ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 57, nr 3: 329-340
1988

Sodium cacodylate as antimitotic agent

JADWIGA A. TARKOWSKA

Department of Plant Anatomy and Cytology, Warsaw University, Krakowskie Przedmieście 26/28, 00-927 Warsaw, Poland

(Received: December 29, 1987. Accepted: February 8, 1988)

Abstract

The effect of pure sodium cacodylate on dividing cells was studied. The root meristematic cells of *Allium cepa* L. (the roots were squashed in acetoorcein) and endosperm cells of *Haemanthus katherinae* Bak. (*in vitro* observations) were used. Serious disturbances in karyokinesis and cytokinesis were found that led most often to the formation of polyploid or multinucleate (*A. cepa*) cells. These results point to damage of the mitotic spindle and phragmoplast. Careful use of cacodylate buffer in ultrastructural studies of microtubules is advised.

Key words: *sodium cacodylate, mitosis, microtubules*

INTRODUCTION

Sodium cacodylate (dimethylarsinic acid monosodium salt) is a compound from which buffers used in electron microscopy (EM) are often made. It contains arsenic, whose harmful effect on the division of animal cells had already been determined in 1929/30 by Piton et al., then confirmed by Dustin (1947, 1978). In the cells of the thymus, spleen and lymph nodes, arsenic compounds cause the accumulation of numerous pathological mitoses (metaphase arrest) and the destruction of cells by pyknosis of the nuclei. Dustin (1978) reports that injecting mice and guinea pigs with sodium cacodylate (2 mg g^{-1} body weight) caused accumulation of metaphase arrest as quickly as 1 hour. Light microscope observations showed analogical chromosome arrangements as seen after colchicine, which may indicate damage of the mitotic spindle.

In spite of numerous publications on the effect of various chemical factors, among others, of several metals on the mitotic spindle microtubules (MTs), there is a lack of studies on the effect of arsenic compounds on dividing plant cells. In the case of sodium cacodylate this is especially important and interesting due to its wide range of uses as a buffering agent in ultrastructural studies, especially on microtubular structures.

The aim of this study is to examine by light microscopy the effect of pure sodium cacodylate on the division of plant cells. The occurrence of disturbances in mitoses may point to the damage of microtubular structures suggested in literature. The results of this study may be of significance in the techniques used in EM.

MATERIAL AND METHODS

Allium cepa L. root tip meristematic cells and *Haemathus katherinae* Bak. endosperm cells were used in this study. The roots were cultured in tap water. When they attained a length of 2-3 cm, they were transferred to aqueous solutions of sodium cacodylate (incubation) of various concentrations from 0.05% (2.3 mM) to 0.5% (23 mM), pH 7.0–7.7. Part of the onions were transferred after 24 hrs of incubation to water again (postincubation) for periods up to 96 hours. The experiments and controls were conducted at a room temperature (22±2°C). The root tips were fixed in acetoalcohol (v/v 1:3), the squashed slides were stained with acetoorcein.

The *Haemanthus* endosperm cells studied *in vitro* were exposed to sodium cacodylate solutions in concentrations: 0.05, 0.1, 0.75 and 1% (2.3-47 mM). The preparations were made according to the method of Molè-Bajer and Bajer (1963, 1968). The dividing cells were observed and photographed under a phase-contrast microscope.

RESULTS

THE ROOT MERISTEMATIC CELLS OF *ALLIUM CEPA*

Sodium cacodylate was seen to be highly toxic to the studied meristematic cells, with its harmful effect becoming visible either during incubation or not until postincubation. The lowest concentration found not to kill the cells within 24 hours was 0.1%. Concentrations higher than 0.1% (0.2% and 0.5%) caused similar changes in dividing cells, although these changes progressed much more quickly and after 24 hours of incubation were irreversible upon transfer of roots to water.

The effects of 0.1% sodium cacodylate were the most characteristic for the studied meristematic cells. The final effect (arrangement and structure of chromosomes) depended on the division phase at which the cell came into

