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# CORTISOL AND MEMBRANE PERMEABILITY FOR $^3H_2O$ OF MUNG BEAN HYPOCOTYL SECTIONS

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**Key Word Index**—*Vigna radiata* Wilczek; Leguminosae; mung bean; membrane permeability; water efflux; cotyledons.

**Abstract**—The membrane permeability of basal and apical hypocotyl segments from mung bean seedlings was studied as a function of time after sowing. Cortisol enhances membrane permeability of the basal hypocotyl segments from the third day and the membrane permeability of the apical hypocotyl segments from the fifth day. When the cotyledons are removed three days after sowing, the permeability of basal hypocotyl segments from seedlings grown in water increases two days, after removal, while the permeability of the apical segments increases one day after removal. Removal of the cotyledons also increases the permeability of apical hypocotyl segments from seedlings grown in cortisol, but has no significant influence on the permeability of the basal hypocotyl segments. Incubation experiments with hypocotyl segments revealed that a three hr incubation in cortisol was sufficient to increase membrane permeability by ±20%.

## INTRODUCTION

Several effects of steroid hormones have been described. Application of steroid hormones stimulates cell elongation growth, cell division and the flowering of certain plants. Geuns [1–4] reported stimulatory effects of various glucocorticoids on the elongation growth of mung bean roots and hypocotyls. Glucocorticoids also increased the number of lateral roots. Cortisol stimulates adventitious root formation on the hypocotyls of intact mung bean seedlings [5] and it is strongly synergistic with IAA in mung bean cuttings [6]. In intact seedlings, both cortisol and the $5\beta$-corticosteroids are active as well as the $5\alpha$-corticosteroids [7]. It is difficult to explain these stimulatory effects on adventitious root formation by a binding of the steroid to a receptor protein, because both $5\alpha$- and $5\beta$-steroids are active, and a difference in binding is to be expected between $5\alpha$- and $5\beta$-steroids. Geuns [7] suggested that some of the stimulatory effects of corticosteroids might be achieved by an altered membrane permeability.

Recently we described a method for measuring the membrane permeability using $^3H_2O$ [8, 9]. The aim of this study is to determine the permeability of selected hypocotyl sections of mung bean seedlings as a function of time, and to study the effects of applied cortisol.

## RESULTS

### *Membrane permeability of basal hypocotyl sections as a function of time*

The half-times ($t_{1/2}$) for the efflux of tritiated water out of the lower hypocotyl sections as a function of time after sowing are shown in Fig. 1a. In control seedlings, the $t_{1/2}$ increases from day 2 to day 3 (from 9.6 to 11.5). After a short stabilization this $t_{1/2}$ decreases again from day 4 to day 6 (from 11.8 to 9.1). The $t_{1/2}$ is inversely correlated with membrane permeability. Therefore, the membrane permeability for $^3H_2O$ of the hypocotyl section just above the root decreases from day 2 to day 3 and increases again from day 4 on.

Also in seedlings grown in cortisol solution the $t_{1/2}$ increases from day 2 to day 3 (from 8.8 to 9.8), illustrating a decrease of membrane permeability for $^3H_2O$ during this period. After day 3, this $t_{1/2}$ decreases to reach a value of 7.2 on day 6. Thus the permeability for water increases from the third day.

Two days after sowing, the permeability of hypocotyl sections from just above the root of seedlings grown in a cortisol solution is not significantly different from the permeability of the control plants. From day 3 on, cortisol enhances the permeability for water (effect between 25 and 35%).

### *Membrane permeability of apical hypocotyl sections as a function of time*

The $t_{1/2}$ for the efflux of $^3H_2O$ out of 5 mm hypocotyl sections taken 4 cm above the root from control seedlings increases from day 4 to day 5 (from 12.8 to 14.5) and remains at that level at least till day 6 (14.4) (Fig. 2a). Thus, the permeability of the apical hypocotyl sections decreases from day 4 to day 5 and remains low at day 6.

