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Effect of Morphine Sulphate on Mitosis of *Allium cepa* L. Root Tips

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Abstract. The cytological effect of one of the alkaloid of Opium, namely morphine sulphate on *Allium cepa* root tips from a qualitative and quantitative point of view were studied. It was found that morphine sulphate caused partial effect on spindle formation and also showed a mito-depressive effects particularly after long treatment.

Various chemical substances were discovered that have the ability to alter the mechanism of mitosis. They vary, however, according to their mode of action (BIESEL 1958 and DEYSSON 1968). Some may inhibit or disturb spindle formation leading to various mitotic abnormalities such as C-metaphase, star metaphase, polyploidy and others. Examples of these chemicals are colchicine (EIGISTI and DUSTINE 1955), organic mercury compounds (RAMEL 1969, FISKEJO 1969 and AHMED and GRANT 1972), insecticides and pesticides (VENKATO and RAO 1969, AMER and ALI 1969). Other chemicals specifically action the chromosomes inducing various types of chromosomal aberrations *e.g.* alkylating agents (HEINER 1971, KIHLMAN *et al.* 1971).

Due to the sensitivity of mitotic mechanism to these chemicals, a number of investigators tried to test the effect of medical substances commonly used by man on mitosis. KABARITY and KHODARY (1967a, b), showed that the three oral contraceptives, Anovlar, Conovid and Lyndiol, have induced different mitotic abnormalities: while anovolar does not affect the formation of the spindle fibres, but affects only their orientation, Conovid inhibits the formation of the spindle fibres producing polyploidy, possibly aneuploidy, star metaphases, anaphases bridges. Lyndiol did not have any effect on metaphase, although anaphase irregularities were observed. The antibiotics chloramphenicol, cycloheximide and actinomycine D, caused a number of mitotic and chromosomal abnormalities (BUIATTI and NUTIRONCHI 1969 and NAGL 1970). Even the artificial sweateners also showed various types of chromosomal aberrations (MAJUNDAR and SOLOMAN 1971).

Morphine sulphate is an alkaloid found principally in opium. It is widely used by man either for medical purposes or as narcotics. The present study was planned to find out the cytological effects of this chemical in root tips of *Allium cepa*.

Material and Methods

Onion bulbs (*Allium cepa* cv. Giza) of the same size and age were used for the present cytological studies, the bulbs were germinated in sandy soil irrigated with tap water. When the roots had reached a length of about 1.5—2.5 cm the bulbs were removed from the pots and the roots were

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washed thoroughly with tap water. Throughout the different experiments in the present study roots were taken having the same length. The roots, while still intact to the bulbs, were immersed in small vials containing the various concentrations of morphine sulphate. Each series of experiments included a simultaneous control treated in an identical way, where tap water was used instead of the alkaloid. All treatments were carried out at room temperature ranging between 20—25 °C. Roots were treated with various concentrations of morphine sulphate ranging from 3.29×10^{-5} to 0.04×10^{-5} mol ml^{-1} details of which are indicated in the text. The roots were kept in the chemical for 4, 12, 24, 48 and 72 h. The culture solution was changed every 36 h according to DAVIDSON and MACLEOD (1966).

The Feulgen squash technique was applied for the cytological work according to DARLINGTON and LA COUR (1969). The root tips (1 cm) were cut after each treatment and fixed in Carnoy's solution (acetic alcohol 1 : 3) for 24 h. The roots were then transferred to 70 % ethyl alcohol and kept in the refrigirator until required. They were stained with leucobasic fuchsine, after hydrolysis in 1 N HCl for 8 min and permanent slides were then prepared after squashing the roots in 45 % acetic acid. For each treatment scoring of the mitotic indices (MI = percentage of dividing cells) was carried out from 10 roots of at least 3 bulbs. Each determination was based from 10 fields at random from each root.