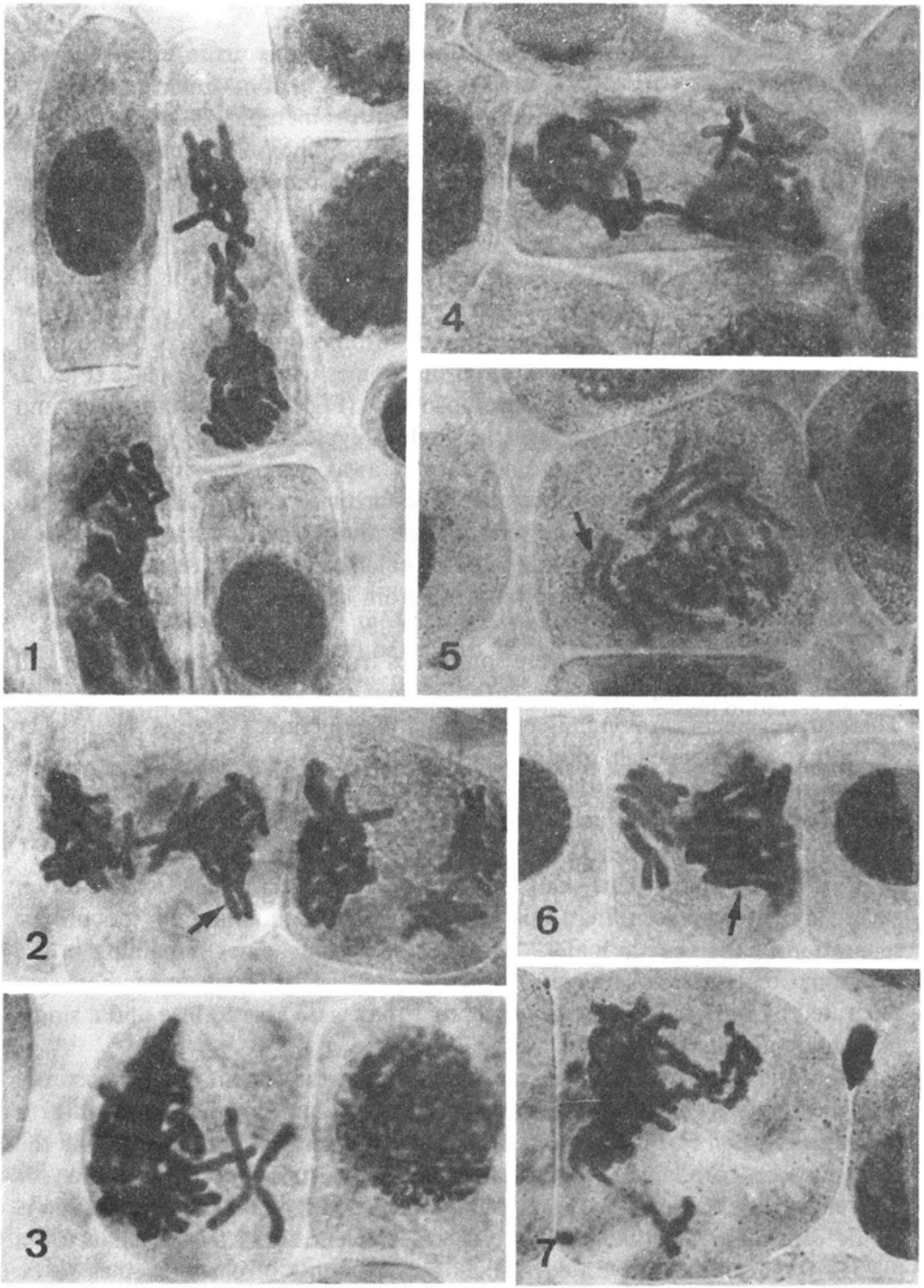
contact with the solution. The abnormalities in the arrangement of the chromosomes were very great. For this reason, taking about their morphology and structure, one should rather speak of prometaphase, metaphase or anaphase chromosomes and not of prophase, metaphase or anaphase which are characterized by a set arrangement of chromosomes. And so, the effect of sodium cacodylate on prometaphase evoked restitution changes (Fig. 8), or the chromosomes did not form a metaphase plate, although their structure was characteristic for metaphase. Thickened and shortened metaphase chromosomes could be seen spread out over the entire cell (Fig. 1, lower cell) or forming one more or less compact group of chromosomes. This is the position in which the longitudinal division of chromosomes usually took place (Figs. 2, 5-7), "X" (Figs. 1, 3, 4) or "ski-chromosomes" (Figs. 5, 6) were formed, and finally through restitution changes, a single polyploid nucleus arose (Fig. 8). Sometimes individual chromosomes stayed away from the main group of metaphase or anaphase chromosomes, and in these cases it is very easy to observe that sodium cacodylate retarded the divisin of the centromere region and the x-chromosomes, characteristic for colchicine, were formed (Figs. 1, 3, 4). Micronuclei (Fig. 10 — arrows) arose from single chromosomes (or their parts).