The $t_{1/2}$ for the efflux of $^3H_2O$ out of the hypocotyl sections, taken 4 cm above the root, from seedlings grown in cortisol solution decreases from day 5 to day 6 (from 13.1 to 11.1), indicating that the permeability increases. In 4-day-old seedlings, there is no difference between sections from control seedlings and seedlings grown in cortisol solution. From day 5, cortisol enhances the permeability for water very significantly.

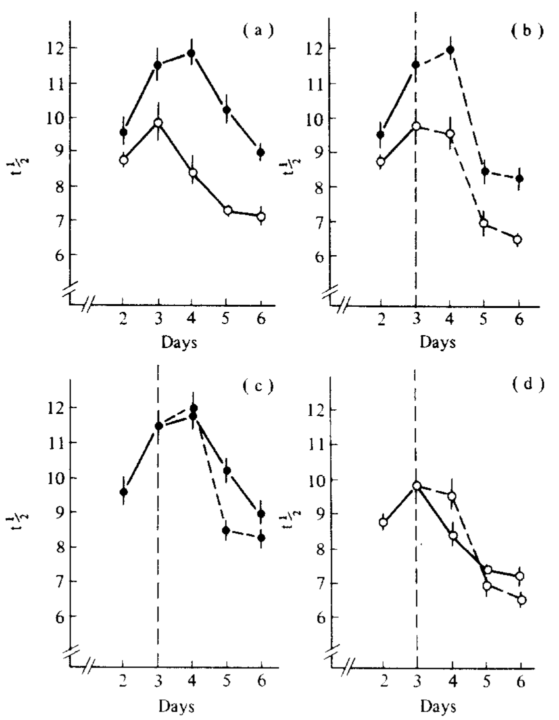

Fig. 1. (a) Half-time for the efflux of tritiated water out of sections just above the root in function of age of the seedlings (in days). Seedlings grown in 50 mg/l cortisol (○) are compared with seedlings grown in water (●). (b) Half-time for the efflux of $^3H_2O$ out of sections just above the root from seedlings with the cotyledons removed at day 3, grown in cortisol (○) or in water (●). (c) Influence of the removal of the cotyledons on the half-time for the efflux of $^3H_2O$ out of sections just above the root of seedlings grown in water (●——● intact and ●--● cotyledons removed). (d) Influence of the removal of the cotyledons on the half-time for the efflux of $^3H_2O$ out of sections just above the root of seedlings grown in cortisol (○——○ intact and ○--○ cotyledons removed). The cotyledons are removed three days after sowing. Mean and s.e. of six experiments.

