Plant Physiol. (1980) 66, 911-916
0032-0889/80/66/0911/06/$00.50/0

Protection of Cellulose Synthesis in Detached Cotton Fibers by Polyethylene Glycol[1]

Received for publication May 12, 1980 and in revised form June 29, 1980

NICHOLAS C. CARPITA[2] AND DEBORAH P. DELMER[3]
MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824

ABSTRACT

Detachment of the cotton fiber cell from the ovule results in loss of over 90% of the *in vivo* capacity for synthesis of [^{14}C]cellulose from [^{14}C]glucose. However, over 50% of the capacity for cellulose synthesis in the detached fiber population is protected when polyethylene glycol 4000 is present during detachment and incubation. Radioautography shows that approximately full capacity is restored in about half the fibers, whereas the other half of the population are incapable of cellulose synthesis from supplied glucose. The rate of cellulose synthesis in such fibers has a pH optimum of 6 and the optimum polyethylene glycol 4000 concentration is 0.06 molal (−9 bars). Cellulose synthesis in such detached fibers is synergistically stimulated by Ca^{2+} and Mg^{2+} and inhibited by K^+.

Evidence is presented which indicates that the protection by polyethylene glycol 4000 is due to its ability to promote membrane resealing, which seems to be required for protecting cellulose synthesis in the detached fiber; however, the requirement for membrane integrity is not exclusively involved in the maintainence of an energy generating system for the synthesis. The possibility that a membrane potential may be required for maintaining an active cellulose synthesizing system is discussed.

Cellulose synthesis has not been demonstrated convincingly in cell-free systems derived from higher plants. Radioactive glucose from UDP-[^{14}C]glucose[4] or GDP-[^{14}C]glucose has been shown to be incorporated into cellulose-like products in cell-free preparations from higher plants, but more rigorous characterization of the reaction products has revealed that most of these products were not cellulose (for review, see ref. 9). The relative ease of obtaining noncellulosic polysaccharide synthesis in such systems compared to the difficulties encountered in obtaining cellulose synthesis *in vitro* may indicate a fundamental difference in their modes of synthesis. Noncellulosic polysaccharide synthesis is thought to be associated generally with an internal membrane system (9, 20, 21), whereas cellulose synthesis is thought to occur at the plasma membrane-cell wall interface[5] (9, 21, 23).

Loss of cellulose synthesis upon disruption of a cell may result from disorientation of the synthetase complex caused by the sudden release of tension on the cell wall and extensive membrane disruption which occurs upon release of turgor. Furthermore, since some cellular activities are partially controlled by turgor (7, 24), one must also consider the possibility that cellulose synthesis may be turgor-dependent. Our goal was to stabilize cellulose synthesis in cotton fiber cells cut from the ovule as a first step toward obtaining a true cell-free system for cellulose synthesis; our idea was to use a high mol wt osmoticum unable to penetrate the cell wall (6): (*a*) to reduce turgor before detachment, and (*b*) to mimic turgor upon detachment since, when a high mol wt solute such as PEG 4000 is used as osmoticum, the inability of the solute to penetrate the cell wall capillaries results in tension which should keep the plasma membrane firmly appressed to the cell wall. In the report here, we present results on the effects of such PEG treatments, and the factors involved in developing a system for *in vitro* synthesis of cellulose are explored further.

MATERIALS AND METHODS

Plant Material. Seeds of cotton (*Gossypium hirsutum* L. cv. Acala SJ-1) were obtained from Hubert Cooper, Jr., United States Department of Agriculture-Science and Education Administration, United States Cotton Research Station, Shafter, Calif., and were planted and grown in growth chambers as described previously (19). Unfertilized ovules were excised from the ovary at the day of anthesis and were transferred aseptically to a medium described by Beasley and Ting (BT medium) (3). The ovules and associated fibers were incubated 15 to 16 days in darkness at 32 C.

Pretreatments and Detachment of Fibers. Ovules and associated fibers were transferred either to fresh BT medium or a buffer of choice in which the glucose concentration was lowered from 120 to 20 mM and which contained, in addition, 0.1 M pentaerythritol as an osmotic substitute for glucose. After two 15-min washes in fresh medium, the ovules were transferred by means of plastic transfer baskets (18) to 15 ml of an incubation medium containing 20 mM [^{14}C]glucose (0.042 μCi/μmol), 100 mM pentaerythritol, and BT medium or buffer ± PEG 4000 (0.06 molal). In some experiments, UDP-glucose was used as a carbon source instead of glucose. The incubation medium was free of glucose and contained instead 1 mM UDP-[^{14}C]glucose (0.042 μCi/μmol). The submerged fibers then were excised from their associated ovules with surgical scissors, leaving only the cut fibers in the incubation medium. Control incubations contained ovules with associated fibers left intact, but the submerged fibers were excised and ovules and aerial fibers were removed at the end of the incubation. Except for the kinetic experiment, all incubations, either with labeled glucose or UDP-glucose, were performed for 1 h at 32 C.

Preparation of Cell Walls. After incubation, the detached fibers were collected with forceps, blotted quickly, and placed in 2 ml $CHCl_3$-CH_3OH (1:1). One ml distilled H_2O was added, and the mixture was agitated by vortexing. Methanol was added dropwise

[1] This work was supported by Department of Energy Contract DE-AC02-76ER01338.

