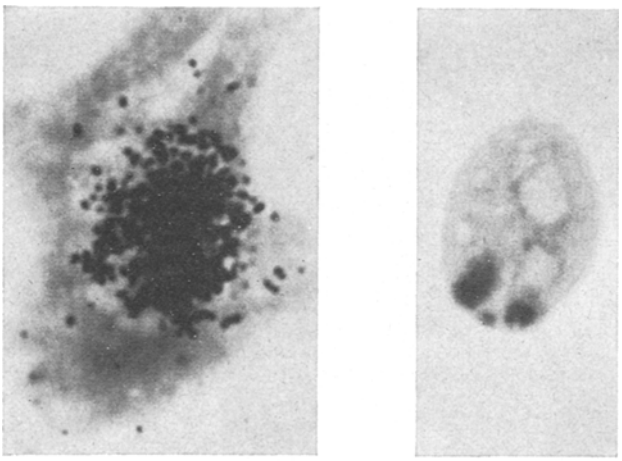
Epithelial cell cultures were set up from trypsinized kidney tissues of female and male *Microtus agrestis* (McCoy's medium 5a with 15% fetal calf serum). The cells were grown on coverglasses and incubated with tritiated uridine (50 µC/ml medium; specific activity 24.9 and 28.3 µC/m*M*) for 3, 10 and 30 min before fixation in 95% ethyl alcohol. They were autoradiographed (Kodak AR 10 stripping film), stained with pararosanilin-methylgreen, and photographed. After removal of the grains they were Feulgen stained and again photographed. Some of the preparations were treated with RNAse (1 mg/ml for 1 h at 37 °C) as controls.

Most of the cell nuclei of kidney epithelial cultures show 2 distinct chromocentres. Sometimes the 2 are fused together resulting in a single large one. In the remaining interphase nuclei the sex chromosomes form either loose structures or are not conspicuous, with the exception of the 'true' sex chromatin part of the *X* chromosomes in female cells[7,8]. If chromocentres are present, they show no or only few grains after labelling with H^3-uridine, whereas the euchromatic regions and the nucleoli are clearly labelled (Figure). No difference in this respect can be observed in preparations which were treated with H^3-uridine for 3, 10 and 30 min. Similar experiments on other cell types, e.g. suspensions of surviving cells from brain and lung tissues, showed also no or only little H^3-uridine incorporation into heterochromatin compared to euchromatin.

Of particular interest are the cells without distinct chromocentres in interphase except a small heterochromatic body, corresponding to sex chromatin in female cells. These cells show a lack of H^3-uridine incorporation in circumscribed nuclear regions which are presumably occupied by the sex chromosomes. This would indicate that heterochromatic chromosomal regions are genetically inactive also in a less or possibly non-condensed state.



a b

Kidney epithelial cell of *Microtus agrestis* in vitro. Absence of grains in heterochromatic regions after H^3-uridine labelling (incubation for 10 min, 40 days exposure). × 2000. (a) Autoradiography, pararosanilin-methylgreen. Chromocentres are stained with methylgreen and appear faint in green filtered photography. (b) After removal of grains and Feulgen staining.

Zusammenfassung. Autoradiographische Untersuchungen nach H^3-Uridineinbau von Nierenepithelzellkulturen der Erdmaus (*Microtus agrestis*) zeigen, dass die Chromozentren nicht oder viel schwächer markiert sind als Euchromatin und Nukleolen. Auch in Zellen ohne sichtbare Chromozentren finden sich Aussparungen im H^3-Uridin-Markierungsbild, die vermutlich den heterochromatischen Geschlechtschromosomen entsprechen. Heterochromatische Chromosomenabschnitte sind demnach genetisch inaktiv oder weniger aktiv, wobei es keine Rolle zu spielen scheint, ob sie in der Interphase zu mikroskopisch sichtbaren Chromozentren kondensiert sind oder nicht.

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A Hypothesis Concerning the Use of Colchicine as a Polyploidy Inducer[1]

It has been demonstrated in a great variety of plant and animal species that colchicine has the ability to arrest the development of the spindle during mitosis[2], and thereby causes the accumulation of cells at metaphase. In woody plants, however, colchicine may fail to produce its mutagenic effects[3]. This has been explained by the lower rate of division of the cells of the woody plants[4]. Taking this into consideration, ZATYKÓ and SIMON[4] have succeeded in increasing the percentage of myxoploids treating black-currant hybrids with gibberellic acid – a hormone which stimulates cellular division[5] – along with colchicine. ZATYKÓ and SIMON have concluded that the higher number of myxoploid cells obtained is partly the result of the activation of cytokinesis caused by the gibberellin. On this basis, it is suggested that any substance physiologically related to the gibberellin may equally intensify the polyploidy activity of colchicine. The experiments described here seem to show that a low dose of colchicine may fulfill the hormonal requirements which have been outlined.

