

Asymmetric Distribution in the Biosynthesis of Cotton Cellulose-[U- ^{14}C]

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ABSTRACT

Autoradiographs of mature cotton bolls which earlier had radioglucose introduced via a thin incision into their peduncles show a marked asymmetry in distribution of the label. Radio-assay shows the specific activity of the cotton fibres on the treated side to be as much as 30-fold that on the opposite side.

INTRODUCTION

The biosynthesis of specifically labelled cellulose- ^{14}C in developing cotton bolls was first investigated by Greathouse (1953), who introduced *D*-glucose-[1- ^{14}C] at the time of optimum sugar translocation into the boll and reported the production of labelled cellulose in 44 per cent radiochemical yield, with 99.9 per cent of the label in the carbon-1 position. Using a potted *Gossypium herbaceum* cotton plant which had been allowed to dry until it lost some turgor, Greathouse made a thin upward incision into the xylem of the peduncle just below a 21-d old boll. The resulting flap was placed into a small cup containing the labelled glucose solution, where it served as a wick to introduce the glucose into the vascular system of the plant. Afterwards the stem was carefully taped to allow normal development of the boll until it was harvested at maturity.

Subsequently, Shafizadeh and Wolfrom (1955) used Greathouse's procedure with a different cotton species (*G. hirsutum*) and not only *D*-glucose-[1- ^{14}C] but the 6-labelled compound as well. Later, Shafizadeh, Wolfrom and McWain (1959) repeated the experiments with *D*-glucose-[2- ^{14}C]. However, in neither case were these investigators able to reproduce either the high specificity or the high yield reported by Greathouse. In particular, they found only about two-thirds of the label in the cellulose at the original position and found radiochemical yields of 23.5, 10.6, and 9.3 per cent with *D*-glucose-[6- ^{14}C], -[1- ^{14}C], and -[2- ^{14}C], respectively.

Because of our interest in obtaining gram quantities of specifically-labelled cellulose for pyrolysis studies, we recently undertook a series of similar experiments. In a preliminary search for appropriate operating procedures, we decided to use uniformly labelled radioglucose introduced under a variety of conditions.

WITH ONE PLATE IN THE TEXT

MATERIALS AND METHODS

Our experiments were performed on greenhouse-grown cotton plants *G. hirsutum* var. Acala 4-42, with an aqueous solution of D-glucose-[U- 14 C] (Amersham/Searle) diluted with unlabelled D-glucose to a specific activity of 1.2 μ Ci/mg. With the following two exceptions the procedure we used for introduction of the radioglucose (0.6 μ Ci in 0.05 ml) was identical with that of Greathouse:

Marx-Figini (1967) not only confirmed the earlier observation (Anderson and Kerr, 1938) that the rate of cellulose production is quite low during the period of growth of the primary cell wall (about 20 d following pollination), but demonstrated that the production rate during secondary thickening (from its inception about the twenty-first day to maturity of the boll some 30-40 d later) is remarkably constant and that the cellulose produced during this period has a nearly uniform degree of polymerization. Thus, to ensure avoidance of any complicating reactions occurring in the final stages of primary cell wall formation, we introduced the radioglucose about 35 d rather than 21 d after pollination.

After a series of experiments with and without partial drying of the plant before introducing the labelled glucose, in which the plants showed no discernible effects of dehydration, no further attempt was made to withhold water.

To determine radiochemical yield in the harvested boll, we ginned, dried, and weighed the cotton. After a sample was removed for radioassay, the remaining fibres were dewaxed, reweighed and again radioassayed. For radioassay, the samples were quantitatively oxidized under reduced pressure by using Van Slyke-Folch reagent (Van Slyke and Folch, 1940). The evolved $^{14}\text{CO}_2$ was collected in a calibrated ionization chamber and the radioactivity measured by a vibrating reed electrometer.

For autoradiographic monitoring of the distribution of radioactivity at harvest, we cut a thin slice from the boll in the plane bisecting the peduncle incision. From each of the two resulting hemispheroids, an axial slice was removed perpendicular to the first cut, and the fibres from the residual quadrants set aside for radioassay. The slices to be autoradiographed were glued onto heavy paper, allowed to air dry for 2 d, then covered with a thin sheet of polyethylene (1 mg/cm 2) and clamped to a sheet of medical X-ray film (Kodak RP Royal X-OMAT) for one week.

RESULTS AND DISCUSSION

Although the average radiochemical yield in these experiments was about 9 per cent, the results obtained were highly variable—even for aliquots of the same boll. The cause of this variability was readily revealed by the autoradiographs. As illustrated in Plate 1, there is a marked asymmetry in the distribution of the label. Blackening of the film is observed only on the side on which glucose was introduced into the peduncle. Radioassay of the various sections of such bolls showed the specific activity on the side under the darkened areas of the film to be as much as 30 times that on the other side. Thus, there appears to be only limited lateral movement of the labelled glucose as it moves through the peduncle and into the boll. (Microscopic examination of tissue sections by Dr. Carl E. Crisp, plant physiologist, Pacific Southwest Forest and Range Experiment Station, also indicate that cotton vascularization is such that specific conduction bundles supply specific areas of the boll.) This must be considered in efforts to obtain homogeneous radio-cellulose or samples with the highest possible radiochemical yields.

