Processes of nuclear degeneration in syncytial latificers have also been observed by MILANEZ[7,8], preceded, however, by nucleolar extrusion. In the laticifers of *C. soldanella* nuclear degeneration takes place without nucleolar extrusion, but with the destruction of the organelle in situ.

A comparison between the frequencies of the altered nuclei (very high) and the frequency of the degenerated nuclei (very low) leads us to suppose that the process of degeneration is quite slow. This could be attributed to the fact that these nuclei have a particular metabolism with a precise functional significance in the process of differentiation and maturation of the articulated laticifers.

Riassunto. Nei tubi laticiferi di *Calystegia soldanella* (Convolvulaceae) sono stati individuati, mediante indagine citologica con il fluorocromo arancio di acridina, quattro tipi di nuclei diversi per dimensioni, presenza o assenza del nucleolo e capacità cromatiche. Il polimorfismo nucleare osservato viene interpretato come un aspetto del processo di differenziazione dei laticiferi articolati.

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Stimulation of Plant Growth by Malformin A

The malformins constitute a small family of cyclic pentapeptides produced by the fungus *Aspergillus niger* van Tiegh.[1,2], which induce severe malformations in the growth of higher plants and pronounced root curvatures[3-5]. Most studies utilized malformin A, a mixture of malformin A_1 and A_2[2]. Although the structure cyclo-D-cysteinyl-L-valyl-D-cysteinyl-D-leucyl-L-isoleucyl was proposed for malformin A_1[6-8], cyclo-D-cysteinyl-D-cysteinyl-L-valyl-D-leucyl-L-isoleucyl was recently proposed[9].

Malformin has several effects on plant growth. It inhibits the elongation of seedlings of *Phaseolus vulgaris* L.[5] and roots of *Zea mays* L.[10], adventitious root formation[11], and elongation and geotropically induced curvatures of *Avena* coleoptiles[12]. The synthesis of major cellular constituents of roots of *Z. mays* and stems of *P. vulgaris* is also inhibited by malformin[10,13]. We report here the first example of the stimulation of plant elongation by malformin.

Table I. Stimulation of growth of etiolated cuttings of *Phaseolus vulgaris* in the light by malformin

Treatment	Growth increment (cm) after 4 days
P. vulgaris cv. Harvester	
In light	
H_2O (control)	4.63
Malformin 10^{-5} *M*	5.80[a]
Malformin 10^{-6} *M*	6.73[a]
Malformin 10^{-7} *M*	5.65[a]
In dark	
H_2O	12.45
Malformin 10^{-5} *M*	9.05
Malformin 10^{-6} *M*	11.20
P. vulgaris cv. Resistant Asgrow Valentine	
In light	
H_2O	6.23
Malformin 10^{-5} *M*	7.30[b]
In dark	
H_2O	13.10
Malformin 10^{-5} *M*	9.30

[a] Significantly different from H_2O controls at 0.01 confidence level, or [b] 0.05 level.

Materials and methods. Malformin A was isolated from *A. niger* strain 58-883 as described[1,3]. Dimethylsulfoxide, used to dissolve malformin, was diluted in the same manner as the malformin solutions and had no effect in the experiments. Seeds of *P. vulgaris* cv. Resistant Asgrow Valentine or cv. Harvester were germinated in vermiculite for 6 and 7 days, respectively, in the dark at 27 to 28 °C. Etiolated seedlings were selected at random, excised 8.0 cm below the top of the hypocotyl hook, transferred to 50 ml beakers containing 25 ml of test solution, incubated in continuous light (1.35×10^5 ergs/cm²/sec, Champion F90T17/w, White Fluorescent) and the length of the stems measured after 4 days. Growth increment was determined by subtracting the original height from the final height of the cuttings. Experiments were performed 5 times employing 30 cuttings per treatment.