[2] Present address: Department of Botany and Plant Pathology, Purdue University, W. Lafayette, Ind. 47907.

[3] To whom reprint requests should be addressed.

[4] Abbreviations: UDP-glucose, uridine 5'-diphosphoglucose; GDP-glucose, guanosine 5'-diphosphoglucose; BT medium, culture medium described by Beasley and Ting (3); PEG, polyethylene glycol 4000.

[5] There are two notable exceptions to these generalizations: (*a*) in a few species of algae, cellulose is synthesized in the Golgi complex (9); (*b*) β-1,3-glucan may be synthesized at the cell surface in some cases as indicated by the localization of a β-1,3-glucan synthetase on the plasma membrane of pea stem cells (2) and probably also in the cotton fiber (18).

until a single phase was obtained, and the mixture was incubated at 37 C for 1.5 h with agitation by vortexing every 30 min. After incubation, 1 ml 0.9% NaCl in 0.01 N HCl and 1 ml $CHCl_3$ were added, and the mixture was agitated again by vortexing. The mixture was centrifuged to separate aqueous and organic phases; the fibers were obtained at the interface. The fibers were removed and washed extensively with CH_3OH followed by H_2O. Three ml acetic-nitric reagent (22) were added, and the mixture was heated for 1.5 h at 95 C, with occasional agitation by vortexing. The insoluble material remaining was pelleted by centrifugation. An aliquot of the supernatant containing hydrolyzed noncellulosic polysaccharides was collected and analyzed for radioactivity. The pellet was washed extensively with H_2O and suspended in about 3 ml H_2O. The suspension was frozen and lyophilized, and the dry material (cellulose) was weighed and assayed for radioactivity. All rates of incorporation have been expressed, therefore, as cpm/mg cellulose derived from the fibers in the assay.

Analysis of Reaction Products by Methylation Analysis. Products before or after acetic nitric digestion were permethylated according to the procedure of Hakamori (12) with the modifications described by Maltby *et al.* (18). Methylated alditol acetate derivatives then were obtained by the procedure of Albersheim *et al.* (1) and separated by GLC according to the methods described by Meinert and Delmer (19). Derivatized radioactive sugars were collected by means of a stream splitter, and the collected samples were assayed for radioactivity.

Autoradiography. The washed fibers before or after acetic-nitric digestion were suspended in a drop of H_2O on a microscope slide and heat-fixed upon the slide by passing it quickly through the flame of a Bunsen burner. The slides were dipped in Nuclear Track emulsion (Kodak) and allowed to dry at ambient temperature, and the coated slides were exposed for 5 days at 4 C. The slides were developed in D-19 (Kodak).

Fluorescence Microscopy. Fibers, attached or detached as indicated, were incubated at room temperature for 5 min in 0.01% fluorescein diacetate in 10 mM Tris-Mes (pH 6.0) ± PEG (0.06 molal). They then were observed and photographed using a Zeiss microscope with a BG-12 excitation and 47 barrier filter.

Measurement of Radioactivity. Dried or aqueous substances were suspended in ACS liquid scintillant (Amersham), and the radioactivity was assayed by liquid scintillation spectroscopy in a Packard Tri-Carb model 3390 spectrometer.

Chemicals. Pentaerythritol was obtained from Pfaltz-Bauer, PEG 4000 was from J. T. Baker Co., the [U-^{14}C]glucose was from either New England Nuclear or ICN, and the UDP-[^{14}C-U]glucose was from New England Nuclear.

Table I. *Incorporation of [^{14}C]Glucose into Polysaccharides by Intact or Detached Cotton Fibers*

Incubations were for 1 h at 32 C in BT medium (pH 6.0) supplemented with 10 mM K-phosphate. "Intact" refers to fibers still associated with their ovules during the incubation. "Detached" refers to fibers cut from their ovules prior to incubation.

Type of Fibers Incubated	Medium Supplement	Polysaccharide Synthesized	
		Cellulose	Noncellulosic Polysaccharide
		cpm/mg cellulose in fibers·h	
Intact		6090	3420
Intact	0.06 molal PEG	8270	4200
Detached		490	1350
Detached	0.06 molal PEG	3340	9700
Detached	0.20 M Mannitol	1520	9250