Metaphase chromosomes were sometimes transported to the potential cell poles in the same way as sister chromosomes are during normal anaphase. These arrangements are reminiscent of somatic reduction, although the number of chromosomes in each group was different (Fig. 6). Disturbances in the movement of chromosomes led to the formation of multinuclear cells in which each of the nuclei (micronuclei) was formed by a random number (always less than 2n) of chromosomes (Figs. 9, 11).

If the metaphase plate had been formed before contact with sodium cacodylate, it was seen that this compound could interfere in the proper separation of the genetic material: bridges or chromosomes remaining in the interzonal region were observed. In many cells, the sister chromosomes were separated by only such a tiny distance, that they were able to fuse and a single polyploid nucleus arose (Fig. 10).

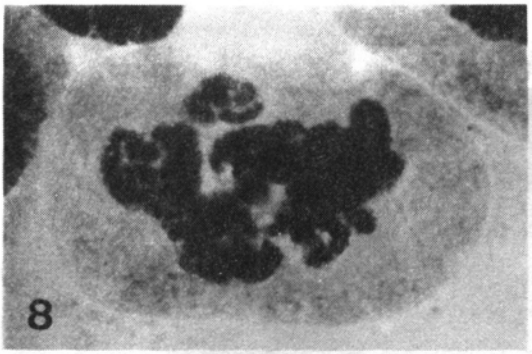
All of the disturbances in the movement and arrangement of chromosomes described above were accompanied by their structural changes, leading to restitution nuclei. For this reason, similar arrangements of chromosomes in the cells may be correlated with different internal structures characteristic for different phases of nuclei division (comp. Figs. 1 and 8, in both the chromatin is in the central part of the cell). The use of concentrations higher than 0.1% (0.25% for example), permitted similar arrangements of chromosomes to be observed, but the duration of their structural changes was shorter. The final pictures,. however, did not differ.

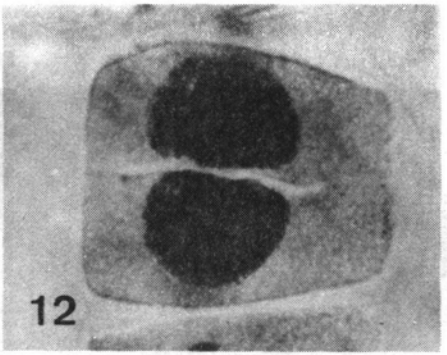
The phragmoplast was more sensitive to sodium cacodylate than the mitotic spindle. Even when the chromosomes were properly segregated to the

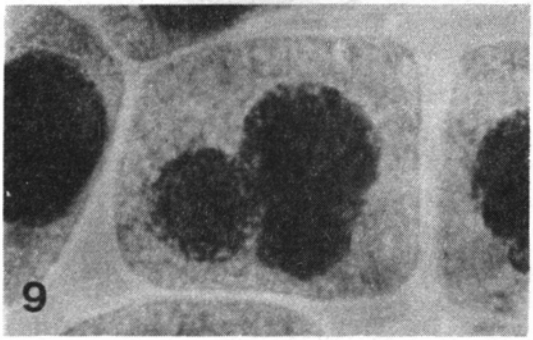


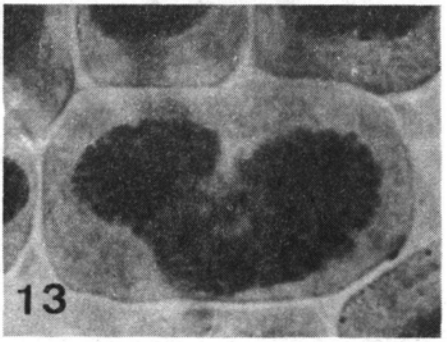
A. cepa root meristematic cells. Squash preparations, acetoorcein, 1000 x. 24 hrs incubation, 0.1% sodium cacodylate with the exception of Fig. 4 (0.2%).

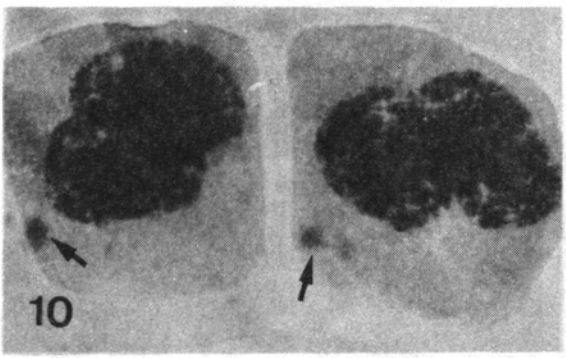
Fig. 1. Lower cell – metaphase chromosomes do not form a plate, upper cell – anaphase chromosomes, X-chromosome in the center. Fig. 2. Longitudinal division of chromosomes can take place after their translocation to the cell poles – arrow. Fig. 3. X-chromosome beyond the group of anaphase chromosomes. Fig. 4. Whole chromosomes at the cell poles, the division of the centromere is clearly delayed. Figs. 5 and 6. "Ski chromosomes" just after longitudinal division – arrows. Fig. 7. One-pole arrangement of anaphase chromosomes

