Serotonin antagonism of the effects of noradrenaline may explain certain of the unusual cardiovascular actions of the former compound. Thus, intravenous serotonin is reported to cause systemic hypotension in neurogenic hypertensive animals and hypertension in neurogenic hypotensive animals (*15*). Similarly, in the perfused dog limb, serotonin increases the net resistance across the vessels in limbs with low neurogenic vessel tone and decreases resistance when the initial tone is high (*6, 16*). Apparently, in these preparations, the direction of systemic pressure and peripheral resistance responses to serotonin depends, at least in part, upon initial neurogenic tone.

The reason for this tone dependence in the dog forelimb has been demonstrated to lie in the differing responses of small and large vessel segments to serotonin. Serotonin constricts the large arteries and veins and dilates small vessels (*5, 6*). The magnitude of constriction in large vessels is largely independent of the initial tone, whereas the dilatation in small vessels is directly proportional to the level of initial neurogenic tone. The addition of a fixed, large-vessel constriction to a small vessel dilatation which increases with increasing tone results in a total resistance change which may be dilator or constrictor, depending upon the initial neurogenic tonic input to the small vessels.

The tone of small vessels is in part related to the concentration of noradrenaline at the nerve endings surrounding them. We suggest that serotonin may decrease small vessel tone through its capacity to antagonize the vasoconstrictor activity of noradrenaline on the small vessels. The diverse effects of serotonin upon total resistance changes in the perfused dog limb and upon systemic blood pressure can be explained similarly.

Serotonin and noradrenaline are located in the same areas of the central nervous system (*17*). Noradrenaline depletion (*4, 18*) and the release of bound serotonin (*19*) from the brain stem have both been suggested as mechanisms for reserpine tranquilization. Extension of noradrenaline-serotonin antagonism to a hypothetical critical area in the central nervous system allows reconciliation of the divergent hypotheses. Thus a functional norepinephrine deficiency might result at such a site if the action of available norepinephrine were inhibited by increases in free serotonin (*20*).

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## Auxin Action on Coleoptiles in the Presence of Nitrogen and at Low Temperature

Coleoptile elongation is thought to be influenced by the plasticity of the pectic matrix of the cell walls. It is also thought that methylation of pectin increases the wall plasticity by reducing the number of carboxyl groups which may be cross-linked by divalent cations. The balance between methylation by methyl transfer reactions and demethylation by pectin methylesterase (PME) controls the methyl content of pectin. Pectin methylesterase activity in the cell wall is probably reduced by the auxin-mediated binding of the enzyme demonstrated by Glasziou (*1*). Under conditions of active methylation, reduced PME activity would allow an increase in the total methyl content of pectin by reducing hydrolysis of methyl groups. This hypothesis accounts for the effects of auxin on methylation (*2*) and cell expansion. Since auxin-mediated binding of PME is thought to be an adsorption reaction (*1*) it is likely to be insensitive to metabolic conditions. It is also likely that PME activity is less sensitive to metabolic conditions than pectin methylation. The sensitivity of auxin-induced expansion to metabolic control may thus reflect the greater metabolic sensitivity of the methylation process. The results presented in this report are consistent with the above interpretation.

Dark grown wheat coleoptiles (var. Federation) were selected for uniformity of length (±0.1 cm) in the range 3.0 to 3.7 cm approximately 95 hours after sowing. Sections 2.2 cm in length were cut 3 mm behind the apex, the primary leaf was removed, and the sections were washed in aerated distilled water for 1 to 2 hours prior to treatment. The coleoptiles were treated for 90 minutes in a basal medium of distilled water or 0.02 molar calcium chloride in the presence or absence of 10 mg/liter of unbuffered β-indolylacetic acid (IAA). Each treatment was also carried out in nitrogen or at 2° to 3°C after prior equilibration of the solutions to these conditions and after prior washing of the sections for at least 10 minutes in distilled water equilibrated to either nitrogen or low temperature. After treatment, the sections were trimmed to 2 cm, and deformation under a constant load of 300 mg for 15 minutes was measured (*3*). All manipulations and deformation measurements for the nitrogen treatments were carried out in an atmosphere of nitrogen in a Perspex box fitted with Polythene sleeves. A refrigerated, constant-temperature room was used for the low-temperature treatments, manipulations, and measurements. Coleoptile expansion during each treatment was determined on ten 1-cm sections. A basal medium of calcium chloride solution was used to prevent expansion during treatment in air at 25°C. With turgid material which had not undergone differential expansion, changes in deformability were taken to reflect changes in wall properties.

Significant coleoptile expansion oc-

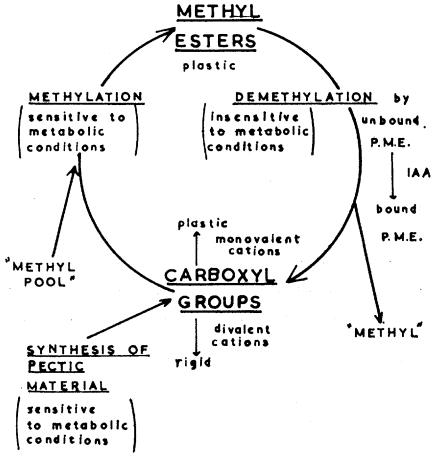


Fig. 1. Possible interconnections between methyl and carboxyl groups of the pectic substances.

