C^{14} with 99.97% of the activity at position one[5]; the culture of *Acetobacter xylinum*, in a medium containing D-glucose-1-C^{14} and ethanol, produced bacterial cellulose-C^{14} in a 3.7% radiochemical yield with 70% of the label at carbon 1 and 28.7% at positions 3 and 4.[3] The *Acetobacter acetigenum*, when grown in a medium containing DL-lactate-1-C^{14}, gave a product cellulose which was labeled mainly in the middle positions of the D-glucose molecules.[4] Conversely, the radioactive D-glucose unit of the cellulose that was biosynthesized from D-glucose-1-C^{14} and D-glucose-6-C^{14} by the growing wheat seedlings, had about 90% of its activity at the terminal positions (approximately 70% at the original terminal position and 20% at the other).[7] Of these products, labeled cotton cellulose was best suited for our requirements.

Small quantities of D-glucose-1-C^{14} were introduced into ten selected cotton bolls according to the method of Greathouse[5] and the treated bolls were allowed to continue in their normal course of development. The crops of radioactive cotton were separated from the seeds and the dried capsules, pooled together and purified; the seeds and dried capsules were found to be radioactive. A representative sample of the radioactive cotton was hydrolyzed by the procedure of Monier-Williams.[8] The resulting D-glucose was chromatographed over carbon[9] and oxidized to potassium D-gluconate.[10]

Sometime later, another like series of experiments was carried out with D-glucose-6-C^{14} but under better growth conditions. Radioactive assay of the C^{14}-labeled potassium D-gluconates indicated a radiochemical yield of approximately 10.6% for the biosynthesis of cellulose-C^{14} from D-glucose-1-C^{14} and 23.5% from D-glucose-6-C^{14}, based on the assumption that purified cotton consists of 100% pure cellulose. The distribution of the label between the terminal and middle positions of the anhydro-D-glucose units, which are given in Table I, was established by periodate oxidation of the potassium D-gluconates according to the method of Eisenberg.[11] As noted above, similar results have been obtained by Hassid and associates for the incorporation of D-glucose-1-C^{14} and -6-C^{14} into the cellulose formed by growing wheat seedlings.

TABLE I

C^{14}-LABELED COTTON CELLULOSE; DISTRIBUTION OF RADIOACTIVITY IN THE COMPONENT ANHYDRO-D-GLUCOSE UNITS

	Activity in μc./mole[a]		
	Cellulose from D-glucose-1-C^{14}		Cellulose from D-glucose-6-C^{14}
Experiment	A[b]	B[c]	C
D-Glucose	29.5	128.5	129.1
Carbon 1	19.1	60.5	28.6
Carbons 2-5	4.4	34.3	13.5
Carbon 6	6.6	38.7	92.0

[a] Microcuries per mole. [b] Preferred greenhouse growth conditions; 10 bolls combined. [c] Less favorable growth conditions; 1 boll.

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in the maturing cotton boll. Our data clearly demonstrate that this is not the sole mechanism for the formation of cellulose-C^{14} in the cotton boll and show that at least a part of the original radioactive sugar enters the structure of the polysaccharide through its scission products. In fact, the exchange of label between the terminal positions of D-glucose could be due to the reversible isomerization of dihydroxyacetone-1-C^{14} 1-phosphate to D-glycerose-3-C^{14} 3-phosphate by the enzyme triose isomerase,[12] in the glycolytic process[13] for the breakdown of D-glucose. This process could account for the conversion of a substantial amount of the radioactive sugar into other constituents of the cotton boll, such as the seed oil; these points will be treated in a succeeding communication.

In early spring, when a longer period of time was required for the development and maturity of the cotton bolls, a single experiment (B, Table I) furnished only a 4.7% radiochemical yield of cellulose with the label distribution shown, demonstrating a more extensive breakdown and resynthesis of the D-glucose-1-C^{14} before its conversion into cellulose. It appears that the period of maximum cellulose formation which, according to Greathouse,[5] should be 21 days after fertilization of the flower, varies with the change in the rate of development of the cotton bolls due to such factors as the season of the year and the general condition of the plants in the greenhouse. This could explain the difference between the yield and distribution of the label in our experiments (A, B and C). It would be logical to assume that should the labeled D-glucose be introduced before the period of maximum cellulose formation, the sugar would have a better chance to participate in other complex functions of the cotton boll.