Results and discussion. At the end of 4 days in light, etiolated *P. vulgaris* cuttings treated with malformin were visibly taller than similar cuttings treated with water (Table I). At 10^{-6} *M*, the optimum concentration, the growth increment of malformin treated cuttings was 45% greater than that of cuttings in water. In the dark, malformin inhibited elongation. Stimulation of elongation of cuttings by malformin was surprizing because similar concentrations of malformin markedly inhibit extension growth of whole bean seedlings in the greenhouse[5]. Thus, the response of etiolated cuttings and green seedlings to malformin is different. In preliminary experiments malformin also stimulated elongation of 4 other cultivars of *P. vulgaris* in the light (Contender, Bountiful, Dwarf Horticultural, and Blue Lake). In most of these experiments malformin retarded leaf expansion and the synthesis of both anthocyanins and chlorophyll.

[1] N. TAKAHASHI and R. W. CURTIS, Pl. Physiol. *36*, 30 (1961).
[2] S. TAKEUCHI, M. SENN, R. W. CURTIS and F. W. MCLAFFERTY, Phytochemistry *6*, 287 (1967).
[3] R. W. CURTIS, Pl. Physiol. *33*, 17 (1958).
[4] R. W. CURTIS, Science *128*, 661 (1958).
[5] R. W. CURTIS, Pl. Physiol. *36*, 37 (1961).
[6] S. MARUMO and R. W. CURTIS, Phytochemistry *1*, 245 (1961).
[7] K. ANZAI and R. W. CURTIS, Phytochemistry *4*, 263 (1965).
[8] A. SCHÖBERL, M. RIMPLER and E. CLAUSS, Chem. Ber. *103*, 3159 (1970).
[9] M. BODANSZKY and G. STAHL, Proc. natn. Acad. Sci., USA, in press.
[10] S. IZHAR, J. BEVINGTON and R. W. CURTIS, Pl. Cell Physiol. *10*, 687 (1969).
[11] R. W. CURTIS and G. FELLENBERG, Pl. Cell Physiol. *13*, 715 (1972).
[12] W. NORRIS JR. and B. BROTZMAN, Advg. Fronts. Pl. Sci. *15*, 121 (1966).
[13] R. W. CURTIS, Pl. Cell Physiol. *10*, 203 (1969).

Because malformin stimulates ethylene production in green cuttings[11], and ethylene inhibits extension growth, we determined ethylene production by etiolated cuttings treated with malformin (Table II). Malformin stimulated ethylene production in etiolated cuttings and stimulated elongation despite these higher ethylene levels.

Although cuttings from green seedlings elongate only slightly we examined the effect of malformin on their growth increment in similar experiments. After 4 days in the light the growth increment of cuttings in water, malformin 10^{-5} *M*, and malformin 10^{-6} *M* was 1.09, 0.61, and 1.44 cm, respectively (average 70 cuttings). Malformin (10^{-6} *M*) stimulated the elongation of green cuttings; however, the absolute increase in growth increment was less than that of etiolated cuttings.

Table II. Effect of malformin on ethylene production by stem segments from etiolated cuttings of *Phaseolus vulgaris* in the light

Treatment	Length of treatment (h)		
	24	48	72
	Ethylene (nl/h/g fresh wt.)		
H_2O	1.23	1.69	1.14
Malformin 10^{-5} *M*	2.96	3.52	1.65
Malformin 10^{-6} *M*	2.78	1.48	1.24

Average 3 determinations, cv. Harvester. Approximately 2.0 cm sections were excized from the hypocotyl hook region, sealed in syringes, incubated 2 to 4 h, and analyzed for ethylene by gas chromatography as described[14].

Light inhibition of etiolated stem elongation is a process mediated by phytochrome[15]. Stimulation of stem elongation by malformin on etiolated cuttings subsequently maintained in light, but not in the dark, suggests an effect of malformin on this process. Until the effect of malformin on other phytochrome mediated responses is known, we are unable to offer an explanation for malformin-induced growth stimulation[16].