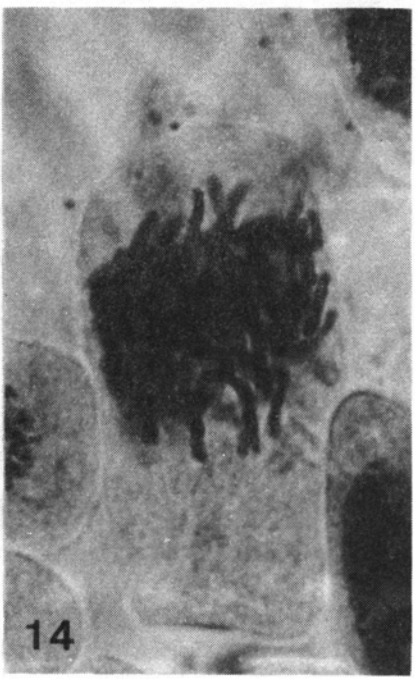


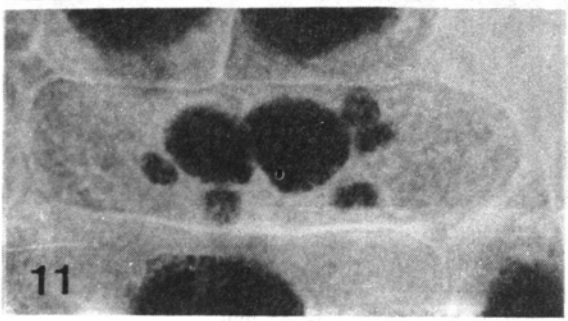








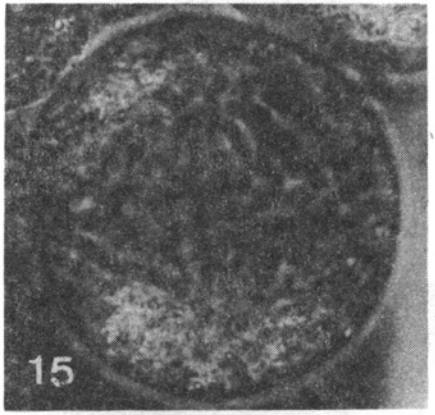


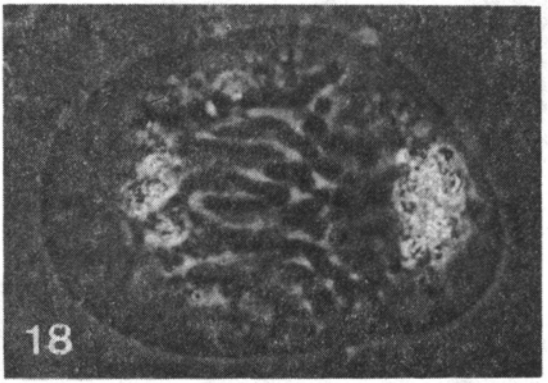


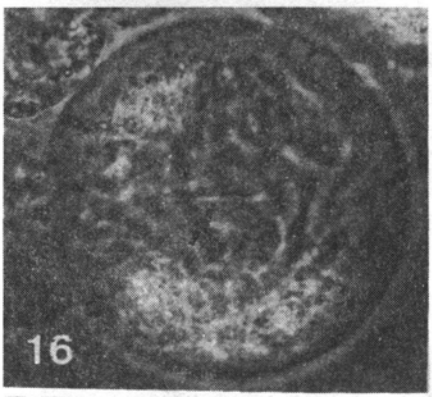
A. cepa, acetoorcein, Figs. 9-14 — 0.1% sodium cacodylate, 1000x