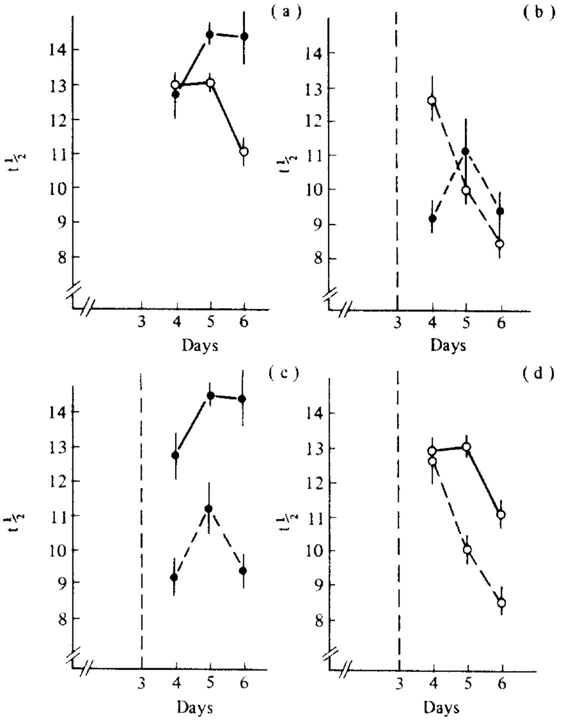

Fig. 2. (a) Half-time for the efflux of tritiated water out of sections 4 cm above the root in function of age of the seedlings (in days). Seedlings grown in 50 mg/l cortisol (○) are compared with seedlings grown in water (●). (b) Half-time for the efflux of $^3H_2O$ out of sections 4 cm above the root from seedlings with the cotyledons removed at day 3, grown in cortisol (○) or in water (●). (c) Influence of the removal of the cotyledons on the half-time for the efflux of $^3H_2O$ out of sections 4 cm above the root of seedlings grown in water (●——● intact and ●--● cotyledons removed). (d) Influence of the removal of the cotyledons on the half-time for the efflux of $^3H_2O$ out of sections 4 cm above the root of seedlings grown in cortisol (○——○ intact and ○--○ cotyledons removed). The cotyledons are removed three days after sowing. Mean and s.e. of six experiments.

### *Influence of the cotyledons on the permeability of basal hypocotyl sections*

The results of this section are described separately, but the experiments took place at the same time. Therefore, the comparisons are valid. The removal of the cotyledons at day 3 decreases the $t_{1/2}$ for the efflux of $^3H_2O$ out of basal sections of plants grown in water two days after the removal (Fig. 1c). This means that the permeability of this zone increases two days after the removal of the cotyledons. Three days after removal, this increase is no longer significant. The removal of the cotyledons has no influence on the $t_{1/2}$ of the efflux of $^3H_2O$ out of basal segments of plants grown in cortisol (Fig. 1d). In lower segments of intact seedlings, cortisol increased the permeability from three days after sowing (see above). However, when the cotyledons are removed at day 3 the effect of cortisol is less pronounced, because in control conditions the permeability is increased whereas with cortisol no change in permeability was observed.

### *Influence of the cotyledons on the permeability of apical hypocotyl sections*

The removal of the cotyledons decreases the $t_{1/2}$ of the efflux out of apical segments of plants grown in water one day after removal, indicating an increase in permeability for water (Fig. 2c). This increase is also shown two and three days, after the removal of the cotyledons.

The removal of the cotyledons also increases the permeability of apical segments of plants grown in cortisol from the second day after the removal (i.e. the effect of the removal appears one day later than in control seedlings) (Fig. 2d). The comparison between the permeability of apical segments of control plants and plants grown in cortisol, both without cotyledons, shows an unexpected result: one day after removing the cotyledons, the $t_{1/2}$ of segments from cortisol treated seedlings is significantly greater than of segments from control seedlings. This happens because in cortisol treated seedlings a delay is observed of the lowering of the $t_{1/2}$ provoked by removing the cotyledons.

### *The effect of cortisol on the permeability of different sections from 4-day-old seedlings*

In a series of experiments the influence of cortisol was investigated in different hypocotyl and root segments. In these 4-day-old seedlings, the only influence of cortisol was found in the lower hypocotyl segments as described above. The $t_{1/2}$ in roots is very low ($8.0 \pm 0.2$ for segments of the root base and $4.0 \pm 0.7$ at 1 cm below the root base). Cortisol was without effect, even if seedlings were grown in a cortisol solution of 100 mg/l (results not shown).

### *The effect of incubating hypocotyl segments in a cortisol solution*

Application of cortisol (200 mg/l) to basal segments cut from seedlings grown in water decreases their $t_{1/2}$ and thus increases the permeability by 18% (Table 1). This is not true for apical hypocotyl segments. We find similar effects with a cortisol-application to hypocotyl segments of seedlings grown in a cortisol-solution. External applied cortisol still lowers the $t_{1/2}$ and thus enhances the permeability of the basal hypocotyl segments, but has no significant influence on the permeability of apical segments.

## DISCUSSION

The sections used for permeability measurements are fully elongated. The apical segments are taken just below the zone of elongation growth. Therefore, the differences observed between apical and basal segments are not due to a difference in elongation growth of the different sections, but are rather a consequence of a difference in differentiation growth and hence of membrane composition. It is known that physiologically younger tissues of mung bean hypocotyls are characterized by a high sitosterol/stigmasterol ratio and physiologically older tissues by a low sitosterol/stigmasterol ratio [10]. Maybe the increase of permeability of basal segments ($t_{1/2} = 9.1$) and the simultaneous decrease of permeability of more apical segments ($t_{1/2} = 14.4$) of 6-day-old seedlings is correlated with a difference in sterol composition.

