References

1. CUNNINGHAM, L., GRIFFIN, A. C., and LUCK, J. M. *J. Gen. Physiol.*, **34**, 59 (1950).
2. DAVIDSON, J. N., and LESLIE, I. *Cancer Research*, **10**, 587 (1950).
3. DOUNCE, A. L., *et al. J. Gen. Physiol.*, **33**, 629 (1950).
4. PRICE, J. M., and LAIRD, A. K. *Cancer Research*, **10**, 650 (1950).
5. HARRISON, M. F. *Nature*, **168**, 248 (1951).
6. LEUCHTENBERGER, C., VENDRELY, R., and VENDRELY, C. *Proc. Natl. Acad. Sci. U. S.*, 37, 33 (1951).
7. LEUCHTENBERGER, C., *et al. Exptl. Cell Research*, **3**, 240 (1952).
8. CAMPBELL, R. M., and KOSTERLITZ, H. W. *Science*, **115**, 84 (1952).
9. SCHNEIDER, W. *Cold Spring Harbor Symposia Quant. Biol.*, **12**, 169 (1947).
10. DAVIDSON, J. N., *et al. Biochem. J.* (*London*), **46**, Proc. xl (1950).
11. ALBERT, S., JOHNSON, R. M., and COHAN, M. S. *Cancer Research*, **11**, 772 (1951).
12. WILBUR, K. M., and ANDERSON, N. G. *Exptl. Cell Research.*, **2**, 47 (1951).
13. HOGEBOOM, G. H., SCHNEIDER, W. C., and STRIEBICH, M. J. *J. Biol. Chem.*, **196**, 111 (1952).
14. SCHNEIDER, R. M., and PETERMANN, M. L. *Cancer Research*, **10**, 751 (1950).

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Biosynthesis of C^{14}-Specifically Labeled Cotton Cellulose[1]

Glenn A. Greathouse[2, 3]

Engineering and Industrial Experiment Station, University of Florida, Gainesville

Cellulose is formed biologically by a process of which the chemistry is almost unknown. In 1949 the author conceived the idea of synthesizing position-labeled bacterial cellulose from sugars labeled specifically with radioactive C^{14}. It was considered that a study of radioactive labeled celluloses synthesized by the microorganism *Acetobacter xylinum*, cultured on, or supplied with, C^{14}-specifically labeled substrates, might help to elucidate the mechanism of normal cellulose biosynthesis. Accordingly, arrangements were made for collaboration with H. S. Isbell and his associates at the National Bureau of Standards and with Milton Harris and his associates at the Harris Research Laboratories. A program was formulated for a joint project, and the Atomic Energy Commission awarded the funds for the proposed basic research. Four reports or publications have resulted from the synthesis of radioactive-labeled sugars and of C^{14}-labeled bacterial cellulose, two from the former (*1, 2*) and two from the latter (*3, 4*).

With the study of specifically labeled bacterial cellulose well under way, the author planned some experiments to learn if radioactive cellulose could be produced in the *Gossypium herbaceum* cotton boll. It is the purpose of this paper to report the success of the experiments concerned with the biosynthesis of C^{14}-specifically labeled cellulose in a cotton boll, while attached to the plant.

Approach. Previous determinations by the author, as well as by other workers, had indicated that the reducing-sugar content of the cotton boll is 60–70% between the time of pollination of the flower and 21 days following fertilization. Likewise, the literature (*5*) showed that the primary cell wall of the cotton fiber is formed and reaches its full length during the first 20 days or so following fertilization of the flower. With this information available, it was reasoned that, if D-glucose-1-C^{14} were introduced into the cotton boll through the vascular system of the plant at the time of optimum sugar translocation and cellulose synthesis, it would diffuse with the other sugars and enter into the cellulose formed by the boll. The importance of introducing the labeled sugar just at the time of optimum sugar translocation cannot be overemphasized. The best results from the standpoint of glucose-1-C^{14} utilization should be obtained at the time the greatest quantity of reducing sugars is being converted to cellulose in the normal boll. This, according to the best prevailing knowledge of cellulose formation, should be approximately 21 days after fertilization of the flower.

Procedure and Results. Four Stoneville 2 B cotton plants were grown from seed in 6-in. pots containing soil in a greenhouse of the U. S. Department of Agriculture at Beltsville, Md. The plants grew and flowered normally. Considerable experimentation was required to develop the best procedure for introducing glucose into the boll without hindering its development. The procedure selected was to slice the stem longitudinally just below the boll with a razor blade and place the cut portion into the sugar solution. The soil in which the plant was growing was first allowed to dry to the point that the plant lost some turgor and was then watered approximately 30 min before introduction of the glucose-1-C^{14}. The sugar solution is taken into the boll within a few minutes, the rate of transfer varying with the sugar content of the boll at the time of experiment. The stem and the boll were carefully taped to prevent injury and to allow normal boll development.

One experiment was performed by introducing the glucose-1-C^{14} into the boll (ovary) the same day the flower was pollinated and harvesting the developing boll on the 21st day. The cotton fibers were removed, dewaxed (*6, 7*), and extracted with 1% NaOH. The small quantity of primary-wall cellulose remaining was assayed for radioactivity. The radioactivity of the cellulose itself was low; the extracts taken of the cellulose and of the remaining parts of the cotton boll contained most of the radioactivity introduced as glucose-1-C^{14}.