[1] Acknowledgments. This work has been supported by a research grant from the National Research Council of Canada.
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Material and methods. Seeds of wheat (*Triticum aestivum* L. em. Thell. var. Rescue) and rye (*Secale cereale* var. Prolific) were germinated on moist filter paper, then incubated in containers with distilled water or an aqueous solution of colchicine. Incubation took place in the dark at 2 °C and 24 °C temperature.

Results and discussion. Figure 1 shows that colchicine stimulates the growth in length of the coleoptile of rye seedlings. This growth effect could be due to the acceleration of the division of the cells. The coleoptile arises as a meristematic ring of cells on the embryonic tissue which later differentiates into the scutellum[6]. Its expansion upon germination starts taking place by cellular division and by the elongation of the cells of the axis above the scutellar node. However, when the coleoptile is longer than 10 mm its growth is generally due solely to elongation of its cells[7,8]. Figure 1 refers to the development of coleoptiles 2 mm long at start of treatment. On the other hand, the coleoptile length did not exceed 10 mm at the end of the incubation period. The experiment illustrated in Figure 1 demonstrates clearly that colchicine stimulates growth at concentrations far below the range which produces its mutagenic effects in plant cells. It does not prove, however, whether or not colchicine is activating preferentially the cells in a state of division. In order to elucidate this matter, the study of the development at 2 °C of roots and coleoptiles of colchicine-treated wheat seedlings has been undertaken. It is remarked that:

(1) Low temperature extends the length of the interphase periods which prepare the cells for mitosis[9]. Furthermore, it disrupts reversibly the spindle microtubules[10]. This brings about the accumulation of cells at metaphase[11], and concomitantly inhibits growth. If colchicine is expected to stimulate cell division, it may cause the cells delayed in interphase – or arrested in metaphase – to enter mitosis. This will increase therefore the rate of growth of the root and the coleoptile.

(2) As an activator of cytokinesis, colchicine would be able to improve the root development when the rate of coleoptile elongation is already declining. This follows from the analysis of the kinetics of growth of these organs: contrarily to the coleoptile, the root grows continuously due to the activity of a meristematic tissue located in its apex[12].

Figure 2 shows that these conditions have been verified. An aqueous solution of colchicine (0.01 μM) not only causes the roots and coleoptiles to grow faster at 2 °C, but still increases root growth when the rate of coleoptile elongation has decreased to a value below the control. This effect on cell division has been investigated further (Table). The results show that colchicine enhances the germination of wheat seeds. Growth of the root upon germination starts primarily as a consequence of the functioning of the apical meristems. So far, this is another indication of the activation of cytokinesis by colchicine.

In this connection, it is of interest to mention that this substance (0.25 μM) is also able to stimulate in vitro the development of a fast growing tumour tissue of *Rumex acetosa*[13].

Gibberellic acid enhances de novo synthesis of α-amylase in barley seeds, as well as RNA synthesis[14]. Moreover, there is evidence that the gibberellin exerts its regulatory role at the gene level[15], and may affect consequently the transcription of the DNA template.

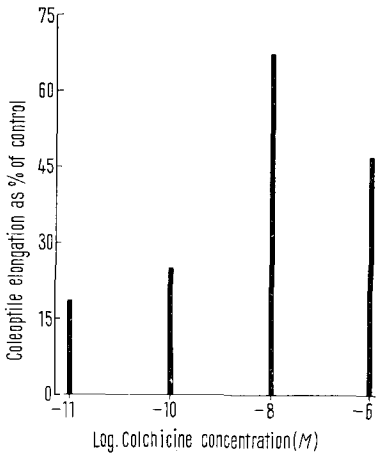

Fig. 1. Effect of colchicine on the elongation in vivo of the coleoptile of rye seedlings. Samples of 20 seedlings with 2 mm long coleoptiles were incubated in the dark in an aqueous solution of colchicine (c) and in distilled water (dw) during 18 h and at 24 °C temperature. L: coleoptile length. % of control $= [L(c) - L(dw)/L(dw)] \times 100/L(dw)$. It is remarked that colchicine stimulates the elongation of the coleoptile at concentrations below the range which produces its mutagenic effects in plant cells.

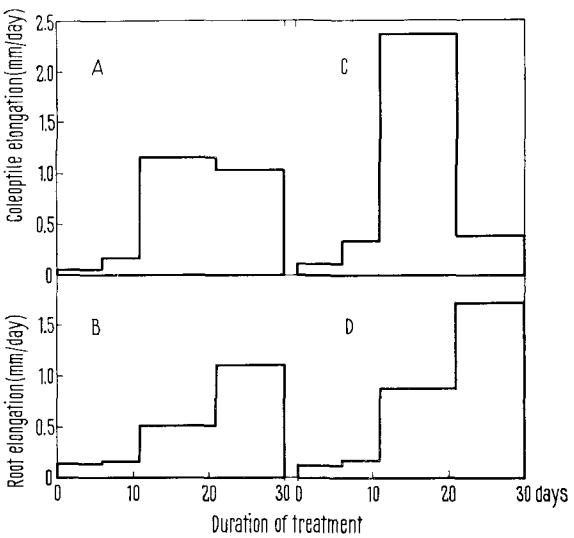

Fig. 2. Effect of colchicine on the development in vivo of the roots and the coleoptiles of wheat seedlings. Samples of 20 seedlings with 1 mm long coleoptiles were incubated in the dark at 2 °C temperature in distilled water (A, B) and in a 0.01 μM aqueous solution of colchicine (C, D). It is seen that colchicine stimulates the development of the roots and the coleoptiles, and still increases root growth when the rate of coleoptile elongation is lower than the rate of the water control.