Table 1. Half-times for the efflux of $^3H_2O$ out of apical or basal hypocotyl segments of 4-day-old seedlings, grown in water or in 50 mg/l cortisol solution

| | Basal segments incubation in | | Apical segments incubation in | |
|---|---|---|---|---|
| Culture medium | $+H_2O$ | +200 mg/l cortisol | $+H_2O$ | +200 mg/l cortisol |
| $H_2O$ | 12.6 ± 0.4 | 11.0 ± 0.4 | 10.6 ± 0.8 | 11.3 ± 0.3 |
| 50 mg/l cortisol | 9.5 ± 0.4 | 8.0 ± 0.41 | 11.7 ± 0.4 | 11.5 ± 0.4 |

The segments were cut and, before loading with $^3H_2O$, incubated in $H_2O$ or cortisol solution (200 mg/l) for 3 hr. Mean and s.e. of six experiments.

The influence of the sterol composition on membrane permeability has been observed by Grunwald [11, 12]. After treatment of red beet disks with methanol, the effectiveness of restoring the membrane constitution (meaured by a decreasing leakage of betacyanin) was in decreasing order: cholesterol > campesterol > sitosterol > stigmasterol. Similar effects were found after an ethanol induced leakage of ions from barley roots. To understand the possible influence of apolar sterols on the permeability of polar compounds such as betacyanin, ions and water, that will probably not migrate through the apolar lipid bilayers, we suggest that an altered sterol composition will influence the positioning of the membrane lipids causing a change in the position of the tunnel proteins. This way a changed sterol composition might influence the permeability of polar compounds. As discussed below an involvement of polyamines is also possible.

In control seedlings the removal of the cotyledons provokes an immediate 30% increase of membrane permeability in apical segments. In basal segments, however, a small increase in permeability is observed only two days after removal. Probably, the removal of the cotyledons causes an immediate deficiency of nutrients and/or membrane components in the apical segments, whereas this deficiency appears much later in basal hypocotyl segments that are probably receiving substances still present in the hypocotyl at the time of the removal.

The removal of the cotyledons has no influence on the permeability of basal hypocotyl segments of plants grown in a cortisol solution. When the cotyledons are removed, the permeability of apical segments increases only two days after removal (lowering of $t_{1/2}$). As compared with control seedlings, cortisol delays the effect of the removal of the cotyledons by one day. So far, we do not understand the effect of cortisol. Possible hypotheses are that cortisol is incorporated (or associated) with membranes (see incubation experiments) or that cortisol induces the synthesis of polyamines which in turn protect the membranes. It is also possible that cortisol influences the distribution of nutrients, salts and membrane components in seedlings growing in cortisol, or even influences the synthesis of membrane components providing the cells with more elements for the membranes.

Cortisol enhances the membrane permeability of the basal hypocotyl segments from day 3 after sowing, and enhances the membrane permeability of the apical hypocotyl segments from day 5 on (Fig. 1a). The effect of cortisol seems to proceed gradually as a function of time after sowing from the basal part of the hypocotyl to the top. Cortisol is taken up by the roots and translocated through the hypocotyls as far as the cotyledons and the leaves [13]. It is known that cortisol is metabolized after its uptake [14]. However, it is unlikely that one of its metabolites causes the effects observed, as a 3 hr application of cortisol to basal segments is sufficient to increase the permeability (Table 1). Because of this rapid effect, a *de novo* synthesis of membrane elements (e.g. membrane proteins, phospholipids and sterols), or a cortisol metabolism seems to be excluded as cause for the changed permeability, which is probably provoked by cortisol itself. It might be possible that cortisol is incorporated in the membranes, most probably in the plasma membranes, or associated with membrane proteins. An alternative possibility is that cortisol changes the amount of polyamines associated with membranes. Indeed Walter and Geuns [15] observed an enhanced polyamine content in mung bean seedlings growing in cortisol. It is known that polyamines are able to change membrane permeability [16] and very quick changes in polyamine content have been observed.

