production est moins touchée dans des conditions d'hypoxie très poussée (2% d'O_2) que dans celles de très forte hyperoxie (80% d'O_2). Ce n'est pas le cas pour l'hydroxyproline: nous avons l'optimum de sa production à la concentration de 20% d'O_2, et la forte hyperoxie (80% d'O_2) se révèle moins nocive que l'hypoxie très poussée (2% d'O_2).

Sur la base de ces résultats, on peut conclure que la production d'hydroxyproline nécessite une quantité d'O_2 supérieure à celle des hexosamines. Ces dernières peuvent participer à la formation des différentes macromolécules de la substance fondamentale du tissu conjonctif (MPS acides sulfatés, MPS acides, glycoprotéines, etc.). Dans notre cas, si nous mettons en rapport la synthèse des MPS sulfatés avec la production des hexosamines, nous pouvons observer que les fibroblastes synthétisent in vitro des MPS sulfatés avec le même rythme à 5% et à 20% d'O_2, tandis que la production des hexosamines baisse fortement à des concentrations d'O_2 supérieures à 5%.

Cette donnée nous suggère l'hypothèse suivante: les hexosamines produites en grande quantité à 5% d'O_2 ne sont pas celles des MPS sulfatés, mais des hexosamines liées à d'autres substances (MPS acides, glycoprotéines, etc.).

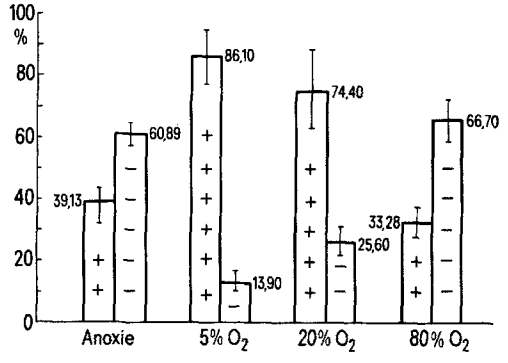

Fig. 2. Pourcentage de cellules marquées (+) et non marquées (−) par le $^{35}SO_4$ dans les cultures de fibroblastes cultivés en différentes concentrations d'oxygène (5%, 20%, 80%) et en anoxie.

Summary. Studies and comparison of the hexosamine and hydroxyprolin production by fibroblasts cultivated in vitro at different oxygen concentrations. The results show that the highest hexosamine production occurs at a low oxygen concentration (5%). The highest hydroxyprolin production occurs at 20% O_2; this oxygen concentration is considered as an hyperoxic one for fibroblasts cultivated in vitro. Besides it has been ascertained that the highest sulphated MPS production occurs between 5 and 20% oxygen without any significant variation.

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Morphological Aspects of the Nuclei in Mature Articulated Laticifers of *Calystegia soldanella*

A salient characteristic of articulated laticifers is the development of a multinucleate protoplast from a chain of longitudinal cells whose transverse walls are either partially or completely resorbed. The functional significance of these coenocytic structures, characterized by internal secretion, is still unknown, even though numerous studies dealing with the fine structure and the composition of the latex have been conducted[1-5]. The morphological aspect of the nuclei during the differentiation of the articulated laticifers is poorly understood, on the one hand because of the limited research on this subject and on the other hand because of the considerable technical difficulties involved in fixation and staining. It has been observed, however, that in *Taraxacum kok-saghyz*, the number and size of the nuclei vary according to the age of the tissue[6], and that in the laticifers of the secondary stem of *Hevea brasiliensis* and of *Manihot glazovii*, the nuclei undergo a degenerative process following nucleolar extrusion[7,8].

Based upon these indications, the purpose of this study is to contribute, using recently acquired techniques of embedding and staining, to the knowledge of the morphological aspects of the nuclei contained in the syncytial mature laticifers of *C. soldanella*.

Segments of the second internode of young stems of *Calystegia soldanella* R. Br. (Convolvulaceae), 5 mm long, were fixed in 10% neutral formalin for 24 h and then embedded in a methyl-butylmethacrylate mixture. The longitudinal sections in sets, 7 µm thick, which were cut using a LKB Pyramitome were hydrated by means of a decreasing alcoholic series and then were placed in a citric acid-$NaHPO_4$ buffer solution (pH 4.1; 0.06 *M*) for 5 min. Next the sections were stained for 15 min with acridine orange (AO), dissolved at a concentration of 10^{-4} *M* in the buffered solution. After a rapid dehydratation in tertiary butyl alcohol, the sections were embedded in a non-fluorescent medium (Entellan, Merck). The use of acridine orange as a fluorochrome is justified because of its demonstrated rapidity in absorption, its intense chromatism even at low concentration, and its specific characteristic of linking with nucleic acids[9-12]. The use of this dye is particularly interesting in laticifer studies because it offers, at the same time, the possibility to stain the cell wall also, therefore permitting an absolute

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identification of the nuclei of these tissues. The sections were viewed with a Zeiss Photomicroscope II equipped with an HBO 200 W lamp as the source of UV- light, inserting an excitation filter with a maximum at 365 nm and a barrier filter at 550 nm.