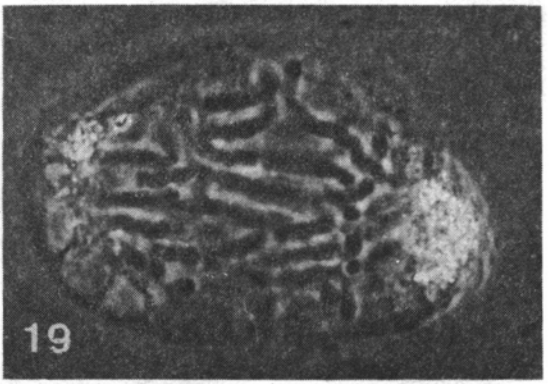
Fig. 8. Structural (restitution) changes in prometaphase chromosomes. 0.25% sodium cacodylate, 24 hrs. incubation. Fig. 9. A binuclear cell, nuclei of unequal size. 24 hrs. incubation. Fig. 10. The joining of telephase groups of sister chromosomes. Micronuclei (arrows) were probably formed from fragments of chromosomes, 24 hrs. incubation. Fig. 11. A cell with 7 micronuclei, 24 hrs. incubation. Fig. 12. An incomplete cell plate, 24 hrs. incubation. Fig. 13. Prophase of a tetraploid nucleus, 24 hrs. incubation and 72 hrs. postincubation. Fig. 14. Tetraploid metaphase, 24 hrs. incubation and 72 hrs. postincubation