Zusammenfassung. Das Wachstum etiolierter, wurzelfreier Bohnenkeimlinge von *Phaseolus vulgaris* war mit Malformin A im Licht, nicht aber in der Dunkelheit stimuliert, und zwar obwohl die Äthylenproduktion durch Malformin-Behandlung gesteigert wird.

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[14] R. W. Curtis, Pl. Physiol. *43*, 76 (1968).
[15] P. Ray, *The living plant* (Holt, Reinhart and Winston, Inc., New York 1972).
[16] Journal Paper No. 5589 of the Purdue Agricultural Experiment Station. Supported in part by grant No. GB-23540 from the National Science Foundation.

On the Absence of Chitin in the Endosternite of Chelicerata

The endosternite of chelicerates is an internal skeletal support for some of the prosomal muscles. Firstman[1] has an excellent review of the structure and derivation of this organ in the various chelicerate classes and orders. It has been contended since Lankester[2] that the endosternite, notably of *Limulus*, contains chitin. However, other authors[3] were unable to find chitin in the endosternites of chelicerates, including *Limulus*. An examination of Lankester's paper[2] shows that the determination of chitin was made by a Prof. Schäfer (footnote on pages 133–134), and that he was cautions in his determination. At one point Prof. Schäfer states, '... I am not prepared to say positively that the substance is 'chitin' or even that it is chiefly chitin; but considering its solubilities, or rather insolubilities, it is probably either that substance or a mixture of that and a substance allied to keratin'. Despite this circumspect comment, Lankester states that chitin is definitely present in the endosternite and draws upon this to make other far-reaching conclusions. Lankester's conclusions were agreed to by Halliburton[4]. However, neither Schäfer's nor Halliburton's methods are currently accepted as documentation for the presence of chitin[5]. Reexamination seemed desirable.

We took endosternites from as many different chelicerate groups as we could muster, and subjected them to the standard van Wisselingh qualitative test for chitin. The specimens were heated in saturated KOH solution in sealed glass tubes at 160 °C for 30 min. Any particulate matter remaining was washed first in 50% ethanol, then in distilled water. In all cases, except for the solpugid apodeme and the positive control, testing ended at this point because the material had entirely dispersed. The positive control and the apodeme gave the characteristic violet color with iodine solution, followed by slow dispersion in 75% H_2SO_4; dispersion in weak acetic acid, followed by precipitation with dilute H_2SO_4; to indicate the presence of chitosan (see page 27[5] for further details).

As a confirmatory test, selected specimens were placed in 1 *N* NaOH for 24 h at 60 °C. This is based on the Hackman and Goldberg[6] procedure for purifying chitin. No structure containing a significant amount of chitin should be dispersed by this method, yet only the positive control retained its structural identity; the others had completely dispersed, with one exception. The exception was the piece of *Limulus* endosternite, which had swollen and started to peel off flakes, but had not completely dispersed. After 72 h the *Limulus* endosternite had dispersed, while the positive control had not changed in appearance, except that it became lighter colored.

Specimens tested by the van Wisselingh method were the endosternites of *Argas persicus* (Oken), *Amblyomma americanum* (L.) (Acarina); *Tarantula* sp. (Amblypygi); *Argiope argentata* (Forskal), *Dictyna* sp., *Dysdera crocata* C. L. Koch, *Lycosa frondicola* Emerton, *Pellenes hoyi*

[1] B. Firstman, J. Arachnol. *1*, 487 (1973).
[2] E. R. Lankester, Q. Jl. microsc. Sci. *24*, 129 (1884).
[3] A. P. Mathews, Z. physiol. Chem. *130*, 69 (1923).
[4] W. D. Halliburton, Q. Jl. microsc. Sci. *25*, 173 (1885).
[5] A. G. Richards, *The Integument of Arthropods* (Univ. Minnesota Press, Minneapolis 1951).
[6] R. H. Hackman and M. Goldberg, J. Insect Physiol. *2*, 221 (1958).