Results

I. General Cytological Observations

The most recognizable effect of morphine sulphate in root tips of *Allium cepa* was its action on metaphase stage. This occurred with variable frequencies according to the period of treatment and the concentration used. The majority of metaphase abnormalities showed typical C-metaphase similar to that induced by colchicine (LEVAN 1938). In such case the chromosomes were scattered all over the entire cell, not gathered at the metaphase plate and each chromosome appeared having its two chromatids fallen apart except at the position of the centromere (Fig. 1, 2). The action of the chemical may be extended causing the daughter chromosomes to fall apart from each other but do not move to the poles forming C-anaphase (Fig. 3). This was

TABLE 1

PROPHASE / METAPHASE RATIO AND FREQUENCY of normal and abnormal phases in mitosis after treating *Allium cepa* roots with morphine sulphate for 4 hours

Concentration $X10^{-5}$ $molml^{-1}$	Total cells examined	Prophase		Metaphase		Ana + Telophase		P/M Ratio
		Normal [%]	Abn. [%]	Normal [%]	Abn. [%]	Normal [%]	Abn. [%]	
3.29	12.000	40.28	1.52	37.42	87.43	22.30	21.10	1.08 : 1
2.96	12.000	38.72	0.61	40.38	74.12	20.90	28.41	0.96 : 1
2.63	12.000	38.55	2.55	40.73	55.82	20.92	26.00	0.94 : 1
2.30	12.000	50.09	2.25	31.71	39.05	18.20	20.62	1.58 : 1
1.97	12.000	47.63	0.00	28.66	31.03	23.71	20.00	1.66 : 1
1.64	12.000	49.27	0.00	23.33	5.59	27.40	6.55	2.11 : 1
0.00	12.000	62.46	0.00	15.48	0.00	22.06	0.00	4.03 : 1

TABLE 2

PROPHASE / METAPHASE SATIO AND FREQUENCY of normal and abnormal phases in mitosis after treating *Allium cepa* roots with morphine sulphate for 12 h

Concentration X10^{-5} molml^{-1}	Total cells examined	Prophase		Metaphase		Anaphase		Telophase		P/M Ratio
		Normal [%]	Abn. [%]	Normal [%]	Abn. [%]	Normal [%]	Abn. [%]	Normal [%]	Abn. [%]	
1.97	7.157	32.97	—	51.10	100	2.20	100	13.74	72.00	0.65 : 1
1.31	9.497	34.42	—	52.03	97.92	5.69	76.19	7.86	27.59	0.66 : 1
0.65	9.914	34.80	—	31.35	46.00	18.18	18.97	15.67	12.00	1.10 : 1
0.16	7.696	62.24	—	17.84	34.88	11.62	10.71	8.30	5.00	3.50 : 1
0.04	7.352	58.09	—	20.95	3.96	11.83	0.00	9.13	0.00	2.70 : 1
0.00	6.544	60.16	—	22.25	3.71	7.42	0.00	10.16	0.00	2.70 : 1

mostly followed by restitution resulting in a tetraploid nucleus. Cells having higher polyploid chromosome number was also observed (Fig. 4).

Anaphases with chromosome bridges were noticed in roots treated with high concentration (Fig. 5, 6).

In roots treated with higher concentrations, micronuclei were noticed in some interphase stages (Fig. 7). Such micronuclei, most probably originate from the noncongression chromosomes, where a nuclear membrane surround them at interphase stage.

II. Effect of Dose and Duration of Morphine on Mitotic Frequency

a) Effect of 4-hours treatment

It is shown in Table 1 that the lowest effect of the chemical upon meristematic cells was found at concentration 1.64×10^{-5} mol ml^{-1}, when

