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Mitodepressive Effects of *Chelidonium* Alkaloids on Root Tip Cells of *Allium cepa* L.

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Abstract. A comparison of the effects of a water extract of the fresh vegetation apex of *Chelidonium majus* L. var. *majus*, with those of berberine sulphate and of chelidonine hydrochloride is reported. The water extract of fresh stems and leaves of *Chelidonium majus* and the berberine sulphate solution had marked mitodepressive effects on onion root tip cells. Chelidonine hydrochloride had similar but less marked effects. On the basis of the results obtained it can be assumed that the most active group of substances with cytostatic effects, hindering the cells from entering mitosis, are protoberberine bases contained in the latex of *Chelidonium majus*. Within the range of the investigated concentrations the extract of fresh stems and leaves was less toxic for the cells than berberine sulphate. The data ascertained provide evidence that the mechanism of the cytostatic action of chelidonine, of berberine, as well as of the extract of fresh stems and leaves of *Chelidonium majus* differs from the mechanism of the action of colchicine. Of the tested *Chelidonium* alkaloids only chelidonine produced a partial inactivation of the mitotic spindle.

Pharmacological and medical research projects have, for many years devoted much interest to substances displaying cytostatic effects. The medicinal herb *Chelidonium majus* has been well known for a long time in the popular treatment of various types of tumours, both benign and malignant. Preparations made from the latex of *Chelidonium majus* have been used equally for medical cancer therapy, especially in superficial neoplasms (BALICKIĬ 1966). However studies into the cytostatic effects of alkaloids contained in the latex of this plant have so far failed to provide any definite conclusions (WIDMANN 1955, SOKOLOFF 1968, BENTURA *et al.* 1969, ŠVEJDA *et al.* 1969, GHEORGHIU *et al.* 1970). ŠVEJDA *et al.* (1974) found that the alkaloids chelidonine and sanguinarine reduced the viability of L-cells grown *in vitro*, however without any demonstrable changes of the ultrastructure of the cells treated. The latex of *Chelidonium majus* contains more than twenty alkaloids, which can be subdivided on the basis of their chemical structure into three major groups: benzophenanthridine, protoberberine and protopine alkaloids (SLAVÍK *et al.* 1965, RUMIŃSKA 1973). It remains to be ascertained to what extent these groups of alkaloids, in particular of the benzophenanthridine and protoberberine types, participate in the cytostatic effect of the latex,

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which was demonstrated repeatedly (ŠVEJDA 1948, 1949, SOKOLOFF 1968, GHEORGHIU *et al.* 1970). Combinations of these two groups of alkaloids are characteristic of the *Chelidoniae* tribe of the *Papaveraceae* family, including also *Chelidonium majus* L. (HEGNAUER 1969). The present communication deals with the comparison of the cytostatic effects of *Chelidonium* alkaloids of benzophenanthridine and protoberberine types with those displayed by the latex of *Chelidonium majus* and thus provides a contribution to the elucidation of the above mentioned problems but on the plant material only.

Material and Methods

A certain number of the plants *Chelidonium majus* L. var. *majus*, transplanted from their original site in a ruderal habitat in Prague 2, to a bed in the Genetic Garden of the Faculty of Science, Charles University in Prague, were used in our experiments for the preparation of extracts of the top of the plants. Chelidonine and berberine alkaloids were tested in the form of saline solutions of chelidonine hydrochloride and berberine sulphate. Mitodepressive effects both of the alkaloids and of the extract were studied on root tip cells of *Allium cepa* L. cv. Všetatská.

1) Preparation of an Extract from the Top of *Chelidonium majus*

Finely chopped fresh stems and leaves were mixed with distilled water in a weight ratio of 1 : 10, and extracted for 17—20 h at room temperature. Subsequently this was decanted and filtered and the freshly prepared water extract of *Chelidonium majus* was used immediately (at a 10 % concentration), or was diluted with distilled water to 1 %, 3.2 % and 6.4 % concentrations, calculated on the basis of the weight ratio of fresh tops to distilled water. Simultaneously another sample of the top of the same plant was used for the determination of the alkaloid content with the use of the acidimetric method (DEBICKA and KACZMAREK 1955). This enabled the comparison of experiments performed in various periods of the vegetation season with differing alkaloid content in the top of the plant (BOSHART 1953). However, it was not possible technically to determine any qualitative changes in the composition of the extract. The actual presence of alkaloids in the acidified water extract was determined with Mayer's reagens (K_2HgIO_4). Positive reactions were associated with the formation of insoluble yellow or orange coloured polyiodide alkaloids.