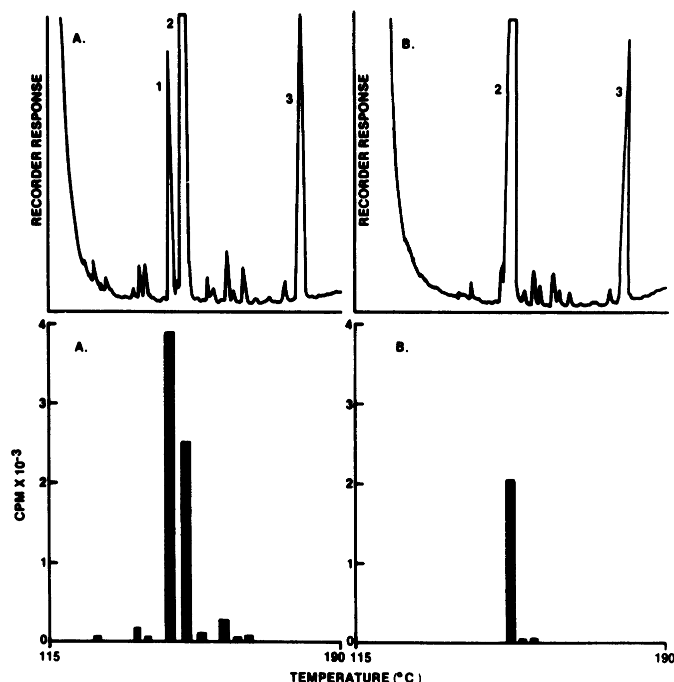

FIG. 1. Methylation analysis of radioactive polysaccharide products derived from incubations of fibers detached in medium containing 0.06 molal PEG. A, before acetic-nitric digestion; B, after acetic-nitric digestion. Separations of partially methylated alditol acetates were made by GLC using a 0.2- × 180-cm glass column packed with 0.2% polyethylene glycol succinate, 0.2% polyethylene glycol adipate, and 0.4% XF-1150 on Gas-Chrom Q (100–120 mesh). Temperature programming was 2 C/min from 115 C to 190 C with a nitrogen flow rate of 30 ml/min. Inositol was used as an internal standard. Derivatized sugars were collected by means of a stream splitter, and radioactivity was measured by liquid scintillation spectroscopy. Peak 1, derivative of 3-linked glucose; peak 2, derivative of 4-linked glucose; peak 3, inositol hexaacetate (internal standard).

RESULTS

Protection of Capacity for Cellulose Synthesis by PEG. When fibers were detached from the ovules, the rate synthesis of [^{14}C]cellulose from [^{14}C]glucose in the fibers dropped to less than 10% of that observed in intact attached fibers. However, when fibers were detached and incubated in the presence of 0.06 molal PEG, over 50% of the capacity for incorporation of glucose into cellulose was maintained compared to intact controls without PEG (Table I). Mannitol at an osmolarity equal to that of PEG protected only about 25% of the activity compared to that of intact controls. PEG also caused some stimulation of cellulose synthesis in the intact controls, indicating that loss of turgor for at least short time periods does not inhibit cellulose synthesis *in vivo*. [The ψ_π of 0.06 molal PEG = −9 bars; ψ_π of cultured fibers of this age = −6 bars (11); it is observed that the fibers collapse when incubated in this molality of PEG.] Detachment of the fibers stimulated the incorporation of glucose into noncellulosic polysaccharides when PEG was added to the incubation medium but, in this case, mannitol was as effective as PEG (Table I).

Characterization of Polysaccharide Products. A methylation analysis of the radioactive polysaccharides derived from fibers incubated intact or detached with PEG demonstrated that most of the incorporation of [^{14}C]glucose into total polysaccharide material was in 3-linked and 4-linked glucose residues (Fig. 1A). Upon acetic-nitric digestion of the fibers, only 4-linked glucose (cellulose) remained (Fig. 1B). Thus, it is concluded that the bulk of the noncellulosic polysaccharide synthesized (acetic-nitric soluble)

was the β-1,3-glucan previously characterized by us in this system (18). The resistance of the 4-linked glucose residues to acetic-nitric digestion indicates that this product is cellulose. (Amylose, another 4-linked glucan of plants, is solubilized by this treatment.) The data shown in Figure 1 are for detached fibers incubated with PEG, but similar results were obtained for intact, attached fibers (data not shown).

Optimal Conditions for Cellulose Synthesis in Detached Fibers. Preliminary experiments with the system reported here revealed that cellulose synthesis in the detached fiber had a pH optimum of 6.0 and a PEG concentration optimum of 0.06 molal (−9 bars) (data not shown). Concentrations of PEG greater than 0.07 molal, however, caused a precipitous decrease in the protection of cellulose synthesis in the detached fiber. Suitable buffers then were sought to replace the complex BT medium used in the pilot studies; K-Mes was found to be the best of those tested, affording incorporation rates about 50% of those observed in the BT medium. K-acetate (pH 6.0) resulted in almost complete loss of all activity in intact fibers as well as detached fibers (data not shown).

Upon examination of the optimal ionic strength of the K-Mes buffer, it was observed that high concentrations (50 mM) were inhibitory to cellulose synthesis in the detached fibers but that cellulose synthesis was unaffected by ionic strength when the buffer was Tris titrated to the appropriate pH with Mes or HCl (Fig. 2A). Addition of varying amounts of KCl to 10 mM Tris-Mes resulted again in the inhibition of cellulose synthesis in the detached fibers (Fig. 2A). These experiments indicate that K^+ inhibits cellulose synthesis in the detached fibers. In contrast, K^+ had no effect on the synthesis of noncellulosic polysaccharides in detached fibers (Fig. 2B). The inhibition by K^+ was not evident in the synthesis of noncellulosic or cellulosic polysaccharides in intact, attached fibers (data not shown). All subsequent incubations were in 10 mM Tris-Mes buffer. Cellulose synthesis in detached fibers had a pH optimum of 6.0 in this buffer, whereas that of intact fibers was 5.5 (Fig. 3); the synthesis of noncellulosic polysaccharides was stimulated in detached fibers at pH above 5.0, and the synthesis of such polysaccharides was stimulated further in both intact and detached fibers at pH above 9.0 (data not shown). As in BT medium, the optimal PEG concentration was 0.06 molal in the Tris-Mes buffer (data not shown).

