

scattered in the nucleoplasm. With Heidenhain's azan, these cells are easily identified as they show strongly orangeophilic reaction (Figure 3).

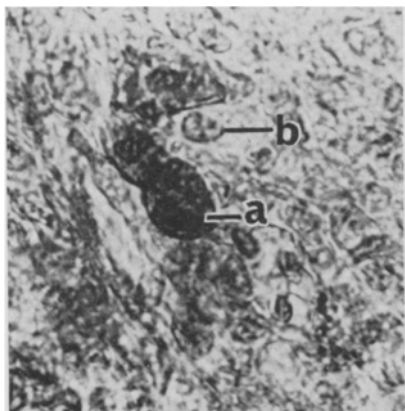


Fig. 3. Transverse section of the Y-organ of *V. litterata* showing selectively stained (Azan) large cells. a) large cell; b) small cell.

According to the earlier literature, the Y-organ of the crustaceans so far studied is composed of only one cell type, but the present study on *V. litterata* revealed 2 cell types in the organ. The Y-organ is known to regulate various physiological activities such as moulting, calcium distribution and reproduction⁶. It is difficult to say whether separate hormones are involved in the regulation of these 3 physiological processes. But the occurrence of 2 types of cell in the Y-organ points to the possibility of the secretion of at least 2 hormones by the Y-organ. Further work on these lines is in progress.

Résumé. Dans l'«organe Y» du crabe *V. litterata*, deux types de cellules ont été observés et leurs caractères morphologiques sont décrits.

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Cytological Effects of Vinblastine in Plants

During the last decade, the cytotoxic action of *Vinca rosea* alkaloids, especially vinblastine and vincristine, has been investigated in several biological systems¹. In this respect, we could mention various animal cells cultivated in vitro², leukemic and bone marrow cells in man³, embryonic cells in vivo⁴ and haematopoietic tissues of chicken embryos⁵. Colchico-mitotic effects i.e. C-metaphases leading to polyploid cells were reported in all cases²⁻⁵.

The cytogenetic effects which could be approximately described as radiomimetic ones, and which are distinct from the C-mitotic activity, are known for vincristine from studies on human leucocytes in leukemic patients⁶; but such effects have been far less studied for vinblastine.

All these investigations led to the use of these alkaloids in leukemia-therapy although it seems that the chemical and cytological basis of this activity is far from being fully understood. Moreover, none of the previous reported works deals with plant cells, in which, however, the action of polyploidizing agents has long been known. These are the reasons why the present investigation was designed with different plant materials.

Material and methods. Seeds of 3 plant species, i.e. *Hordeum sativum* Jess (var. Pirolina), *Vicia faba* L. (ssp. minor var. Åkerböna Weibull) and *Nigella damascena* L. (var. Miss Jekyll) were germinated in petri dishes on moistened filter paper (21°C) for 5-6 days. Root tips were immersed (3 h) in solutions of increased concentrations ($1.10^{-6}M$ – $1.10^{-4}M$) of vinblastine (Velbe®, Eli Lilly and Co, Indianapolis, USA). After treatment, roots were washed with distilled water and then replaced in the above-described experimental conditions till fixation (Carnoy) at different times from 0 to 24 h after the end of treatment. Slides were prepared from Feulgen squashes.

Results. The main data for anaphase investigation are given in Table I. The radiomimetic effects consist in chromosome bridges and fragments. The amount of such aberrations is low (less than 1%) in barley and a bit higher in broad bean. *Nigella damascena* was found to be the most

sensitive species. At $1.10^{-4}M$, the maximum amount of aberrations was scored 4 h after treatment; the amount decreased at longer durations after the end of the treatment. Paradoxically, the maximum amount was scored at

Table I. Percentages of anaphase aberrations

Material	Concentration (M)	Time after treatment (h)				
		0	4	8	12	24
<i>Hordeum sativum</i>	1.10^{-4}	—	0.0	0.0	0.5	0.0
	1.10^{-5}	0.7	0.7	0.0	0.0	0.7
	1.10^{-6}	0.3	0.0	0.3	0.3	0.0
	Control	0.3	not investigated			
<i>Vicia faba</i>	1.10^{-4}	2.0	3.7	—	—	1.0
	1.10^{-5}	0.7	2.7	1.0	0.0	0.7
	1.10^{-6}	0.7	0.0	0.0	1.3	0.7
	Control	2.3	not investigated			
<i>Nigella damascena</i>	1.10^{-4}	5.5	8.5	5.5	2.5	1.5
	1.10^{-5}	12.5	4.5	1.5	0.5	0.5
	1.10^{-6}	3.5	1.0	2.5	0.0	0.0
	Control	1.0	not investigated			

300 anaphases analyzed in *Hordeum* and *Vicia*, 200 anaphases in *Nigella*. —, Few or no anaphases owing to metaphase accumulation.