2) Other Tested Substances

The range of the tested chelidonine hydrochloride concentration was limited by the water solubility of this substance. It was used in concentrations of 0.1 %, 0.05 % and 0.01 %. Berberine sulphate which is readily soluble in water was used in 0.1 %, 0.01 % and 0.005 % concentrations. Because of the assumed analogous effects of chelidonine and colchicine (WIDMANN 1955) the latter was tested in a 0.05 % concentration.

3) Experimental Procedure and Assessment of Cytostatic Effects

Onion bulbs were cultivated in water at room temperature. The roots of several onions were immersed in a given solution, while some other onions kept in distilled water served as controls. Root tips varying in length from two to four cm were removed after an exposure of 1, 2, 5, 8, 10, 12 and 24 h.

Long-term effects of tested solutions were investigated on root tips which were removed after longer time periods. Some onions the roots of which were immersed for 24 h in a given solution were subsequently retransferred to distilled water for observations of an eventual recovery of the treated cells. Each sampling consisted of a random selection of three onions incubated in the tested solution, as well as of three control onions. One root tip was removed from all six onions. This procedure was repeated two or three times for each concentration of all individual tested substances.

The removed root tips were fixed in acetic alcohol (3 : 1), macerated in a mixture of HCl — alcohol (1 : 1), washed in distilled water and stained with aceto-orcine. Squash preparations were made in a saturated levulose solution. Fifteen microscopic fields at a magnification 10×60 were examined in each single root tip. The numbers of nuclei in the interphase, as well as in individual mitotic phases were counted in each microscopic field. Impaired nuclear division (inhibition of the function of the mitotic spindle, binucleate cells, bridge formation *etc.*) were recorded as well.

The data obtained during the studies of three root tips (three controls and three root tips incubated in the tested solution) from each sampling were used for the calculation of mitotic indices, as well as of the proportions of individual mitotic phases (the so—called phase indices). The numbers of nuclei ascertained in individual samplings and in individual tested substances ranged from 1500 to 2000.

Results

a) Chelidonine Hydrochloride

Mitodepressive effects of chelidonine were evident as early as from the eighth hour of exposure to the solution (Fig. 1). An expressive reduction of mitotic activity was observed in root tips cultivated in 0.1% and 0.05% concentrations. The frequency of individual mitotic phases within 24 h of exposure was characterized by their maximum values expressed in terms of per cent in Fig. 2. Continued observations within the time interval between 24 and 100 h of exposure failed to disclose any further reduction of the mitotic index. There were, on the contrary, signs of a gradual repair of mitotic activity. The transfer of treated root tips from the chelidonine hydrochloride solution into distilled water resulted in a sharp increase of mitotic activity; there was particularly a marked increase of the percentage of prophases.

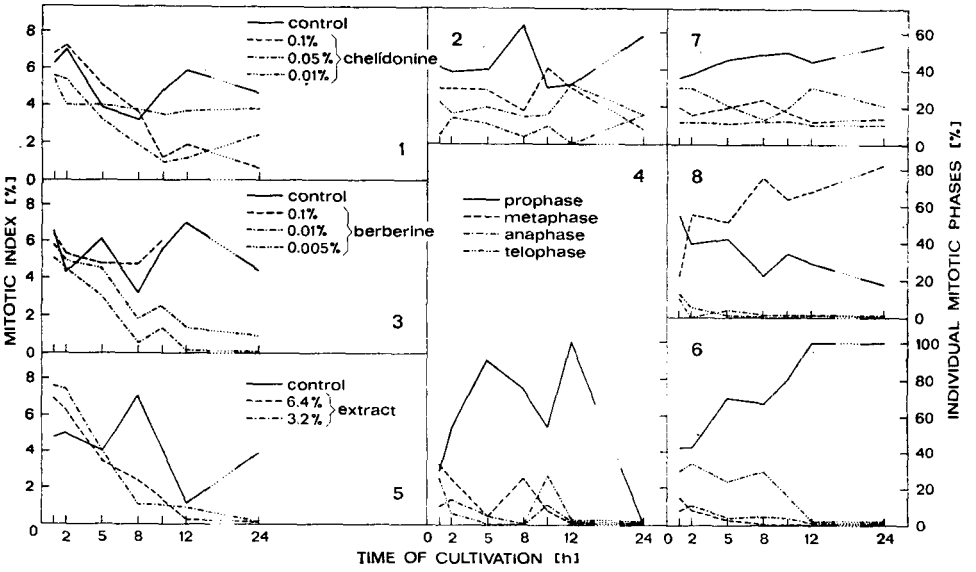
Deviations from normal mitosis: impaired nucleolar division occurred, though rather uncommonly, after exposure to all three concentrations. Chromosomes showed the formation of free groups in the equatorial plane, *i.e.* on both poles of the mitotic spindle, occurring during metaphase and anaphase. A tetrapolar anaphase was recorded as well. Chromosomes showed a trend towards stickiness and bridge formation during the anaphase. All these aberrations were evident from the 2nd to 5th hour of exposure to the tested solution. Binucleate cells were seen only rarely.