Addition of Mg^{2+} and Ca^{2+} enhanced the rate of cellulose synthesis synergistically, indicating that both ions stimulated cellulose synthesis in the detached fibers (Fig. 4). In contrast, the synthesis of noncellulosic polysaccharides from [^{14}C]glucose was independent of the exogenous Mg^{2+} or Ca^{2+} (data not shown). The synthesis of both cellulose and noncellulosic polysaccharides was approximately linear with respect to time for up to 2 h (data not shown). Under optimized conditions [10 mM Tris-Mes (pH 6.0), 0.06 molal PEG, 3 mM $CaCl_2$, and 3mM $MgCl_2$)], the rate of incorporation of radioactivity into cellulose approximately equaled that obtained in BT medium + 0.06 molal PEG.

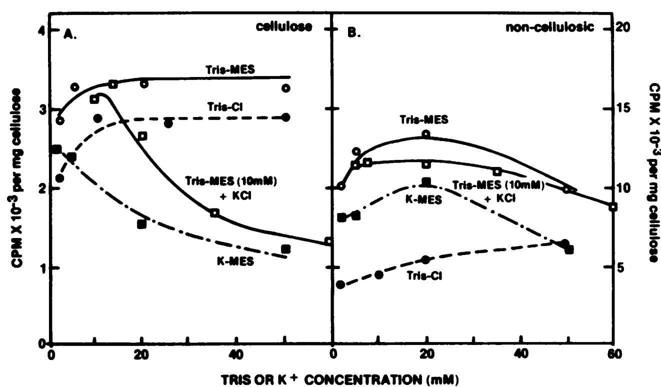

FIG. 2. Comparison of [^{14}C]glucose incorporation into cellulose and noncellulosic polysaccharides of detached cotton fibers (in 0.06 molal PEG) as a function of buffer ionic strength. A, cellulose; B, noncellulosic polysaccharides. The concentration plotted refers to that of Tris for the Tris-Mes and Tris-Cl and to that of K^+ for the K-Mes or Tris-Mes + KCl.

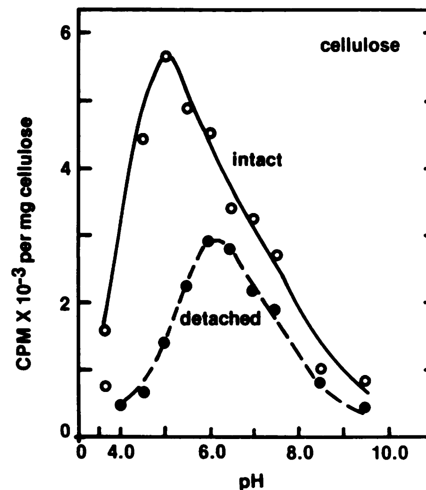

FIG. 3. Incorporation of [^{14}C]glucose into cellulose by intact and detached cotton fibers as a function of external pH. All incubations were 1 h at 32 C in 10 mM Tris-Mes + 0.1 M pentaerythritol, 3 mM $CaCl_2$, and 3 mM $MgCl_2$. The medium for detached fibers also contained 0.06 molal PEG.

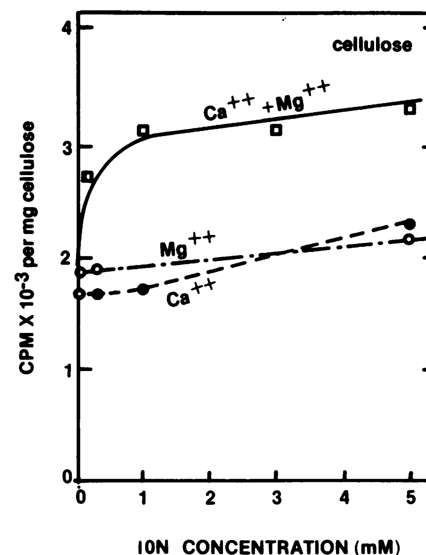

FIG. 4. Influence of Mg^{2+} and Ca^{2+} (as $MgCl_2$ and $CaCl_2$) on the incorporation of [^{14}C]glucose into cellulose in detached cotton fibers. All incubations were in 10 mM Tris-Mes (pH 6.0) + 0.1 M pentaerythritol, 0.06 molal PEG, for 1 h at 32 C. The ion concentration indicated is for each species of cation.