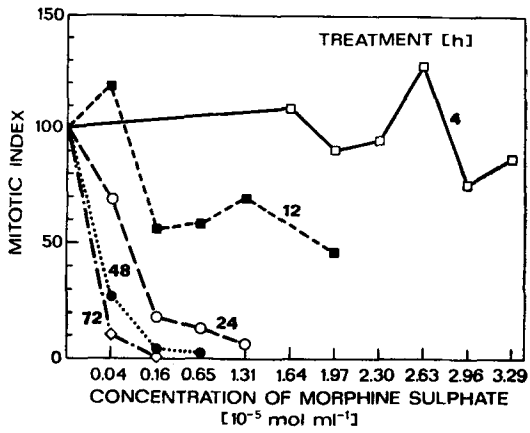

Fig. 8. The effect of morphine sulphate on the mitotic index in *Allium cepa*

abnormal metaphase has reached a minimum frequency of 5.59 %. This frequency increases linearly with the increase of morphine concentration until it reached a maximum of 87.43 % for roots treated with the highest concentration of 3.29×10^{-5} mol ml^{-1}. The abnormal anaphase and telophase on the other hand increases with slight fluctuation. In general, however, their frequency are far less than that of metaphase. The maximum frequency of metaphase stage 40.73 % (normal + abnormal) was obtained at concentration 2.63×10^{-5} mol ml^{-1} at which prophase stage attain the lowest frequency 38 35 %. Metaphase and prophase frequencies almost parallel each other in roots treated with the higher concentration. Thus while the metaphase percentage increases with the increase of morphine concentration, the percentage of prophase decreases. This becomes more clear when the prophase/metaphase ratio was calculated. In case of the untreated roots it represents the highest ratio 4.03 : 1, then decreases with the increase of chemical concentrations until it almost become equal in values with higher concentrations (Table 1). Such results indicate that there is accumulation of metaphase on the expense of prophase by treating the roots with high concentrations of the alkaloid.

The frequency of abnormal prophase is very low and it only appears in roots treated with higher concentrations.

Morphine sulphate shows very little effect on the mitotic indices for roots treated with different concentrations for four hours. The lowest MI value is 3.51 produced in roots treated with one of the highest concentration 3.29×10^{-5} mol ml^{-1} compared with the untreated roots which has MI value of 4.68 (Fig. 8).

b) Effect of 12-hours treatment:

More diluted series of morphine sulphate concentrations than the previous experiment were used $1.97 - 0.04 \times 10^{-5}$ mol ml^{-1}. Similar types of abnormalities to the previous observations were obtained. The numbers and percentages of the normal and abnormal mitotic stages after treatment with various concentrations are shown in Table 2. It is clear from the table that

TABLE 3

PROPHASE / METAPHASE RATIO AND FREQUENCY of normal and abnormal phases in mitosis after treating *Allium Cepa* roots with morphine sulphate for 24 h

Concentration X10^{-5} molml^{-1}	Total cells examined	Prophase		Metaphase		Anaphase		Telophase		P/M Ratio
		Normal [%]	Abn. [%]	Normal [%]	Abn. [%]	Normal [%]	Abn. [%]	Normal [%]	Abn. [%]	
1.31	6.492	31.82	—	68.18	100	0.00	—	0.00	—	0.47 : 1
0.65	6.767	28.00	—	52.00	100	6.00	100	14.00	28.57	0.54 : 1
0.16	8.874	52.13	—	24.47	78.26	8.51	12.5	18.89	0.00	2.10 : 1
0.04	8.304	35.53	—	34.10	5.04	12.32	00.0	18.05	1.59	1.00 : 1
0.00	5.822	48.46	—	22.69	2.40	15.12	00.0	13.72	0.00	2.14 : 1

TABLE 4

PROPHASE / METAPHASE RATIO AND FREQUENCY of normal and abnormal phases in mitosis after treating *Allium Cepa* roots with morphine sulphate for 48 h

Concentration X10^{-5} molml^{-1}	Total cells examined	Prophase		Metaphase		Anaphase		Telophase		P/M Ratio
		Normal [%]	Abn. [%]	Normal [%]	Abn. [%]	Normal [%]	Abn. [%]	Normal [%]	Abn. [%]	
0.65	6.934	0.00	—	75.00	100	0.00	—	25.00	100	—
0.16	9.869	9.68	100	74.19	100	0.00	—	16.13	100	0.1 : 1
0.04	7.809	49.66	0	23.49	25.71	13.42	5	13.42	5	2.0 : 1
0.00	6.591	45.28	0	22.32	0.00	15.45	0	16.95	0	2.0 : 1

there is a reverse relationship between the prophase and metaphase stages frequencies similar to the four hours treatment. The percentage of metaphase reached maximum of 52.03 % and 51.1 % at concentrations 1.31 and 1.97×10^{-5} mol ml^{-1} and 1.5 %; where prophase appeared at its low frequencies of 34.42 % and 32.97 %. This relationship was also clear from the P/M ratio.

The frequencies of anaphase and telophase, on the other hand, fluctuate slightly but in general their frequencies were less than that of metaphase.

Mitosis was inhibited in this experiment in roots treated with high concentration. The MI was depressed to 2.54 compared with the control value 6.56 (Fig. 8).