b) Berberine Sulphate

The concentration 0.1% proved excessively toxic for the cells. The use of two lower concentrations (0.01% and 0.005%) resulted in a marked mitodepressive effect (Fig. 3) associated with a relative increase of the prophase index (Fig. 4). The toxic effect of berberine sulphate was manifested by

pycnosis with following disintegration of cell nuclei. This disintegration was recorded as early as after two hours of exposure to the 0.005% solution, beginning at the periphery and proceeding into the central part of the root. After 24 h of exposure to the 0.005% solution the predominant majority of nuclei appeared to have been killed and no recovery followed after the transfer of the root into distilled water.

Deviations from normal mitosis: a characteristic and common anomaly after exposure to berberine sulphate consisted of chromosomal bridges observed from the fifth hour of exposure onwards. Binucleate cells were also rather frequent, sometimes the two nuclei were connected by a bridge. Some of the treated cells contained micronuclei.



Figs. 1, 3, 5: Values of the mitotic index of root tip cells of *Allium cepa* cultivated for 24 h in chelidonine hydrochloride solutions (1); in berberine sulphate solutions (3); in 3.2% and 6.4% extracts of the top of *Chelidonium majus*. The stems and leaves were sampled on August 27, 1974, alkaloid content 1.21% (5).

Figs. 2, 4, 6, 7, 8: The percentage of individual mitotic phases in root tip cells of *Allium cepa* cultivated for 24 h in a 0.1% solution of chelidonine hydrochloride (2); in a 0.01% berberine sulphate solution (4); in a 6.4% extract of the top of *Chelidonium majus*. The stems and leaves were sampled on August 27, 1974, alkaloid content 1.21% (6); in distilled water — controls (7); in a 0.05% colchicine solution (8).

c) Colchicine

The results of the investigated effects of 0.05 % colchicine solution were in full agreement with reported experience. The mitotic index did not differ from controls during root tip cultivation in the above mentioned colchicine solution, and showed an increase from the twelfth hour onwards. A steep rise of the metaphase index occurred as early as after two hours of cultivation, while the anaphase and telophase indices were very low and later disappeared completely (Fig. 8). Mitosis was further associated with anomalies which

were typical of colchicine: *c*-metaphase, polyploid nuclei, multipolar mitosis *etc.*

d) Extract from the Fresh Top of *Chelidonium majus*

Exposure to all four tested concentrations of the extract (1 %, 3.2 %, 6.4 %, and 10 %) resulted as early as after two hours in a steady decrease of the mitotic index (Fig. 5). Disintegration of nuclei was ascertained after 24 h of cultivation in a 10 % extract. The percentage of prophases increased after 2 to 5 h of exposure to the extract, while the other mitotic phases were supressed (Fig. 6). However the mitodepressive effects of the extract were only temporary and the division of treated cells was resumed after an exposure of 24 h. No changes occurred within the first three hours after the transfer of roots from a 6.4 % extract into distilled water. First signs of an increased mitotic index were recorded after four hours of renewed cultivation in distilled water.

Deviations from normal mitosis: from the 2nd h of root cultivation in 6.4 % and 10 % concentrations of the extract it was possible to record a frequent formation of anaphase and telophase bridges, as well as a high frequency of binucleate cells. These anomalies persisted even after the retransfer of roots into distilled water following their exposure to the extract for 24 h. High concentrations of the extract (10 %) resulted in changes suggestive of tetrapolar anaphase and of the production of micronuclei.