It was observed that the nuclei of both the medullar and cortical laticifers appear to be preferentially arranged in groups of 3–4 in the same segment, whereas the adjacent segments seem anucleate and are always surrounded by a dense matrix with a vesicular structure, which represents, following lipidic extraction, the proteic and polysaccharidic substratum of the latex. Single nuclei were only rarely observed and nuclei in mytosis were never seen; this may be due to the mode of development of the syncytial laticifers[13]. The laticifer nuclei of *Calystegia soldanella* consistently have a spheroidal form, but differ in size, chromatic capacity and presence or absence of the nucleolus. Based upon these characteristics, they have been divided into 4 types (Figure):

a) *Nuclei with metachromatic nucleolus.* These nuclei have a frequency of 22.5% and are of notable dimensions (11.7 $\pm$ 1.05 µm), usually larger in size than the nuclei of the surrounding parenchymatic cells. In the nucleus, large chromatinic masses (0.5–0.6 µm), 6 or 7 in number, assume a yellow-green color. The nucleolus of considerable size (2.5–3.0 µm), always larger than that in the nuclei of the surrounding cells, responds to fluorochrome with a metachromatic colouring.

b) *Nuclei with orthochromatic nucleolus.* The frequency of these nuclei is 21.3% and their size is inferior (8.8 $\pm$ 0.74 µm) to those of the preceding group, whereas the chromatinic masses maintain the number, size and chromatic capacity described for type a). The nucleolus shows reduced dimensions (averaging 1.5–2.0 µm) and is coloured green by AO.

c) *Nuclei without nucleolus.* The dimensions of these nuclei are quite reduced (6.06 $\pm$ 0.28 µm) and their frequency corresponds to 53.7%. Although the number of the chromatinic masses remains at 6–7, their size is usually greater than those of the 2 types of nuclei previously described. Furthermore, they show a different chromatic capacity since, with AO, they are intensely yellow in colour.

d) *Nuclei in degeneration.* Nuclei in degeneration were also observed with a frequency of 4.3%. Their diameter was extremely variable (6–10 µm). Inside these nuclei large chromatinic masses, which are barely visible, are weakly coloured dark green.

In the 4 groups considered, the diametrical values of the nuclei observed show a statistical diversity of $p < 0.01$.

The chromatic variations observed in the nucleoli stained with AO, resulting in an orange colour in some nuclei and a green colour in others, may be considered in relationship to the alteration of the RNA. In fact, the molecular significance of the orthochromatism of the RNA-AO complex lies in a modified structure of the helicoidal chain[14]. The change of fluorescence observed in the chromatinic masses of the nucleic may also be interpreted as a manifestation of a mutated macromolecular structure of the DNA[15, 16].

In conclusion, the formation of a multinucleate protoplast in the laticifers of *C. soldanella* may be the determining cause of the appearance of nuclei with different morphological characteristics; this in turn may be related to the process of differentiation of the tissue. The observations made during this study seem to indicate that, while a few nuclei increase in size and chromatic intensity, others face a slow process of degeneration which results in the reduction of the diametrical value, alteration and disappearance of the nucleolus, denaturation of the DNA and finally, nuclear fragmentation.

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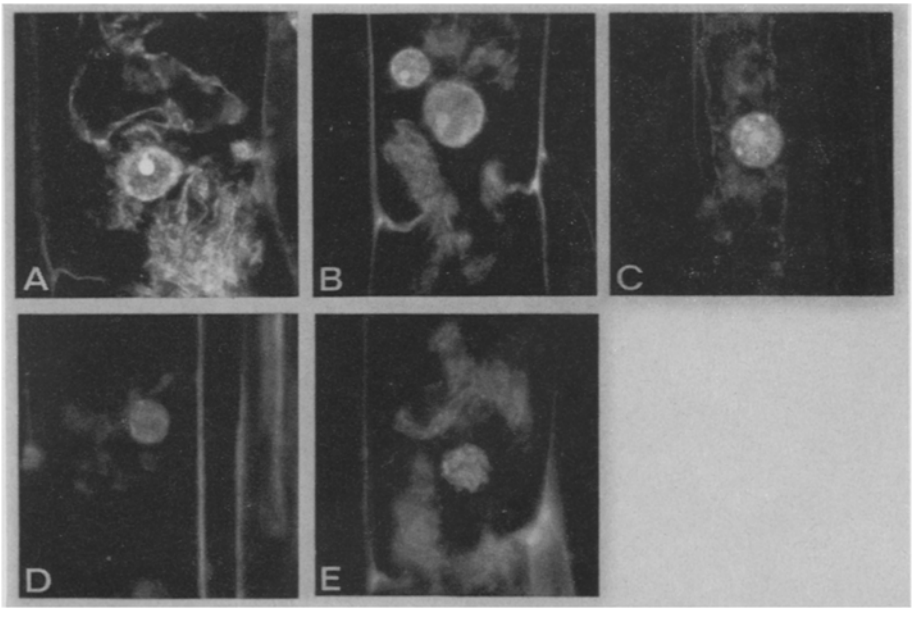

Nuclear polymorphism in differentiated laticifers of *Calystegia soldanella*, made evident by a fluorochromic staining (acridine orange): A) Nucleus with a metachromatic nucleolus. ×650. B) Nucleus with 2 nucleoli: 1 metachromatic (right) and 1 orthochromatic (left). ×760. C) Nucleus with a scarcely visible orthochromatic nucleolus. ×720. D) Nucleus with no nucleolus. ×760. E) Nucleus in degeneration. ×800.

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.

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