Callose: Lateral Movement of Assimilates from Phloem

Abstract. *Brief heating of cotton petioles increased phloem callose and decreased lateral movement of assimilates containing carbon-14. The heat-induced phloem callose appears to have been responsible for part of this decrease; we conclude, therefore, that lateral movement from sieve tubes was along protoplasmic connections.*

Callose, a plant polysaccharide characterized by its staining properties (*1*) and by rapid formation after a stimulus (*2, 3*), has a wide distribution within the plant kingdom (*4*) and can probably be formed by all living cells of vascular plants. Callose from different sources may differ in structure (*5*), but results of recent studies (*6*) have all been consistent with the β-1,3-glucan structure established for phloem callose of *Vitis* (*7*). Callose may be deposited both externally and internally to the plasmalemma and in a great variety of geometric forms (for example, *5, 8*).

Callose deposits at pit fields or sieve plates might be expected to restrict movement of assimilates. This expectation is based on the assumptions (i) that cell-to-cell movement of assimilates is primarily along plasmodesmata [the term used in the broad sense (*9*) to include strands connecting sieve elements] and (ii) that this movement would be restricted by a sufficient increase in length, by a decrease in diameter of these protoplasmic connecting strands, or by both. Heavy deposits of either pit callose or sieve-plate callose would constrict or lengthen the connecting strands because both deposits are external to the plasmalemma. If the second assumption is valid, and if experimental difficulties can be overcome, studies of the effects of callose on movement of assimilates offer a way to test the first assumption. A major difficulty is the separation of the direct effects of treatment from the effects of callose; the treatments used to induce callose formation may affect movement of assimilates more than does the callose which they induce. Such direct effects of treatment may account for the differences between the results discussed here and those of Eschrich *et al.* (see *10*).

The fact that in our experiments phloem callose apparently restricted lateral movement of assimilates leads to the conclusion that their lateral movement from sieve tubes was along protoplasmic connections. This conclusion is based on the reasoning that lateral-pit callose would be expected to restrict movement if plasmodesmata of the lateral walls of sieve elements were major routes of movement from sieve tubes. Sieve-plate callose would not be expected to affect lateral movement.

The following general methods, where not specified otherwise, apply throughout. Cotyledonary petioles of cotton (*Gossypium hirsutum* L. var. Acala) were heated at 45°C for 15 minutes by a flow of water directed over a petiole length of approximately 1 cm (plant intact). Water flow was restricted at its outlet to this length of petiole by flexible rubber flaps positioned so as to straddle it. Callose was detected by fluorescence microscopy (*2*) of sections cut from plants frozen intact in crushed dry ice. The distribution of assimilates containing C^{14}, derived from the urea-C^{14} which was applied to the blade (*11*), was determined by gross autoradiography (*12*) and counting. The translocation period did not include the period of heating; urea-C^{14} was applied to blades 1 to 4 hours after they were heated, for translocation periods of usually 3 hours.

Heating led to a localized increase in phloem callose and to a decrease in lateral movement of C^{14}-assimilates. The effect on tracer distribution, that is, the decrease in lateral movement, was restricted to the heated portion. Amounts of phloem callose returned to normal within 1 day after heating. Lateral movement also returned to normal within this time; the petioles of plants given urea-C^{14} 1 day after heating for a translocation period of 3 hours had uniformly dark autoradiographic images.

To test the hypothesis that part of the decrease in lateral movement was a consequence of increased phloem callose, a way was sought to avoid the formation of phloem callose in heated petioles. Pretreatment by storage in the dark was one method; there was little or no increase in phloem callose when plants were stored in darkness for 16 or more hours and heated directly when removed from darkness. Dark-stored plants and plants not dark-stored were heated as usual and urea-C^{14} was applied 2 to 4 hours later for translocation periods of 3 to 4 hours. There was more lateral movement in heated low-callose plants (dark-stored) than in heated high-callose plants. This difference is consistent with the hypothesis that callose affected lateral movement of assimilates.

Another explanation was that the decrease in lateral movement might be due entirely to reduced sink (*13*) activity and that there was more lateral movement in dark-stored (low-callose) plants, in spite of this low sink activity, because dark-storage caused low carbohydrate status. This explanation appears inadequate, since lateral movement differed in plants which had different amounts of phloem callose but presumably equally depleted sinks. Since no other explanation for this difference is apparent, we concluded that phloem callose was responsible for part of the decrease in lateral movement.

When dark-stored plants were held in light for 4 hours before being heated they produced as much phloem callose as plants not dark-stored. By heating some dark-stored plants as soon as they were removed from darkness (low-callose plants) and heating others after 4 hours in light (high-callose plants), and by applying urea-C^{14} to all after 5 hours in light, we could compare movement in plants with equal hypothetical starvation sinks (since all plants were in light an equal time before urea-C^{14} application). The high-callose dark-stored plants showed a low lateral movement typical of plants not dark-stored. The difference in time between heating and application of urea-C^{14} was not responsible for the difference in lateral movement; dark-stored plants heated directly when removed from darkness and given urea-C^{14} after 1 hour in light also had the higher lateral movement typical of low-callose plants.

The formation of parenchyma callose differed in many ways from that of phloem callose. It was more variable and took about a week to return to normal, and in the process changes in form of the callose deposits took place. In the context of this study the most important difference between the two was that the formation of parenchyma callose was little affected by dark-storage. Therefore, we concluded that any effect

parenchyma callose may have had was slight.