Table 1. Effect of treatment with IAA, in the presence of nitrogen or at low temperature in basal media of distilled water or calcium chloride, on deformation of wheat coleoptile sections. Each result is the mean of the deformations of ten sections per treatment. Differences between means were taken as significant at the 5-percent level.

| Expt. No. | Basal medium | Mean total angle of deformation (deg) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Before treatment (25°C) | After treatment in | | | | | |
| | | | Basal medium alone | Basal medium + IAA | Basal medium + $N_2$ | Basal medium + $N_2$ + IAA | Basal medium at 3°C | Basal medium at 3°C + IAA |
| 1 | Distilled water | 25 | 27 | 40 | 11 | 23 | | |
| 2 | Distilled water | 23 | 25 | 38 | | | 18 | 26 |
| 3 | 0.02*M* $CaCl_2$ | 15 | 12 | 16 | | | 6 | 14 |
| 4 | 0.02*M* $CaCl_2$ | 32 | 12 | 17 | 8 | 16 | | |

curred only in water or water plus auxin. Deformation data from four representative experiments are shown in Table 1. Treatment with calcium chloride solution caused a significant stiffening of the sections compared with the initial values. In both basal media deformation of auxin-treated coleoptiles was significantly greater than that of sections treated in the absence of auxin. The deformation of coleoptiles treated in nitrogen or at 3°C in a basal medium of calcium chloride solution was significantly less than the deformation of coleoptiles treated in the basal medium alone. Nitrogen also caused a significant stiffening of sections treated in a basal medium of distilled water. The deformation of coleoptiles treated in nitrogen or at 3°C in the presence of auxin was significantly greater than the deformation of such tissue treated in the absence of auxin and was not significantly different from that of coleoptiles treated in basal media alone.

The effect of auxin in reducing wall stiffening in nitrogen and at low temperature suggests an action of auxin which is independent of these conditions, which reduce metabolic activity. The increased wall plasticity and cell expansion which result from auxin action are, however, metabolically dependent. Changes in cell-wall plasticity under different conditions may be interpreted in terms of pectin methylation and demethylation reactions, as is illustrated in Fig .1.

The activity of PME is insensitive to a large number of inhibitors (*4*), and pectin demethylation may occur independently of metabolic conditions. Because of a requirement for adenosine triphosphate, methylation is sensitive to metabolic conditions. A greater sensitivity of methylation compared with demethylation would result in a decrease in the methyl content of pectin in the presence of nitrogen or at low temperature. The increased number of carboxyl groups available for divalent cation binding would result in the observed wall stiffening under such conditions.

The effect of auxin in reducing wall stiffening at low temperature or in nitrogen may be due to an action of auxin which reduces the rate of demethylation and thus conserves the methyl content of pectin. The metabolic insensitivity of the action of auxin which conserves wall plasticity is consistent with Glasziou's hypothesis of an auxin-mediated adsorption reaction which reduces pectin demethylation by reducing PME activity.

It is suggested that the metabolic sensitivity of auxin-induced wall plasticization and cell expansion reflects the sensitivity of the methyl incorporation into pectin and that an auxin action which conserves wall plasticity is independent of metabolic conditions (*5*).

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## Separation of Tobacco Leaf Proteins by Centrifugation across a Density Boundary

Studies by Wildman and coworkers (*1*) have shown that two major protein fractions are present in green tobacco leaves. The two fractions, I and II, are characterized by sedimentation constants of about 19 and 4 Svedberg units, respectively. Attempts to isolate the fractions have not been wholly successful. A partial resolution can be obtained by salt precipitation (*2*), and fairly pure fraction I has been obtained by repeated ultracentrifugation, but in very low yield and in a highly aggregated form (*3*). This report (*4*) describes a simple procedure for separating the two fractions by ultracentrifugation across a density boundary (*5*).

In principle, a stable boundary is formed by layering a tobacco extract over a buffer-salt solution of greater density. In view of the difference in the sedimentation constants of the two fractions, it is then possible to choose centrifugation conditions such that the faster moving fraction I is completely sedimented, whereas the more slowly sedimenting fraction II moves only a short distance below the initial boundary. After centrifugation the upper portion of the tube should contain only fraction II, and fraction I should be concentrated in the pellet. The middle portion—that is, the region from the initial boundary to that just above the pellet—should contain some fraction II and probably some fraction I because of convection. The procedure and the results obtained in an actual separation run are described below.

The laminar portion of a sample of mature Burley tobacco leaves was lyophilized to a moisture content of 7.66 percent and ground in a Wiley mill. An extract was prepared by grinding 0.3 g of the tissue with 10 ml of 0.2*M* potassium maleate buffer, *p*H 7.5, in a Ten Broeck homogenizer. The buffer contained 0.2 g of $Na_2S_2O_4$ per liter as a color inhibitor, and the extract was maintained at about 7°C throughout the grinding and subsequent manipulations. Seven such extracts were pooled, and cell debris and other insoluble material were removed by centrifugation in a Spinco C rotor at 42,040 rev/min (av. 130,000 *g*) for 1 hour. A thin, oily, green layer at the surface of the liquid was removed by slicing off the upper portion of the Lusteroid tubes with a Spinco tube slicer; the faintly hazy supernatant fluid was then filtered through a 0.5-μ Millipore filter (*6*) to remove the suspended particulate matter. This clarified extract was clear and light brown in color and contained 0.85 mg of N per milliliter, which constituted approximately 60 percent of the total nitrogen of the original sample. A portion of the filtrate was mechanically dialyzed for 6 hours against three portions (two changes) of extraction buffer (without $Na_2S_2O_4$) and then analyzed for total nitrogen and examined in the ultracentrifuge.

For the separation run, seven tubes were prepared. Four milliliters of the clarified extract were added to each tube. A syringe, filled with extraction buffer, which was also 0.2*M* in $K_2SO_4$, was carefully inserted so that the tip of the needle was close to the bottom of the