and whether other polysaccharides were formed by the same cell-free enzyme system. The experiments reported here demonstrate that both these processes are brought about by enzymes isolated from the developing cotton boll.

Bolls at various stages of development were obtained from cotton plants (*Gossypium hirsutum*) through the courtesy of Drs. R. E. Johnson and F. T. Addicott, of the Department of Agronomy, University of California, Davis. The ages of the bolls were calculated from the time the flowers opened. The bolls were picked in the late afternoon, refrigerated overnight and sent to this laboratory in crushed ice the following morning. In each case enzymes were isolated from the plant material and incubated with the substrate on the day the material was received. Carpel walls were peeled from the bolls, and the segments, composed of seed hairs and immature seeds, were removed. In some experiments hairs were separated from the seeds by teasing out the seeds from the fibrous mass. The material was ground in a chilled mortar with sea sand and an equal weight of a buffer which contained 0·1 M *tris* HCl, *p*H 7·5, and 5 per cent polyvinylpyrrolidone (PVP)*. The homogenate was squeezed through two layers of cheesecloth and centrifuged at 1,000*g* for 5 min; the precipitate was taken up in a small volume of the same buffer. The supernatant solution was centrifuged at 20,000*g* for 20 min and that precipitate was also suspended in a small volume of the PVP buffer. Both centrifugal residues were used as sources of the enzyme in most of the experiments.

^{14}C-labelled substrates were synthesized and purified, and the incorporation of radioactivity into the product was estimated as described in previous publications[1,2].

Like the cell-free preparations from other plant species and organs[2], particles obtained from developing cotton bolls catalysed the incorporation of D-glucose-^{14}C into cellulose only from GDP-D-glucose-^{14}C. No incorporation of radioactivity could be observed when GDP-D-glucose was replaced in the reaction mixture by any of the following ^{14}C-labelled compounds: UDP-D-glucose, ADP-D-glucose, CDP-D-glucose, D-glucose 1-phosphate or D-glucose.

The extent of incorporation of radioactivity from GDP-D-glucose-^{14}C into alkali-insoluble polysaccharide is shown in Table 1. The most active preparations were from bolls between 4 and 8 days old. The catalytic activities of particles precipitated with quite diverse centrifugal forces were about the same. This suggests that no discrete organelle is involved in this process.

In all cases the incorporation of label from GDP-D-glucose-^{14}C into the product was stimulated by the addition of GDP-D-mannose to the reaction mixtures. Furthermore, the data in Table 2 demonstrate that when particles prepared from seed hairs alone were used, GDP-D-mannose still enhanced the incorporation of ^{14}C from GDP-D-glucose-^{14}C into the alkali-insoluble product. By analogy with the results obtained earlier[2] this stimulation indicates the formation of another polysaccharide in addition to cellulose. The properties of the polysaccharide-synthesizing system from the cotton boll thus appear to be about the same as those of similar systems isolated from the roots and hypocotyls of mung bean seedlings[2].

Cotton fibres are formed from specialized cells in the outer layer of the seed coat and are made up almost entirely of cellulose; thus it is surprising that enzyme preparations from these cells appear to catalyse the extensive synthesis of one or more polysaccharides other than cellulose. This phenomenon may be an artefact of the process *in vitro*, that is, the enzyme responsible for cellulose synthesis is perhaps unable to distinguish GDP-D-mannose from GDP-D-glucose, whereas GDP-D-mannose is not encountered by the enzyme *in vivo*, and hence that compound does not act as a glycosyl donor. If purification of the enzyme or enzymes involved in this synthesis can be achieved and the nature of the D-glucosyl acceptor can be elucidated, undoubtedly a more heuristic hypothesis will offer itself.

* When PVP was omitted from the homogenizing buffer no synthesis of polysaccharide by the cell-free preparations could be demonstrated. Since cotton bolls contain large amounts of phenolic substances, PVP probably neutralizes the inhibitory effect of these compounds on isolated enzymes. This subject has been discussed at length by Hulme, Jones, and Wooltorton[4].

Table 1. Effect of Age of the Boll, Particle Size, and Addition of GDP-D-mannose on Synthesis of Polysaccharide *in vitro*

Age of bolls (days)	Alkali-insoluble polysaccharide (c.p.m.) 1,000*g* fraction − GDP-D-mannose	1,000*g* fraction + GDP-D-mannose	20,000*g* fraction − GDP-D-mannose	20,000*g* fraction + GDP-D-mannose
4	190	380	200	655
6	170	340	220	610
8	305	560	220	545
12	85	330	120	415
15	120	315	180	520
21	120	200	30	95

Reaction mixtures contained 0·2 ml. of the particle suspension (derived from 2–4 g of immature cotton seeds and hairs) in 0·1 M *tris* HCl/5 per cent PVP buffer, *p*H 7·5, 2×10^{-4} μmole (0·02 μc; 6,000 c.p.m.) GDP-D-glucose-^{14}C, and, when added, 1×10^{-3} μmole GDP-D-mannose in a total volume of 0·25 ml. Mixtures were incubated for 1 h at 23°–25° and the reaction stopped by immersing the tubes in boiling water for 2 min. Alkali-insoluble polysaccharide was isolated and its radioactivity estimated as described previously[1].

Table 2. Synthesis of Polysaccharide *in vitro* by Enzymes prepared separately from Seeds and Seed Hairs

Source of enzyme	Alkali-insoluble polysaccharide (c.p.m) − GDP-D-mannose	+ GDP-D-mannose
Seeds	230	500
Seed hairs	180	460

The seeds (with most of the fibre removed) and the seed hairs from 14-day-old cotton bolls were homogenized separately in PVP buffer as described in the text. The reaction mixtures contained 0·2 ml. of particles precipitated at 20,000*g* and derived from 2 g of seed hairs or 4 g of seeds. The other conditions of the experiment were as given in Table 1.

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Nucleoside Incorporation in Animal Cell Cultures

In a recent paper Cleaver[3] has investigated the uptake of nucleosides by tissue culture cells in physiological saline. From the results he states: "This suggests that the metabolism of these cells is markedly disturbed by brief exposures to a physiological saline and in particular the biosynthesis of the acid-insoluble fractions (for example, DNA and RNA) comes to a standstill. It has been assumed that under these conditions tritiated thymidine is incorporated into DNA and tritiated cytidine is incorporated into both DNA and RNA[1,2]".

This reference to my experiments[1,2] is misleading, and may give the impression that I incubated cell cultures in saline. In fact, as I stated clearly, in the procedure described[1] in my original papers[1,2] on nucleoside uptake, the cells were incubated in a mixture of three parts growth medium (containing 20 per cent serum plus amino-acid supplement) with one part Tyrode containing the nucleoside; thus the final concentration of serum in the medium was 15 per cent, which is in fact higher than that used (10 per cent) by Cleaver[3] in his control experiments.

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