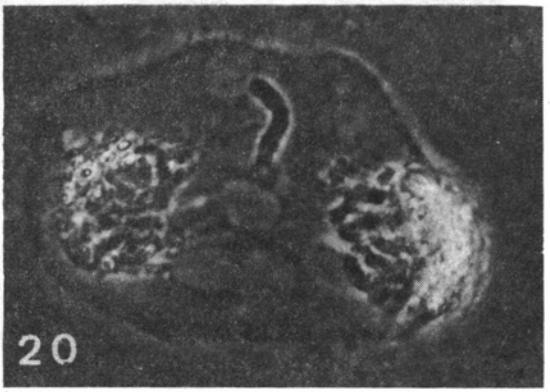
PLATE III









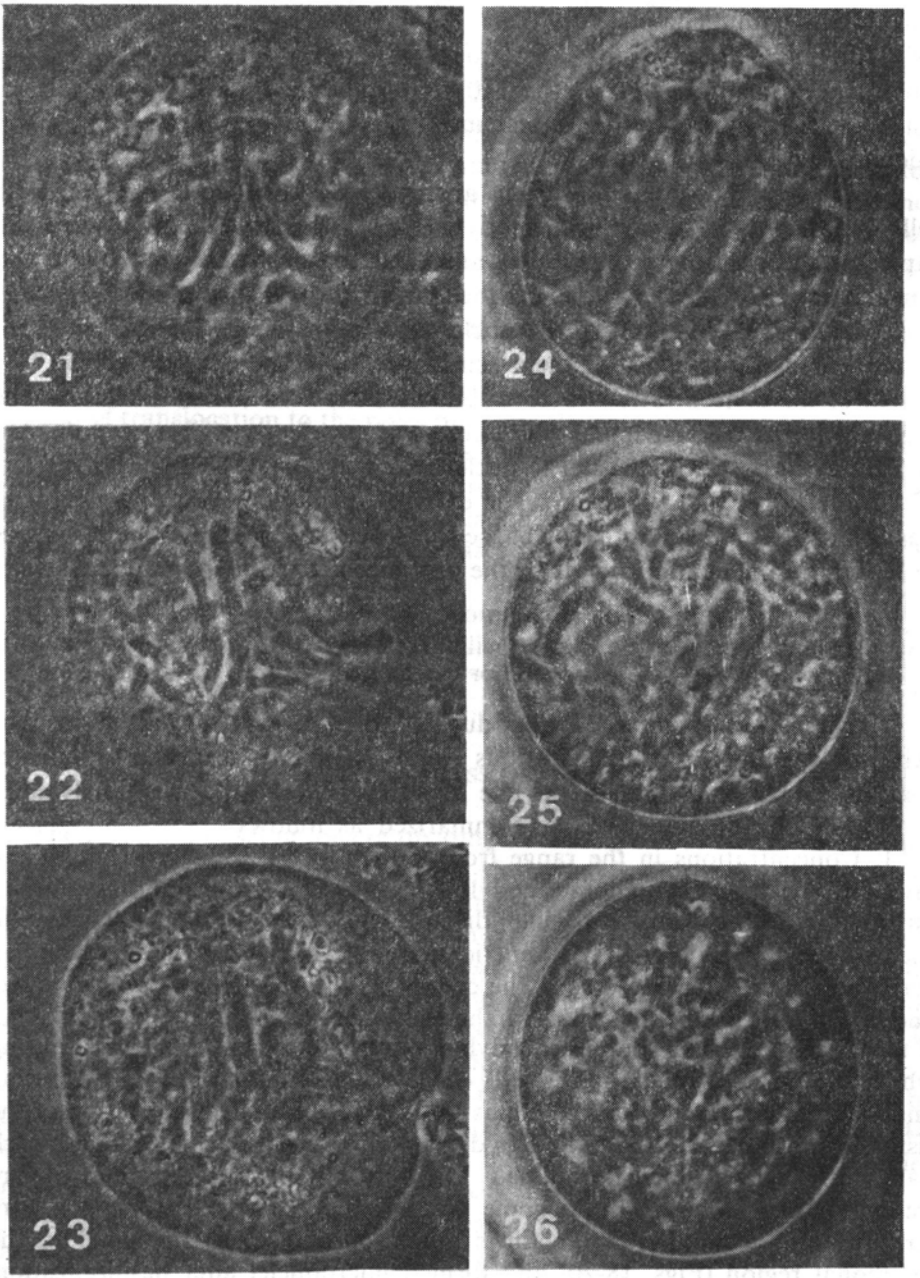


H. katherinae endosperm cells, *in vitro*, approx. 700x

Figs. 15-17 — the same cell, sodium cacodylate, 0.1%. Fig. 15. Anaphase, 20 min. of sodium cacodylate action. Fig. 16. The start of the development of the cell plate (arrow), 50 min. Fig. 17. Incomplete cell plate, spilt at both ends, 1 hr 45 min

Figs. 18-20 — the same cell, sodium cacodylate 0.05%. Fig. 18. Prometaphase, 15 min. Fig. 19. Anaphase, unsynychronous translocation of chromosomes to the poles, 2 hrs. 30 min. Fig. 20. Telophase, lack of phragmoplast and cell plate, a chromosome is in the vacuolized interzonal region, 3 hrs. 20 min

PLATE IV



H. katherinae, in vitro, approx. 700x

Figs. 21-23 — sodium cacodylate 1%, the same cell. Fig. 21. Prometaphase, 1 hr. 30 min. Fig. 22. Metaphase, 3 hrs. Fig. 23. Restitution changes, 4 hrs

Figs. 24-26 — the same cell, sodium cacodylate 0.75%. Fig. 24. Anaphase, unsynchronous translocation of chromosomes, 1 hr. Fig. 25. Distinct disturbances in the anaphase movements of chromosomes, 1 hr. 50 min. Fig. 26. A restitution nucleus. 2 hrs 30 min

cell poles, abnormalities in cytokineses were found. The number of binucleate cells or cells with incomplete cell plates (Fig. 12) was very high. The lack of the cell plate made it possible for sister groups of chromosomes to fuse, which has already been mentioned above (Fig. 10). It. also seems that it was possible for sister nuclei to fuse after the nuclear membranes had been formed, but direct evidence for this is lacking.

In spite of the distinct and serious abnormalities in the course of mitosis in cells exposed to 0.1% sodium cacodylate for 24 hours, the roots were still capable of growth upon transfer to water. The first divisions of polyploid and binucleate cells did not occur until the third day of postincubation (Figs. 13 and 14). Of course, not all of the nuclei with chromosome numbers over 2n underwent division, most of them remained above the division region.

All of the concentrations over 0.1% killed the cells within 24 hours. The degeneration of the nuclei and whole cells was not clearly visible until the 2nd or 3rd day of postincubation. The nuclei were pycnotic or disintegrating (clots of chromatin in the cytoplasm). The external manifestation of these changes was the loss of turgor and rotting of the roots. The appearance of these changes so late in postincubation points to the prolonged effect of sodium cacodylate.

THE ENDOSPERM CELLS OF *HAEMANTHUS KATHERINAE*

The cells were studied *in vitro* during three periods of vegetation. The behavior of numerous cells in various phases of division, exposed to different concentrations of sodium cacodylate (from 0.05% to 1%), was analysed.

The results obtained can be summarized as follows:

1. Concentrations in the range from 0.05% to 0.1% did not inhibit cell division. The action of sodium cacodylate on metaphase and anaphase (that is, on the fully formed mitotic spindle) did not cause any visible disturbances in the proper separation of the genetic material. However, abnormalities in cytokinesis were often observed. The cell plates formed were often incomplete and could be split at the ends in the shape of the letter V (Figs. 15-17). If the harmful effect of sodium cacodylate on prometaphase (the period during which the mitotic spindle is formed) occurred, it only became visible during anaphase. The mitosis could be almost normal, only the transportation of the sister chromosomes to the cell poles did not occur at the same time, but each daughter nucleus finally obtained the full set of chromosomes. More frequently in anaphase, chromosome bridges were formed, usually they were streched and torn apart. Sometimes whole chromosomes remained in the interzonal region (Figs. 18-20) and formed micronuclei after despiralization. The formation of cell plates in such cells was much delayed or did not occur at all. The cell remained binuclear. Both of the abnormalities observed above (translocation of chromosomes and formation of cell plates) were seen often enough to be sure that they could not be spontaneous disturbances or result from the techniques employed in preparing the material.