In a separate experiment with three developing bolls on a single branch, we found that when radioglucose was introduced into the terminal peduncle, cotton of similar specific activities was obtained in all three bolls, although asymmetry was evident only in the terminal boll. Thus, results obtained when several bolls are close together should be interpreted with caution. Indeed, such situations must be avoided if several bolls are to be used for different labelling experiments.

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EXPLANATION OF PLATE

PLATE 1. Photograph (left) and autoradiograph (right) of transection of radioglucose-fed cotton boll shows the extreme asymmetry of radiocellulose distribution.

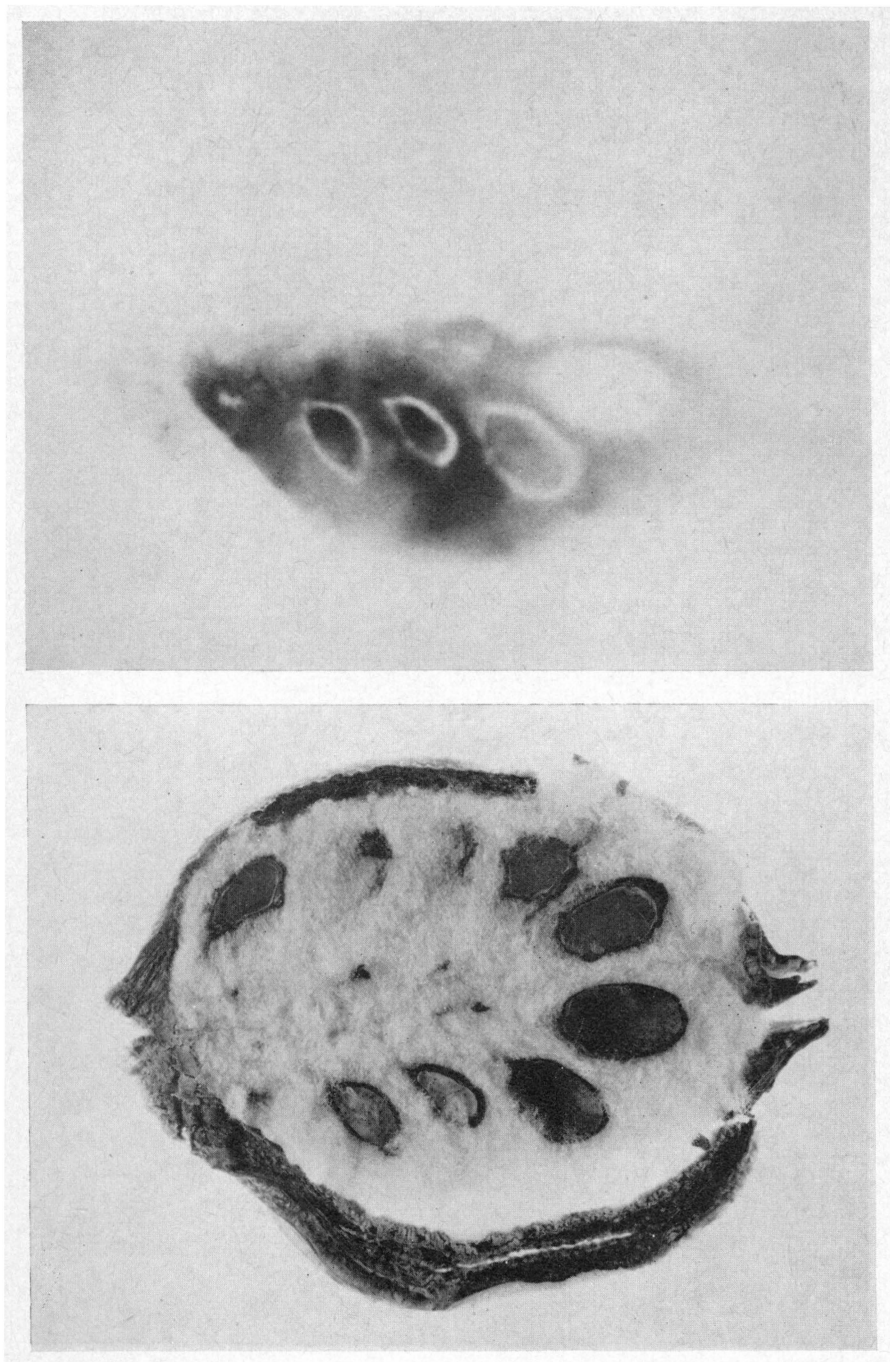


PLATE 1