Experimental

Preparation of C^{14}-Labeled Cotton Cellulose.—A number of cotton plants, *Gossypium hirsutum* var. *Paula*, were cultivated in 7-inch pots in the greenhouse of the Botany Department of The Ohio State University, and the dates of their flowerings[14] were carefully recorded. After some preliminary experiments (including expt. B of Table I) to find the best conditions, 15.7 μc. of D-glucose-1-C^{14} (150 mg., prepared according to Isbell and co-workers[15]) was introduced in equal quantities into 10 well developed 21-day old cotton bolls according to the method of Greathouse.[5] This experiment was performed during the month of July (expt. A, Table I). The plant was first segregated and not watered for 1 day. Then 1 cm. of the stem immediately below the boll was thinly sliced upward and the cut portion was introduced into a small glass cup of 1-ml. capacity sealed to a glass rod held in a clamp. The cup contained a solution of 15 mg. of D-glucose-1-C^{14} in 3 drops of water. After complete absorption of the sugar solution by the plant, the cup was washed twice with 2 drops of water and the washings were allowed to enter the plant in the same manner. This process was normally over within a few hours. The sliced stem was then carefully taped and the plant was watered and allowed to continue its normal course of development. The treated boll matured after 40 to 50 days. The resulting radioactive cotton was separated from the seeds and the dried capsule and its radioactivity was roughly counted at infinite thickness. It was then mixed with other crops, dewaxed and purified according to Greathouse[5]; yield 9.19 g. of dry cellulose (1.672 μc.).

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During September another 10 selected 21-day old cotton bolls, growing in a more suitable environment (Expt. C, Table I), were treated with 33.0 μc. of D-glucose-6-C^{14} (150 mg.[16]). They matured after 30 to 35 days; yield 9.77 g. of purified, dry radioactive cellulose (7.784 μc.).

Hydrolysis of Cellulose-C^{14}.—The purified radioactive cellulose from each experiment (Table I) was divided into 400-mg. portions which were radioassayed at infinite thickness and found to have about the same degree of activity. One gram from each experiment was then selected as a representative sample and was hydrolyzed with 72% sulfuric acid by the method of Monier-Williams.[8] The hydrolyzate was neutralized with a solution of barium hydroxide, centrifuged and filtered. The filtrate was passed through a column containing 40 ml. of an equal mixture of Amberlite resins IR-120 (H) and IR-4B (OH)[17] and concentrated under reduced pressure to 10 ml. In order to separate the partially hydrolyzed materials, the concentrated solution was passed through another column containing 20 g. of an equal mixture of activated carbon and Celite[18] according to Whistler and Durso,[9] and washed with 200 ml. of water. The solution was then lyophilized and the remaining sirup was crystallized from methanol and 2-propanol; yield 0.64 g. of D-glucose-C^{14}.

Radioactivity Determination.—D-Glucose-C^{14} from the hydrolysis of radioactive cellulose was oxidized to potassium D-gluconate by the procedure of Moore and Link[10] and counted after recrystallization from methanol and water. The distribution of label within the anhydro-D-glucose unit of the cellulose-C^{14} was obtained by degradation of the potassium D-gluconate with sodium periodate according to the method of Eisenberg,[11] and further oxidation of the resulting formic acid and formaldehyde to carbon dioxide. Thus the carbon dioxide from positions 1, 6 and 2–5 (combined) of the cellulose anhydro-D-glucose units was converted to barium carbonate and assayed for radioactivity (counted).

Counting Methods.—All samples were counted as solids at infinite thickness using a mica window Geiger tube[19] connected to a decade scaler,[20] and compared with a standard of the same material. The potassium D-gluconate used as a reference was standardized by conversion to barium carbonate[11] which was in turn compared with standard barium carbonate obtained from Tracerlab.[21] The samples were counted long enough to reduce the random counting error to ±3%, and the background radiation was kept to a minimum with a 2 in. thick lead shield.

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(20) Potter Instrument Co., Inc., Flushing, N. Y.

(21) Tracerlab, Inc., Boston 10, Mass.

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The *para*-Claisen Rearrangement. II. The α- and γ-Methylallyl Ethers of Methyl *o*-Cresotinate[1,2]

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Earlier work[3] on the preparation of the α-ethyl-

(1) Taken from the Ph.D. dissertation of Robert L. Crecelius, University of Wyoming, 1954.

(2) This work was supported by a Frederick Gardner Cottrell Grant from the Research Corporation.

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