2. Sodium cacodylate at concentrations of 0.75% and 1% made the division of most nuclei and entire cells impossible. Late-prophase or prometaphase chromosomes could be translocated, their arrangement in the cell could be similar to that at metaphase, the longitudinal division of chromosomes into chromatids could also take place, but their separation to two poles never occurred. Due to the anomalities, either polyploid nuclei were formed through restitution processes (from a complete set of chromosomes) (Figs. 21-23), or several variously sized micronuclei arose, which usually fused into one nucleus. In extreme cases late-prophase or prometaphase chromosomes did not change their position, and the gradually proceding intrachromosomal process, that is, despiralization of chromatin, led to the onset of interphase (polyploid nuclei).

If cacodylate (0.75% or 1.0%) began to act on chromosomes which were in the process of translocation to the poles (mid-anaphase), a single polyploid nucleus was also formed (Figs. 24-26). This points to the very strong inhibitory effect of the studied compound on the movement of chromosomes. The abnormalities in the division of the endosperm cells (in each of the applied cacodylate concentrations) caused each of the phases as well as the entire mitosis to be very protracted in time.

Concentrations exceeding 1% completely inhibited the division of *Haemanthus* endosperm cells, and killed most of the cells within a few minutes.

DISCUSSION

The results of observations of dividing *Allium cepa* root meristem cells and *Haemathus katherinae* endosperm cells show that sodium cacodylate causes significant disturbances both in cytokinesis and karyokinesis. The types of disturbances seen in the divisions of both types of cells seem to indicate that the mitotic spindle and phragmoplast are damaged. Sensitivity to sodium cacodylate is seen both during the period when the spindle is formed (mainly prometaphase) as well as during the period when it is functioning (anaphase). In the endosperm polyploid cells are formed most often. In meristematic cells, a wide variety of abnormal chromosome patterns are observed, which may be indicative of the degree of damage to the mitotic spindle (Östergren 1944, 1950, Tarkowska 1981). However, on the basis of light microscopic studies only, one cannot draw conclusions about the nature of the damage.

The numerous bi- and multi-nuclear onion cells can be the result of damage to or lack of the phragmoplast. These kinds of cells are still capable of division.

The unpublished data of Łobodzińska also points to the destructive effect of sodium cacodylate on cytoplasmic MTs. She found that it deorganizes the proper formation and thickening of the secondary cell wall in xylem vessels in *Allium cepa* roots, and that it destroys the MTs responsible for maintaining the proper shape of the generative cell in *Tradescantia virginiana* pollen grains.

A characteristic of the effect sodium cacodylate is its prolonged action. The disturbances which arose during its direct action remained present in the onion cells during long (3 days) postincubation. It may be that cacodylate is retained in certain of the cell's structures or damages its internal processes. It is known from literature (Dustin 1947) that various mitotic poisons act as enzyme inhibitors, making it impossible for mitosis to begin or the normal polarization of the spindle to occur. Arsenic compounds are among the very numerous compounds known to "poison" various enzyme systems. However, appropriate studies are lacking which would allow any conclusions to be drawn in this respect.

When the data from literature and this study are taken into consideration, the question arises if sodium cacodylate may and should be used in electron microscopy investigations. As a buffer, it is used most often at a concentration of 2.14% (0.1 M). This concentration is, for both studied cell types, not only highly toxic, but lethal (for *Allium cepa* over 0.1% (4.7 mM), for *H. katherinae* over 1% (47 mM)).

The sensitivity of cells to sodium cacodylate is different for different plants and depends on their physiological state. This became very clear during the experiments repeated several times on onion roots. Market onions are not of a uniform variety, hence the varied results, which however, did exceed one-tenth of one percent (and therefore were always below the 2% buffer concentration). In *Haemanthus*, no variance correlated with the individual plant or period of blooming (the observations were conducted during 3 vegetation periods) was observed in the reaction of the cells to the concentration of sodium cacodylate used.