The global results of the studies of the cytostatic effects of chelidonine alkaloids and the experimental procedures were described in more detail in my diploma thesis (FRESLOVÁ 1976).

Discussion

Chelidonine and berberine alkaloids, as well as extracts of fresh stems and leaves of *Chelidonium majus* exerted mitodepressive effects on root tips of *Allium cepa* during an exposure of 24 h. Both berberine and the extract of fresh tops of *Chelidonium majus* resulted in the most marked supression of cell division. Both substances produced similar anomalies of mitosis. The similar course of the changes of mitotic indices, as well as of indices of individual mitotic phases provided evidence that an identical, or at least chemically and biologically similar substance was responsible for these effects in both cases. Shorter mitodepressive effects of the extract compared to berberine were most probably due to the lack of stability of the effective components of the crude extract. Within the range of the investigated concentrations the toxicity of the extract to cells was less marked than that of berberine, since cells exposed to the extract recovered during recultivation in distilled water, while berberine resulted after 24 h in the death of all cells. The application of 0.1 % solution of berberine sulphate resulted probably in cell fixation beginning immediately after immersion into the solution. Chromosomes in dividing nuclei were mostly pycnotic and root tips were completely macerated after 10 h of solution treatment. Departures of mitotic index in this case (Fig. 3) were perhaps due to variability among individual roots as well as among individual samplings. ŠVEJDA *et al.* (1969) observed L-cell death after exposure to another protoberberine base-koptisine, which exerted equally marked toxic effects, and produced similar

structural changes of chromatin and in addition certain chromosome aberrations.

Repeated experiments disclosed that an extract prepared in November (alkaloid content 0.81%) exerted less marked effects than an extract prepared in August (alkaloid content 1.21%). This was most probably due to the lower alkaloid concentration in autumn. However, another factor is obviously of importance as well, *i.e.* the changes of stylopine content which occurs in summer predominantly in the leaves and in autumn mostly in the roots (SLAVÍK 1954, SLAVÍK *et al.* 1965). The chemical structure of this protoberberine base is very similar to that of berberine and it most probably exerts a similar cytostatic effect.

The mechanism of action of berberine obviously is not completely identical with that of chelidonine. The maximum values of individual indices of mitotic phases (Fig. 2) show that chelidonine produces a retardation of individual mitotic phases but only in part of nuclei completing mitosis. At the beginning of the experiment these nuclei were further than at the end of the S phase. (An expressive reduction of mitotic activity was observed after 8 h of experiment). The cell cycle in other nuclei was also retarded, which could be due to the blocking of high energy substances which are essential for mitosis (BENTURA *et al.* 1969). WIDMANN (1955) believed that the effects of chelidonine are analogous to those of colchicine. The results of our experiments refute this claim, since contrary to colchicine, chelidonine did not stimulate cells to enter mitosis and did not block mitosis in the metaphase. It was possible to ascertain only a slight degree of inhibition of the mitotic spindle activity after exposure to chelidonine, which was manifested by multipolar mitosis.

Berberine hindered the cells from entering mitosis. The increase of the prophase index occurring from the very beginning of exposure to a berberine sulphate solution can be explained by the prolongation of the prophase. In spite of a dramatic decrease of mitotic activity a certain number of the cells completed division, though at a slow rate. The prophase was prolonged approximately to three hours (Fig. 4) compared to 68 min under normal conditions (LÓPEZ-SÁEZ *et al.* 1966). It may be that this assumption is rather deformed by evaluation of curves of individual phase indices (Figs. 4, 6). The low frequency of mitotic nuclei results in less exact determination of individual phase indices. However, the total count of investigated nuclei (1500 to 2000 per sampling) probably provides sufficient exactness, though the statistic evaluation was not performed.

The bridges which were found during the cultivation of root tips in berberine sulphate solutions and in extracts from the leaves and stems, were most probably due to pycnosis and the chromosome stickiness, since no fragments were disclosed. Binucleate cells developed probably in the anaphases associated with formation of numerous bridges preventing the development of a new cell wall. Micronuclei developed obviously in distinct groups of sticking chromosomes.

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