Utilization of UDP-[^{14}C]glucose by Detached Fibers. The data presented above indicate that cellulose synthesis from glucose can be protected when fibers are detached from their ovules in the presence of PEG. Since each fiber is a single cell, it was assumed that cutting a fiber should render it permeable to a phosphorylated derivative of glucose such as UDP-glucose, the most likely candidate for precursor to cellulose in cotton (5, 9, 21). It was therefore of interest to find that UDP-[^{14}C]glucose was not utilized as a substrate for cellulose synthesis in fibers detached and incubated in PEG (Table II). Consistent with previous work from this laboratory (10), it was found that UDP-[^{14}C]glucose could serve as substrate for noncellulosic glucan synthesis in detached fibers

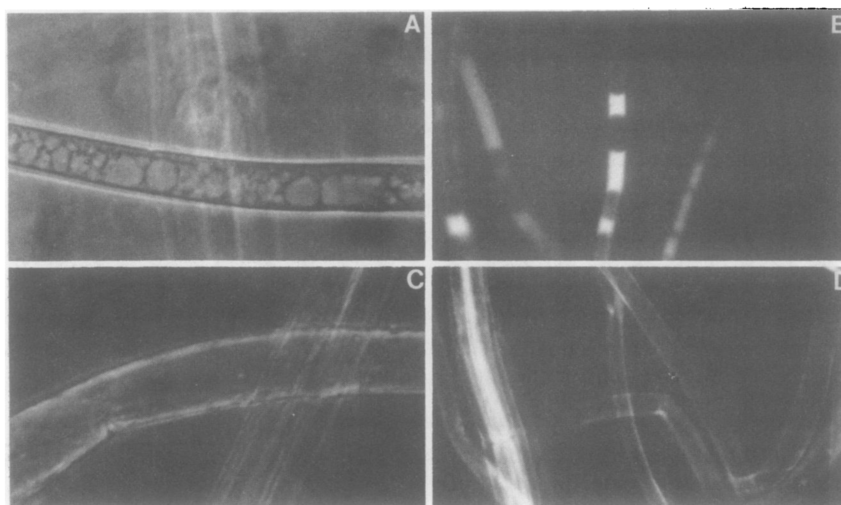

FIG. 5. Microscopic examination of fibers detached in the absence or presence of PEG. Fibers were detached in 10 mM Tris-Mes (pH 6.0) ± 0.06 molal PEG. A, fibers detached in the absence of PEG and observed by phase microscopy; B, fibers detached in the absence of PEG, incubated for 5 min in 0.01% fluorescein diacetate, and observed by fluorescence microscopy. C and D are similar to A and B, respectively, except that the fibers were detached and incubated in the presence of 0.06 molal PEG.

Table II. *Comparison of Incorporation of Radioactivity from [^{14}C]-Glucose or UDP-[^{14}C]Glucose into Polysaccharides of Intact or Detached Fibers as a Function of Medium pH*

Incubation was for 1 h at 32 C in BT medium and 0.06 molal PEG.

Treatment	pH 6.0		pH 7.5	
	Cellulose	Noncellulosic Polysaccharides	Cellulose	Noncellulosic Polysaccharides
	cpm/mg cellulose in fibers·h			
[^{14}C]Glucose				
Intact	5180	3520	1330	1940
Detached	490	1410	70	2370
Detached in PEG	2880	4350	530	3150
UDP-[^{14}C]Glucose				
Intact	60	190	10	410
Detached	20	1340	80	4530
Detached in PEG	50	440	20	1440

and that this activity is greater at pH 7.5 than at pH 6.0. However, inclusion of PEG, which stabilizes the capacity for both noncellulosic and cellulosic glucan synthesis from glucose, actually depressed the capacity for utilization of UDP-[^{14}C]glucose for noncellulosic glucan synthesis. When fibers are progressively cut into finer pieces in the presence of PEG, the capacity for cellulose synthesis from glucose decreased, although significant incorporation was still observed in finely cut fibers (Table III). Such progressive cutting did not allow cellulose synthesis from UDP-[^{14}C]glucose but did substantially stimulate noncellulosic glucan synthesis.

Table III. *Incorporation of Radioactivity from [^{14}C]Glucose and UDP-[^{14}C]Glucose into Polysaccharides of Fibers Detached in PEG as a Function of Degree of Cutting of Fibers*

Fibers about 20 mm long were detached and incubated without further excision (one cut) or cut with scissors three or six additional times. Finely chopped fibers were excised further into pieces 1 to 2 mm in length. All incubations were in BT medium (pH 6.0) plus 0.06 molal PEG for 1 h at 32 C.

Treatment	Polysaccharide Synthesized	
	Cellulose	Noncellulosic Polysaccharides
	cpm/mg cellulose in fibers·h	
[^{14}C]Glucose		
Detached (1 cut)	2470	8000
Detached (4 cuts)	1630	7990
Detached (7 cuts)	1480	6530
Detached (finely chopped)	1070	5990
UDP-[^{14}C]Glucose		
Detached (1 cut)	10	830
Detached (4 cuts)	20	1330
Detached (7 cuts)	20	1830
Detached (finely chopped)	20	3590

Microscopic Examination of Fibers. Examination by phase microscopy showed that detached fibers appeared structurally quite different, depending upon whether they were cut and incubated in the presence or absence of PEG. Fibers cut in the absence of PEG showed notable disruption of cellular organization and extensive vesiculation of the cells' membrane systems (Fig. 5A). Examination of such fibers by fluorescence microscopy following detachment and incubation in fluorescein diacetate showed that only localized vesicles within the fiber wall were capable of retaining hydrolyzed fluorescein (Fig. 5B). By contrast, most of the fibers cut in the presence of PEG resembled intact, attached fibers in morphology (Fig. 5C) and in their ability to accumulate fluorescein throughout the length of the fiber, although there was some variability in the extent of accumulation among the fibers (Fig. 5D). The ability to hydrolyze and retain fluorescein has been used in the past as a viability test in plant cells (16).