In the deliberations on the use of sodium cacodylate in the preparation of material for electron microscopy, one should take into account that in the first phase, it acts jointly with the fixing solution, which is characterized by a very fast rate of diffusion into tissues and cells. Thereby, its harmful effect may be diminished to a great extent. This is of great importance in the submicroscopic studies of MTs fixed with glutaraldehyde buffered with sodium cacodylate. The results of such studies were presented by Luftig et al. (1977) who used glutaraldehyde buffered by various compounds, including cacodylate, to visualize MTs in HeLa cells. In the study by these authors it was seen that cacodylate caused a decrease in the visibility of MTs in HeLa cells cultured *in vitro*. This was probably due to the depolimerization of the MTs. The authors concluded that a final interpretation of the results requires the use of labeled glutaraldehyde. According to the suggestions of Dustin (1978) this would be the reason for the occurrence of so many "metaphase arrests". From the above data it can be seen that sodium cacodylate can be, according to Deysson's definition (1968), considered an antimitotic substance.

Similarly as cacodylate buffer, phosphate buffers also do not good preserve MTs in HeLa cells fixed with glutaraldehyde (Luftig et al. 1977). Studies on the effect of pure phosphate buffer on dividing cells have been undertaken in our laboratory. The results of that study will make a wider discussion of this very important question possible.

The results presented in this paper show unquestionably that informed caution should be used when sodium cacodylate is chosen as the buffer for submicroscopic studies. This is especially true for studies on microtubular structures, but above all the mitotic spindle and phragmoplast. And so, Dustin's comments (1978) are valid and justified.

Acknowledgment

I would like to thank mgr Mirosława Ważyńska for help in preparing the photographic documentation.

REFERENCES

Deysson G., 1968. Antimitotic substances. Int. Rev. Cytol. 24: 99–148.
Dustin P., 1947. Some new aspects of mitotic poisoning. Nature 159: 794-797.
Dustin P., 1978. Microtubules. Springer, Berlin-Heidelberg-New York.
Luftig R. B., McMillan P. N., Weatherbee J. A., Weihing R. R., 1977. Increased visualization of microtubules by an improved fixation procedure. J. Histochem. Cytochem. 25: 175-187.
Molè-Bajer J., Bajer A., 1963. Mitosis in endosperm techniques of studies *in vitro*. La Cellule 63: 399-407.
Molè-Bajer J., Bajer A., 1968. Studies of selected endosperm cells with the light and electron microscope the technique. La Cellule 67: 257-265.
Östergren G., 1944. Colchicine mitosis, chromosome contraction, narcosis and protein chain folding. Hereditas 30: 429-467.
Östergren G., 1950. Cytological standards for the quantitative estimation of spindle disturbances. Hereditas 36: 371-382.
Piton R., 1929-30. Recherches sur les actions caryoclasiques et caryocinétiques des composés arsenicaux. Arch. Intern. Med. Expér. 5: 355-411.
Tarkowska J. A., 1981. Relations between ultrastructure of mitotic spindle and chromosome translocation. Acta Soc. Bot. Pol. 50: 585-594.

Kakodylan sodu jako związek antymitotyczny

Streszczenie

Badano działanie czystego kakodylanu sodu (dwumetylowy arsenian jednosodowy) na dzielące się komórki merystematyczne wierzchołków korzeni cebuli (*Allium cepa* L.), oraz komórki bielma krasnokwiatu (*Haemanthus katherinae* Bak.). Stosowano różne stężenia kakodylanu: dla korzeni w granicach od 0.05% (2.3 mM) do 0.5% (23 mM), dla komórek bielma od 0.05% (2.3 mM) do 1% (47 mM). Wierzchołki korzeni gnieciono w acetoorceinie. Obserwacje bielma *in vitro* prowadzono w kontraście fazowym wg metody opisanej przez Molè-Bajer i Bajer (1963, 1968).

Wyniki badań wskazują, że kakodylan sodu wywołuje istotne zaburzenia w kario- i cytokinezie. Można przypuszczać, że uszkodzone zostaje zarówno wrzeciono podziałowe, jak i fragmoplast. W komórkach bielma powstają, zwykle w drodze przemian restytucyjnych, jądra poliploidalne. W komórkach merystematycznych nieprawidłowe układy chromosomów prowadzą do powstania komórek wielojądrowych lub poliploidalnych, związanych często z zaburzeniami w cytokinezie (całkowity brak lub tylko częściowe

przegrody pierwotne). Kakodylan sodu charakteryzuje przedłużone działanie. Zgodnie z definicją Deysson (1968) należy go zaliczyć do związków antymitotycznych.

Z uzyskanych własnych wyników i danych z literatury wynika jednoznacznie, że w badaniach nad strukturami mikrotubularnymi, przede wszystkim wrzeciona podziałowego i fragmoplastu, należy zachować dużą ostrożność.