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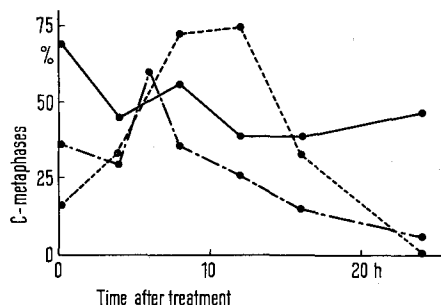


Fig. 1. C-metaphases induced by vinblastine ($1.10^{-4}M/3$ h) in *Hordeum* (—), *Vicia* (---) and *Nigella* (...).

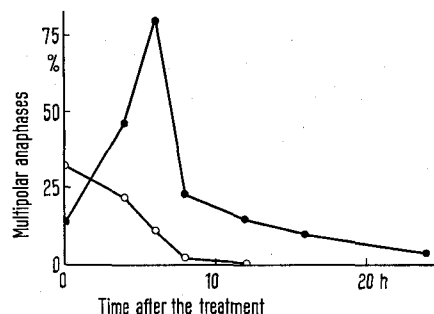


Fig. 2. Multipolar anaphases induced in *Nigella* by vinblastine $1.10^{-4}M$ (●) and $1.10^{-5}M$ (○).

Table II. Percentages of metaphase aberrations in *Nigella damascena*

Concentration (M)	Time after treatment (h)				
	0	4	8	12	24
1.10^{-4}	1.7	0.3	0.7	3.0	0.0
1.10^{-5}	0.3	1.0	—	—	—
Control	0.3	not investigated			2.0

300 cells analyzed. —, Absence of C-metaphases.

$1.10^{-5}M$ immediately after treatment, but decreased quickly with the recovery period. The lowest concentration only yielded aberrations immediately after treatment.

Besides the radiomimetic aberrations, we also observed stathmokinetic effects which confirm for plant materials the data obtained in animals so far. We noticed colchico-metaphases which were sometimes so numerous that anaphase analysis was prevented. This was the case for barley and broad bean at the highest concentration but it should be pointed out that this effect occurred somewhat later in *Vicia* (Figure 1).

The modification of C-mitotic effects runs parallel to the radiomimetic ones in the 3 species. Merostathmokinetic effects (incomplete C-mitosis) were observed all the time which consisted in multipolar anaphases (Figure 2), metaphases-anaphases suggesting a partial disorganization of spindle fibers.

The sensitivity of *Nigella damascena* incited us to analyse further metaphase aberrations. In root tips the C-mitotic induction is roughly proportional to the concentration, no C-metaphases remaining at the lower concentration for duration longer than 4 h after treatment. In Table II, the chromosomal aberrations observed mainly consisted in breaks, giving rise to acentric fragments. The amount of such aberrations is very low as compared with that obtained for anaphases. These results suggest that

the anaphase aberrations were induced subsequently to the metaphase, probably at early anaphase. They can be related to disturbances of the spindle apparatus. Their origin is the same as that of multipolar anaphases.

Discussion and conclusions. *Nigella damascena* has always been reported to be a very sensitive plant material suitable for investigations in mutagenesis. Thus it is not surprising that the highest amount of chromosomal aberrations was observed with that species, on the one hand. On the other hand, the radiomimetic effect is all the time low, if existing at all, in the two other species. Recent investigations using dominant lethal mutations in mouse⁷ failed to detect mutagenic effects. It can thus be concluded that in all biological systems so far investigated the mutagenic action of the vinblastine is slight. However, the C-mitotic effect could result indirectly in mutations by giving rise to polyploid cells⁸.

Résumé. Des racines de *Hordeum sativum*, *Vicia faba* et *Nigella damascena* ont été traitées par des solutions ($1.10^{-4}M$ à $1.10^{-6}M$) de vinblastine. Chez les trois espèces, nous avons pu observer des effets stathmocinétiques: C-métaphases et anaphases multipolaires. Chez *Vicia* et *Hordeum*, les taux d'aberrations sont très faibles; par contre, *Nigella* s'est montrée plus sensible à l'action radiomimétique de l'alcaloïde.

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Characterization of DNAs from *Coprinus lagopus* and *Mucor azygospora*

Very little information exists on the characteristics of fungal DNAs. Since DNA serves as the basis for the functional and genetical differentiation of an organism, its characterization becomes necessary for any study of dif-

ferentiation at molecular level. BRITTEN and KOHNE¹ have shown that the studies of DNA:DNA dissociation and reassociation reactions give very reliable information regarding the nature of DNA and genome size (total DNA/