Autoradiography revealed that essentially all attached, intact fibers accumulated radioactivity from glucose into cell wall material (Fig. 6A) which included cellulose (radioactivity remaining after acetic-nitric treatment; Fig. 6B). The accumulation of radioactivity was relatively uniform throughout the length of the fibers. Essentially all fibers detached in the presence of PEG synthesized some wall polysaccharide (Fig. 6C). Most of this synthesis was uniform throughout the length of the fiber but, in about 10% of the detached fibers, regions of intensive labeling were observed (see arrow Fig. 6C). These may represent regions of enhanced deposition of β-1,3-glucan since such regions are not observable in fibers subjected to acetic-nitric digestion. Of most interest was the observation that, after acetic-nitric digestion, there are clearly

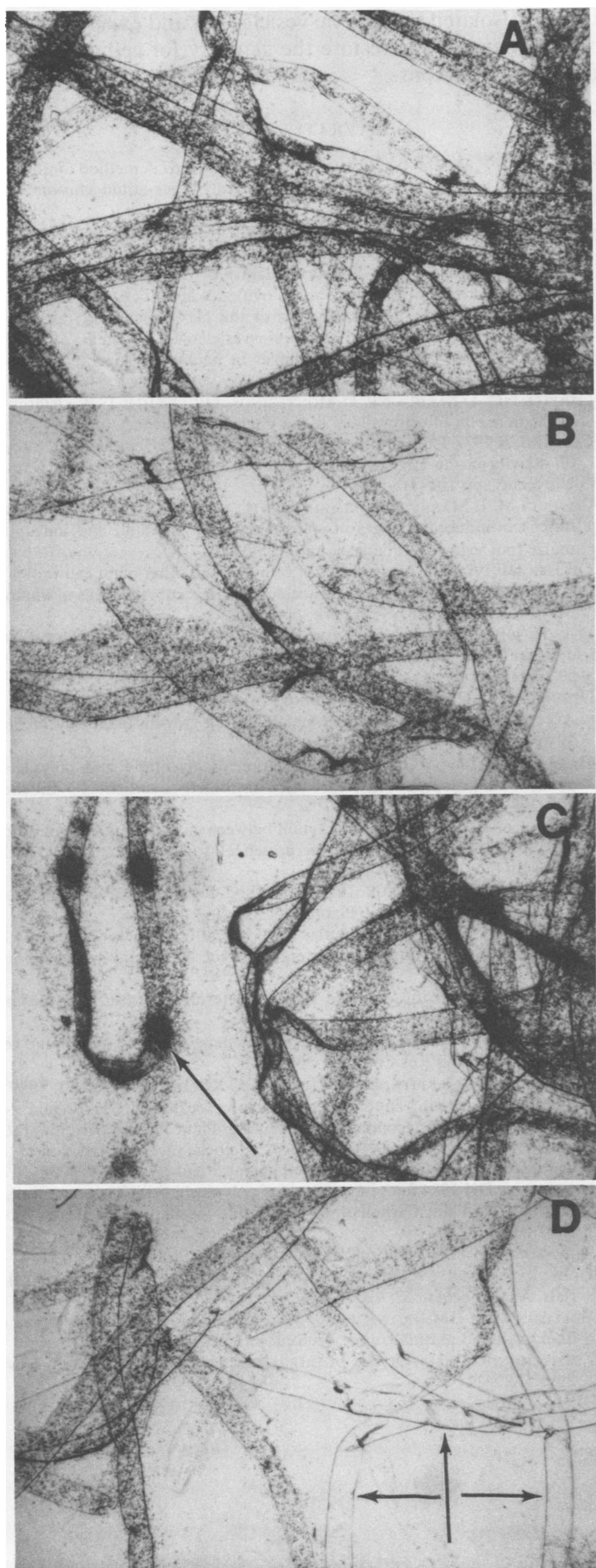

FIG. 6. Autoradiograms of intact and detached (+PEG) cotton fibers after incorporation of [^{14}C]glucose into polysaccharides A, before acetic-nitric digestion (intact fibers); B, after acetic-nitric digestion (intact fibers); C, before acetic-nitric digestion (detached fibers); D, after acetic-nitric digestion (detached fibers). Prior to radioautography, incubation was in 10 mM Tris-Mes (pH 6.0) + 0.1 M pentaerythritol, 3 mM $CaCl_2$, 3 mM $MgCl_2$, and 125 μCi [^{14}C]glucose for 1 h, 32 C. The medium for detached fibers also contained 0.06 molal PEG.

two groups of detached fibers distinguishable with respect to their ability to synthesize cellulose (Fig. 6D). About 50% of the fibers detached in PEG showed no observable incorporation of label into cellulose (see arrows Fig. 6D), whereas the other 50% showed incorporation comparable to that obtained with attached, intact fibers.