Application of cortisol to apical hypocotyl segments has no influence on membrane permeability. Obviously, a definite membrane structure and composition are required to interact with the applied cortisol.

So far we cannot explain the differences in permeability observed between lower and higher hypocotyl sections as a function of age of the seedlings, nor do we understand the influence of cortisol on these phenomena. Cortisol increases the membrane permeability of hypocotyls from 4-day-old seedlings only in the basal zone. Geuns and Loeys [13] found an accumulation of cortisol and its metabolites in these basal segments, in which also the adventitious root formation is strongly stimulated by applied cortisol [5]. Perhaps this enhanced adventitious root formation is preceded by an increase in membrane permeability of the zone in which the adventitious root formation takes place. Cortisol enhances this membrane permeability from day 3, corresponding with its influence on the initiation process of adventitious root formation.

This increased permeability might lead to an enhanced translocation of certain substances. It is in fact known that glucocorticoids increase the amino acid transport in isolated rat hepatocytes [17]. Gower [18] also found an enhanced amino acid uptake by cortisol. Cotyledons were shown to be a major source of inherent root promotion in mung bean cuttings [19]. Physiological processes like adventitious root formation are regulated by the relative concentration of growth substances, for instance auxins and cytokinins. It is possible that this relative concentration is regulated by an altered membrane permeability.

It is difficult to understand the physiological significance of increasing or decreasing water permeability. In preliminary experiments it was found that the permeability of ions (measured as a change in conductance) was altered in a similar manner. Our results demonstrate that within the living plant, differences in membrane permeability exist that are under the control of endogenous factors coming from, e.g. the cotyledons. Membrane permeability in different hypocotyl segments can also be altered by applied substances such as cortisol. Although we have no definite proof we suggest that water crosses the membrane through hydrophilic pores.

## EXPERIMENTAL

*Plant material.* Mung bean seedlings [*Vigna radiata* (L.) Wilczek] were grown in moist vermiculite in the dark at 25°. Cortisol was dissolved in 0.1% *N*,*N*-dimethylformamide (DMF). At this concn DMF has no influence on the elongation growth of hypocotyls or roots nor on the adventitious root formation, nor on lateral root formation. The cortisol concn was 50 mg/l (*ca* $1.4 \times 10^{-4}$ M). The plants were grown in the cortisol soln. The control seedlings were grown in 0.1% DMF soln, further called water ($H_2O$).

Two different 5 mm sections of the mung bean hypocotyls were used in our experiments: basal segments between 0 and 0.5 cm above the root and apical segments between 4 and 4.5 cm above the root. Seedlings younger than 2 days are too small to examine the permeability of the section just above the root: since the hypocotyls are less than 5 mm! For the experiments with segments 4 cm above the root, we could take only seedlings 4 day old and older, as in younger seedlings the sections 4 cm above the root are still in elongation growth.

In the experiments with external applied cortisol (200 mg/l), hypocotyl segments were taken from 4-day-old seedlings grown in $H_2O$ or in a cortisol-soln (50 mg/l). They were incubated in cortisol for 3 hr before the $^3H_2O$ application.

In experiments to study the influence of the cotyledons on the permeability of the hypocotyls, the cotyledons were removed 3 days after sowing. The hypocotyl segments were taken as indicated above.

*Permeability measurements.* The water permeability of tissues saturated with $^3H_2O$ was measured. The efflux of the $^3H_2O$ is followed during 30 min using a flow-through cell, taking samples every 30 sec. A home-made computer program allowed us to split up the diffusion curve obtained into 3 components, reflecting 3 different diffusion processes. Our results are only correlated with the slowest diffusion process (third curve), probably the efflux through the membranes themselves [8]. The results are expressed as differences in $t_{1/2}$ of the 'third' diffusion curves. Each experiment was repeated at least 6 times. Very reproducible results were obtained (s.e. between 3 and 6%).

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