Accordingly, a second experiment was performed in which 12.5 μc of glucose-1-C^{14} were introduced

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[3] Present address: Prevention of Deterioration Center, National Research Council, Washington, D. C.

through the sliced stems just below the 21-day boll. The treated boll matured 30 days later. The fiber from the treated boll was separated from the seeds and extracted with ethyl alcohol in a Soxhlet extractor for 5 hr (*7*) to remove the waxes quantitatively (*6*). The extracted fiber was air-dried and then boiled in 300 ml of 1% NaOH for 1 hr, the fiber being tied with thread to a glass rod to keep it well under the surface and prevent oxidation. On removal from the alkali, the fiber was rinsed in dilute acetic acid, then in water repeatedly until rinsings no longer were acid, and the sample was dried at 50° C for 2 hr in a circulating-air oven. Yield: 406 mg of purified cellulose.

The purified cellulose was hydrolyzed by the method of Monier-Williams (*8*). A yield of 337 mg of unpurified sugar was recovered from the cellulose hydrolyzate. The crude sugar was made up to 5 ml with water, and a 1/100-ml portion was removed for direct determination of C^{14} activity according to the method of Schwebel, Isbell, and Karabinos (*9*). This procedure indicated that 5.49 μc C^{14} were present in the 5 ml of sugar solution. Thus, approximately 44% of the radioactive C^{14} was converted to cellulose and recovered in the glucose molecule.

The 5 ml (less the 1/100 ml used for analysis) of sugar solution were passed through approximately 5 g of Darco G-60 and Celite according to the method of Whistler and Durso (*10*) and washed with water (10 times the volume of adsorbent) to recover the glucose. This procedure separates the monosaccharides from the disaccharides, the monosaccharides coming off in the water wash and the disaccharides being eluted with 20% ethanol. This separation indicated 95% of the cellulose had been hydrolyzed to glucose by the Monier-Williams method.

The water wash solution of glucose from the carbon-Celite column was freeze-dried and crystallized from methyl and isopropyl alcohol. A yield of 298 mg of crystallized glucose was recovered with a specific activity of 0.0192 μc/mg or 3.459 μc/mM as determined by the vibrating reed electrometer. A 75-mg sample of the radioactive glucose was oxidized by oxygen in aqueous potassium hydroxide[4] to remove carbon 1. The resulting potassium D-arabonate was separated and recrystallized. A 5.82-mg sample of the purified potassium D-arabonate was oxidized and the activity of the resulting CO_2 determined by use of a vibrating reed electrometer. The drift rate was equivalent to 1.1 dps for the 5.82 mg sample. This corresponds to 1.04×10^{-3} μc/mM. Dividing 1.04×10^{-3} by 3.459 (activity of original glucose from cotton) $\times 100 = 0.03\%$. Hence 99.97% of the activity was in carbon 1 of the glucose molecule.

These data are most interesting, since they constitute strong evidence that the glucose-1-C^{14} was synthesized to cellulose directly by an enzyme system of the cotton boll. No one has previously traced the synthesis of cellulose in the cotton boll through the introduction of glucose-1-C^{14} and thus there are no prevailing theories as to the mechanism involved in cellulose synthesis. From the above data one might theorize that the glucose is polymerized directly, possibly receiving the essential energy through phosphorylation.

Later publications will compare the data obtained on the biosynthesis of C^{14}-specifically labeled cellulose in the cotton boll and on that of the cellulose produced by *A. xylinum*.

[4] An unpublished procedure of H. S. Isbell and associates of the National Bureau of Standards.

References

1. Isbell, H. S., *et al. J. Research Natl. Bur. Standards*, **48**, 163 (1952).
2. Isbell, H. S., and Karabinos, J. V. *Ibid.*, 438.
3. Greathouse, G. A., *et al.* The Biosynthesis of C^{14}-Specifically Labeled Cellulose by *Acetobacter xylinum*. Abst., 2nd Biochem. Congr., Paris (1952).
4. Minor, F. W., *et al.* The Biosynthesis of C^{14}-Labeled Cellulose by *Acetobacter xylinum*. Abst., 12th Intern. Congr. Pure Appl. Chem., N. Y. (Sept. 1951).
5. Anderson, D. B., and Kerr, T. *Ind. Eng. Chem.*, **30**, 48 (1938).
6. Marsh, P. B., *et al. Textile Research J.*, **20**, (5), 288 (1950).
7. Conrad, C. M. *Ind. Eng. Chem., Anal. Ed.*, **16**, 745 (1944).
8. Monier-Williams, G. W. *J. Chem. Soc.*, **119**, Part 1, 803 (1921).
9. Schwebel, A., Isbell, H. S., and Karabinos, J. V. *Science*, **113**, 465 (1951).
10. Whistler, R. L., and Durso, D. F. *J. Am. Chem. Soc.*, **72**, 677 (1950).

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A Cage for Rearing Predator-Prey Populations of Mites[1]

Thomas Burnett

Dominion Parasite Laboratory, Belleville, Ontario, Canada

At the Belleville laboratory, studies are in progress on the establishment of a predator-prey system, the grain mite *Acarus siro* L. being used as the prey species and other mites as predators. The initial phase of this work dealt with the development of a universe that would provide the following requirements for the studies: (*a*) the mites should develop in as natural a state as laboratory conditions would allow; (*b*) the populations of predator and prey should be spread in a single plane so that most of the individuals could be observed and counted; (*c*) it should be possible to record the distribution of the predator and prey populations; (*d*) physical factors such as temperature, humidity, and the surface over which the predator searches for prey should be regulated; (*e*) if necessary, it should be possible to add fresh food as required; and (*f*) it should be possible to replicate each experiment. A description of a cage designed to satisfy these conditions forms the basis of this note.

The cage consists of 2 square sheets of glass held ½ in. apart by strips of acrylic resin plastic on the four sides (Fig. 1). The base of the cage consists of a sheet of double-diamond glass with smoothed edges.

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