DISCUSSION

We have found that the capacity for cellulose synthesis from [^{14}C]glucose by cotton fibers can be protected when the fibers are detached from the ovule and incubated in the presence of PEG. This protection could result partially from tbe reduction of turgor prior to cutting since mannitol at equal osmotic concentrations could also partially protect the capacity for cellulose synthesis (Table I). However, PEG was about twice as effective as mannitol in stabilizing the synthesis to detachment (Table I). Two major possibilities for such an increased stabilization are: (*a*) facilitation of membrane resealing upon detachment or (*b*) direct protection of the cellulose-synthesizing complex. Halper and Srere (13) have reported that PEG could stabilize a specific interaction of citrate synthase and mitochondrial malate dehydrogenase *in vitro*, thus providing a precedent for the stabilization of multisubunit complexes by PEG.

However, although a direct protective effect of PEG on the synthesizing complex cannot be excluded, the results presented here would seem to favor the alternate interpretation, that PEG restores the capacity for cellulose synthesis by promoting membrane resealing. The results which support this conclusion are as follows. (*a*) Since glucose can be effectively used as a substrate for polysaccharide synthesis in such detached fibers, the energy-producing and glucose-phosphorylating systems must be to a certain extent still functional. (*b*) Examination of fibers detached in PEG showed that most fibers possess normal cellular integrity, at least at the level detectable by phase microscopy, and exhibit the ability throughout the fibers to retain fluorescein produced from fluorescein diacetate. (*c*) UDP-glucose cannot be used to any significant extent for glucan synthesis by intact fibers (ref. 10 and Table II). When such fibers are cut in the absence of PEG, UDP-glucose can be utilized for the synthesis of β-1,3-glucan, but not cellulose. However, if fibers are cut in the presence of PEG, the capacity to utilize UDP-glucose for β-1,3-glucan synthesis is decreased by more than 50%. This could be explained by assuming that PEG can cause a resealing of the plasma membrane of at least half of the fibers to the extent that UDP-glucose can no longer permeate to the active site of the β-1,3-glucan synthetase. (*d*) PEG has been found to promote fusion of protoplasts in other plant systems (14).

Rather strong, albeit indirect, arguments can be made that UDP-glucose is a precursor to cellulose *in vivo* in cotton fibers. It has been previously demonstrated in this laboratory that UDP-glucose is the predominant nucleotide sugar in cotton fibers and that carbon in UDP-glucose turns over *in vivo* at a rate sufficient to account for the synthesis of both β-1,3-glucan and cellulose (ref. 5 and N. C. Carpita and D. P. Delmer, manuscript in preparation). Furthermore, UDP-glucose levels increase markedly in fibers just prior to the onset of maximum rates of secondary wall cellulose synthesis (5). Yet it has been shown here that UDP-glucose supplied to detached fibers cannot serve as a significant source of carbon for cellulose synthesis even in those fibers (approximately 50% of the total population as judged by radioautography) detached in PEG which are competent to synthesize cellulose from glucose and which, therefore, possess an active synthetase complex. Such a result can only indicate one of two things: (a) either UDP-glucose is not a precursor, a possibility which is considered unlikely, or (*b*) the UDP-glucose is not accessible to the site of cellulose synthesis in those fibers which retain the capacity to synthesize cellulose from glucose.

From the results presented above, it is concluded that detach-

ment of fibers in PEG results in two populations of cells which are roughly equal in number, one population has resealed to sufficient extent to restore the capacity for cellulose synthesis from glucose; the other population remains sufficiently leaky to allow penetration of UDP-glucose for use in β-1,3-glucan synthesis but cannot use this substrate for cellulose synthesis because the complex is active only in cells possessing an intact membrane. It is important to reiterate that the rate of incorporation of radioactivity from glucose into cellulose in fibers detached and incubated in PEG is about 50% of that of intact fibers; the radioautography shows that about half of such fibers is incapable of cellulose synthesis, whereas the other half of the population is comparable in capacity to that of intact fibers. Thus, it seems that the protection by PEG is an all or none phenomenon requiring a certain degree of "intactness." Since all fibers detached in the presence of PEG can synthesize polysaccharide from glucose (Fig. 6 C), both populations must retain adequate energy supplies for polysaccharide synthesis. This indicates that an additional requirement is necessary for those fibers which retain the capacity to synthesize cellulose.

Could this additional requirement involve the necessity for a certain threshold potential across the plasma membrane? Such a potential could be necessary to drive the movement of substrates across the membrane or for the proper organization or movement of the synthesizing complex within the plasma membrane. Perhaps relevant to this hypothesis is the recent observation of Komor *et al.* (15) that only energized membranes of *Chlorella*, yeast, or *Escherichia coli* are rendered permeable by certain detergent treatments, a result which clearly indicates substantial differences in the structural organization of energized and nonenergized membranes. This conclusion is also supported by studies which showed that changes in transmembrane potential can cause changes in membrane rigidity of lipid membrane vesicles (17). Also pertinent may be the observation that K^+ inhibits cellulose synthesis in detached fibers at concentrations that reduce membrane potentials in other plant systems (8). (Although K^+ inhibition was not observed in intact fibers in the experiments reported here, the authors have recently observed that such inhibition by K^+ can be demonstrated if the Cl^- anion is replaced by the relatively more impermeant SO_4^{2-} anion.) Also relevant is the observation that cotton fibers rendered permeable to UDP-glucose by a variety of procedures (cold shock, detergents, or dimethyl sulfoxide) can always use this substrate for β-1,3-glucan synthesis but never for cellulose synthesis (D. P. Delmer, unpublished data). Such treatments would certainly dissipate normal membrane potentials.