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To date, the molecular mechanisms of colchicine stimulation of growth have not been studied. The observations of KING[16] seem to indicate that they could be similar to the gibberellin-induced α-amylase biosynthesis. This author has shown that colchicine (1.0 μM) stimulates the liberation of sugars in the wheat seed during germination. In addition, the demonstration that colchicine binds in vitro to DNA molecules[17] points out again that its site of action may be at the gene level. Besides, it is plausible to foresee that colchicine produces its effect of growth stimulation in a way similar to the induction of c-mitosis. That is to say, due to its capacity to bind to subunit protein of microtubules of the mitotic apparatus[18].

In brief, the mechanisms underlying the stimulatory effect of colchicine remain unclear. Nevertheless, the afore-discussed would seem to indicate that colchicine – at low concentration – may well be effective as a promoter of cell division, and thereby prepare the tissues for its ulterior mutagenic activity at an higher concentration. It may be concluded therefore, that the cellular mechanism of colchicine action has a twofold function: the stimulation of the nuclear division and of cytokinesis, followed by the induction of polyploidy. One practical consequence from these findings might be the use of colchicine in a dosage sequence for increasing the percentage of polyploid cells in the tissues.

Effect of colchicine on the germination of wheat seeds

Colchicine concentration (μM)	Duration of treatment (h)	
	48	56
0	58	69
0.1	65	72
10.0	69	86
1000.0	76	93

Samples of 30 seeds were incubated in the dark at 24 °C temperature in an aqueous solution of colchicine and in distilled water. The results are given as percentage of seeds germinated at the end of the incubation periods.

Résumé. Le développement de la racine et de la coléoptile est stimulé lorsque des plantules de Graminées sont incubées avec la colchicine à des concentrations au-dessous du seuil qui produit ses effets mutagéniques. Cette stimulation de la croissance – qui semble être due à une activité méristématique accrue – met en évidence un double aspect du méchanisme d'action de la colchicine: la stimulation de la prolifération cellulaire, suivie de l'induction de la polyploïdie. En outre, ceci suggère que le traitement des organes avec des doses de colchicine chaque fois plus élevées devrait permettre d'augmenter le nombre de cellules polyploïdes dans les tissus.

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Fixation of Elementary Nitrogen by *Nostoc punctiforme* (Kütz.) Hariot and *Scytonema bohneri* Schmidle in Pure and Unialgal Cultures

A number of blue-green algae have been shown to fix elementary nitrogen in pure cultures. Association of other micro-organisms, especially bacteria, with these algae have been shown to result in increased nitrogen fixation, and in most of the cases the total nitrogen fixed was higher than that fixed by either of the organisms when grown singly[1-3].

During a survey of the soil algae from the rice fields, a large number of blue-green algae have been isolated in unialgal cultures. Many of these are found to thrive well on a culture solution free from nitrogen. In the present investigation the nitrogen fixation capacity of 2 algae has been studied and compared in both unialgal and pure cultures. Unialgal cultures of *Nostoc punctiforme* (Kütz.) Hariot and *Scytonema bohneri* Schmidle, isolated from rice field soils of Ballia and Ghazipur (U.P., India), respectively, were raised in pure bacteria-free cultures by use of UV-irradiation coupled with streaking on silica gel plates after the methods of GERLOFF et al.[4], and WATANABE[5]. Purity of cultures was tested with peptone solution (1%), mixture of glucose (0.5%) and peptone (1%) solutions, meat extract ('Oxo-Lablemco') solution (1%) and DE's[6] liquid solution containing glucose (0.5%).

The cultures were grown at 28–30 °C under continuous fluorescent light (180 Lux) in 250 ml Pyrex Erlenmeyer flasks, containing 100 ml of DE's[6] nitrogen-free medium, supplemented by modified HUTNER's[7] micronutrient solution, in which $(NH_4)_6Mo_7O_{24}$ was replaced by MoO_3 in order to make it nitrogen-free as described earlier[3]. Initial pH of the solution was adjusted to 7.0.

The cultures were harvested after 35 days of growth and the purity of the cultures was retested. The algal mat of each replicate was separated by centrifugation. Nitrogen was estimated both in the supernatant and the algal mat

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