In spite of substantial increases in sieve-plate callose, the autoradiographs showed no indication of a restriction of longitudinal translocation. No unequivocal interpretation of this can be made at present. It may mean that sieve-plate callose, in the individual sieve tube, has no effect. This would imply that movement of assimilates across the sieve plate was not limited to connecting strands but was through the sieve plate as a whole. Alternatively, it may mean that the conductive capacity of the phloem exceeded the demands made upon it. There were always some sieve plates with small amounts of callose. It is conceivable that either removal of assimilates at sinks or some other process limited movement in these petioles with the result that the conducting capacity of the few functional sieve tubes was not exceeded. It is clear, at any rate, that sieve-plate callose was not limiting translocation in the untreated plants, since amounts far in excess of normal had no effect on longitudinal translocation.

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13. The term "sink" means tissue to which assimilates and other solutes move and are absorbed; sink is receiving tissue in contrast to source or supplying tissue.
14. Modified from a Ph.D. thesis presented to the Graduate Division, University of California, Davis. A more detailed report is in preparation. We thank Dr. S. Yamaguchi for advice on methods of autoradiography. This study was supported in part by NSF grant G-17825.

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Steroid Stimulation of Beating of Cultured Rat-Heart Cells

Abstract. *The addition of cortisol acetate and deoxycorticosterone acetate to cultured heart cells from rats, 4 to 6 days old, stimulated and prolonged beating. Protein synthesis was slowed down in the presence of steroids. Addition of steroids to cells that had stopped beating caused the reestablishment of the beat.*

Primary rat-heart cells in culture not only grow but continue to beat (*1*). This phenomenon was not expected since cells in culture usually lose their specific function as well as their distinguishing morphology. Recently (*2*) it was reported that serum lipids or certain fatty acids were essential for the maintenance of this beat. We now report on the relation between steroid hormones and the beat of heart cells in culture. Cortisol acetate and deoxycorticosterone (DOC) acetate stimulated the beat; cholestrol had no effect. While treatment with cortisol acetate was more effective than with DOC acetate, both caused prolonged beating and resulted in a higher percentage of beating areas. Cortisol acetate and DOC acetate also reestablished beating of cells which had stopped beating after 7 days in control growth medium. The reestablishment of beating was accompanied by morphological changes which, along with the beating, were maintained for as long as 2 months.

Heart cells were obtained from rats 4 to 5 days old. Thirty to forty rats were anesthetized with ether, and the hearts were removed aseptically. The techniques and medium for culturing the cells were those reported (*1*), with the following exceptions. The culture medium contained 20 percent bovine serum instead of 10 percent human serum and 10 percent fetal-calf serum. The washed and minced tissue was placed for treatment with trypsin in a 500-ml continuous-flow flask (Bellco Glass, Inc., Vineland, New Jersey) containing a magnetic stirring bar. The flask was surmounted by a separatory funnel containing 0.1 percent trypsin in Hanks saline solution. Both were attached to a ring stand and placed over a magnetic stirrer. Stirring was maintained to prevent the formation of bubbles. This whole assembly was kept sterile and was housed at 37°C. The trypsin-treated heart cells were removed from the bottom of the flask at 15-minute intervals. This cell suspension was allowed to collect in a roller tube contained in an ice bath. Fresh trypsin was added from the separatory funnel, and the treatment was continued for another 15-minute period. Five transfers of trypsin were usually sufficient to fragment the heart tissue into isolated heart cells.

The cell suspension was centrifuged at low speed for 4 minutes. Trypsin was then decanted, and 3 ml of fresh, cold, complete growth media (CGM) was added to the packed cells. The cell pellet was resuspended by mixing with a Vortex Junior Mixer and then placed on ice.

Cell suspensions were pooled, stirred slowly, and poured through two sterile stainless steel screens (1-mm² mesh) to remove clumps of undissociated heart tissue and to insure uniform distribution of cells. In most cases cells

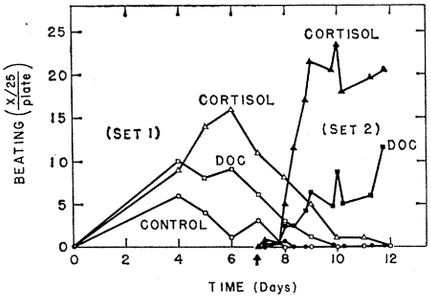

Fig. 1. Beating areas from 25 areas (per plate) randomly selected as a function of time after addition of cortisol acetate and DOC acetate. Zero represents 24 hours after culturing on control medium (CGM). Steroid-supplemented medium added at 0 days and 7 days (arrow) in set 1 and set 2, respectively.

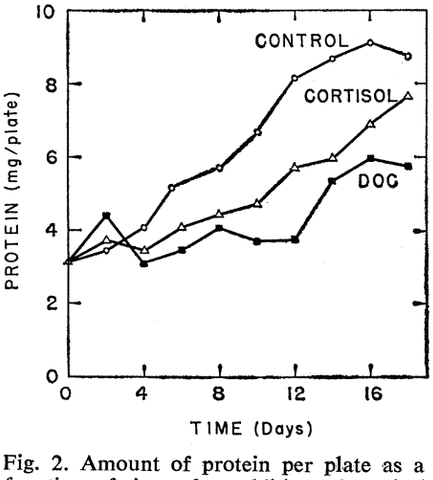

Fig. 2. Amount of protein per plate as a function of time after addition of cortisol acetate and DOC acetate. The zero time is 24 hours after culturing on control medium (CGM).