The requirement of an intact membrane system for active cellulose synthesis may explain the lack of previous reports of *bona fide* cellulose synthesis in cell-free preparations from higher plants. Thus, a paradox may exist, membrane integrity must be disrupted in order to allow access of an impermeant substrate to the site of synthesis, but such disruption destroys the catalytic activity of the complex. Techniques are available to restore potentials across isolated membrane vesicles (4), and experiments are in progress to attempt to restore the capacity for cellulose synthesis under such conditions.

LITERATURE CITED

1. Albersheim P, DJ Nevins, PD English, A Karr 1967 A method for the analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography. Carbohydr Res 5: 340–345
2. Anderson RL, PM Ray 1978 Labeling of the plasma membrane of pea cells by a surface-localized glucan synthetase. Plant Physiol 61: 723–730
3. Beasley CA, IP Ting 1974 Effects of plant growth substances on *in vitro* fiber development from unfertilized cotton ovules. Am J Bot 61: 188–194
4. Beck JC, B Sacktor 1975 Energetics of the Na^+-dependant transport of D-glucose in renal brush border membrane vesicles. J Biol Chem 250: 8674–8680
5. Carpita NC, DP Delmer 1978 Changes in nucleotide sugar pools in cotton fibers. Plant Physiol 61: S-116
6. Carpita NC, D Sabularse, D Montezinos, DP Delmer 1979 Determination of the pore size of cell walls of living plant cells. Science 205: 1144–1147
7. Cleland, R 1977 The control of cell enlargement. *In* D Jennings, ed, Integration of Activity in the Higher Plant. Proc 31st Symp Soc Exptl Biol, Cambridge University, pp 101–115
8. Cocucci M, E Marré, A Ballarin-Denti, A Scacchi 1976 Characteristics of fusicoccin-induced changes of trans-membrane potential and ion uptake in maize root segments. Plant Sci Lett 6: 143–156
9. Delmer DP 1977 The biosynthesis of cellulose and other plant cell wall polysaccharides. *In* FA Loewus, VC Runeckles, eds, Recent Advances in Phytochemistry, Vol 11. Plenum, New York, pp 45–77
10. Delmer DP, U Heiniger, C Kulow 1977 UDP-glucose: glucan synthetase in developing cotton fibers I. Kinetic and physiological properties. Plant Physiol 59: 713–718
11. Dhindsa RS, CA Beasley, IP Ting 1975 Osmoregulation in cotton fiber. Accumulation of potassium and malate during growth. Plant Physiol 56: 394–3980
12. Hakamori S 1964 A rapid permethylation of glycolipid and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide. J Biochem 55: 205–208
13. Halper LA, PA Srere 1977 Interaction between citrate synthase and mitochondrial malate dehydrogenase in the presence of polyethyleneglycol. Arch Biochem Biophys 184: 529–534
14. Kao KN, MR Michayluk 1974 A method for high-frequency intergeneric fusion of plant protoplasts. Planta 115: 355–367
15. Komor E, H Weber, W Tanner 1979 Greatly decreased susceptibility of nonmetabolizing cells towards detergents. Proc Natl Acad Sci USA 76: 1814–1818
16. Larkin PJ 1976 Purification and viability determinations of plant protoplasts. Planta 128: 213–216
17. Lelkes PI 1979 Potential dependent rigidity changes in lipid membrane vesicles. Biochem Biophys Res Commun 90: 656–662
18. Maltby D, NC Carpita, D Montezinos, C Kulow, DP Delmer 1979 β-1,3-glucan in developing cotton fibers: structure, localization, and relationship of synthesis to that of secondary wall cellulose. Plant Physiol 63: 1158–1164
19. Meinert MC, DP Delmer 1977 Changes in biochemical composition of the cell wall of the cotton fiber during development. Plant Physiol 59: 1088–1097
20. Northcote DH 1974 Sites of synthesis of polysaccharides of the cell wall. *In* JB Pridham, ed, Plant Carbohydrate Chemistry, Academic Press, New York, pp 165–181
21. Robinson, DG 1977 Plant cell wall synthesis. Adv Bot Res 5: 89–151
22. Updegraff DM 1969 Semimicro determination of cellulose in biological materials. Anal Biochem 32: 420–424
23. Willison JHM, JM Brown Jr 1977 An examination of the developing cotton fiber: wall and plasmalemma. Protoplasma 92: 21–41
24. Zimmermann U 1978 Physics of turgor and osmoregulation. Annu Rev Plant Physiol 29: 121–148