No prophase abnormalities were noticed. Metaphase and telophase abnormalities frequencies increases linearly with the increase of morphine sulphate concentration until it reached a maximum of 100 % at the highest concentration applied.

c) Effect of 24-hours treatment:

This period of treatment was found to have more drastic effect on mitosis (Table 3). The percentage of prophase decreases while metaphase percentage increases with the increase of concentration. A drop in the anaphase and telophase frequencies were detected with the increase of chemical concentration. Complete anaphase inhibition was observed in roots treated with the concentration 1.31×10^{-5} mol ml^{-1}. The MI drops sharply from 6.13 of the control to 0.34 for the highest concentration applied Fig. 8.

No prophase abnormalities were observed in this experiment. Metaphase abnormalities, on the other hand, reached 100 % at lower concentrations from the previous experiments 1.31×10^{-5} mol ml^{-1}. The lowest concentration used showed no effect on anaphase except in lowering its frequency.

d) Effect of 48-hours treatment:

C-metaphase and stickness were the most prominent abnormalities. Three concentrations were only used, the highest was 0.65×10^{-5} mol ml after

which the roots died. The percentages of normal and abnormal cells in the different stages of mitosis are shown in Table 4. MI was sharply reduced to 0.12 in roots treated with high concentration. Compared to the control value 7.07, even when the lowest concentration used, MI was still low (Fig. 8). Thus longer period of treatment with low alkaloid concentration show more

TABLE 5

PROPHASE / METAPHASE RATIO AND FREQUENCY of normal and abnormal phases in mitosis after reating *Allium Cepa* roots with morphine sulphate for 72 h

Concentration $X10^{-5}$ $molml^{-1}$	Total cells examined	Prophase		Metaphase		Anaphase		Telophase		P/M Ratio
		Normal [%]	Abn. [%]	Normal [%]	Abn. [%]	Normal [%]	Abn. [%]	Normal [%]	Abn. [%]	
0.16	8.180	—	—	—	—	—	—	—	—	—
0.04	5.535	51.34	—	31.43	27.27	8.57	—	8.75	—	1.6 : 1
0.00	7.584	44.97	—	92.38	—	11.70	—	20.94	—	2.01 : 1

inhibitory effect on mitosis, than high concentration for short period. The distribution frequencies of mitotic states are shown in Table 4. Normal metaphase was only seen in roots treated with low concentrations, while in higher concentrations 0.65×10^{-5} mol ml^{-1} and 0.16×10^{-5} mol ml^{-1} the abnormalities reached 100 % (Table 4). At the same concentrations complete inhibition of anaphase was detected. Few cells, however, were found at the telophase stage and they were abnormal.

e) Effect of 72 hours treatment:

In spite of the low concentrations of the chemical used in this treatment (0.16×10^{-5} mol ml^{-1} and 0.04×10^{-5} mol ml^{-1}) mitosis was highly affected (Table 5). The mitotic index drops to 0.63 when treated with 0.16×10^{-5} mol ml^{-1} and to zero when treated with the second concentration compared with that of the untreated which had a mitotic value of 6.3 (Fig. 8). The percentage of abnormalities in metaphase was low in comparison to the previous experiments and no abnormalities were noticed in prophase, anaphase and telophase.

Discussion

The results obtained in the present investigation indicate that morphine sulphate has the ability to induce a number of mitotic abnormalities. The main conspicuous effect is the formation of C-metaphase. In fact most of abnormalities produced may be related to this effect. Such C-metaphase was produced with variable frequencies in all concentrations used and after various periods of treatment. The induction of C-metaphase is an indication of the action of morphine sulphate on spindle formaion. In roots treated with the alkaloid for long duration, complete inhibition of spindle was noticed, resulting in 100 % C-metaphase. In other cases, partial inhibition was observed where a low percentages of C-metaphase were produced accompanied with normal anaphase and telophase stages. Thus it could be concluded that morphine sulphate acts on the spindle fibres similar to colchicine (LEVAN 1938 and 1954), but it is less effective than this

alkaloid. Similar results were observed using the antibiotics (NAGL 1970), penetrazol (KABARITY 1968) and many others (DEYSSON 1968).

The presence of chromatic bridges in some cells treated with morphine sulphate are most likely due to stickness exerted by the action of the chemical on the chromosomes. At anaphase spindle fibres are formed quite normally. Thus separation of the daughter chromosomes to the poles become incomplete and remained connected together by these chromatic bridges. Such abnormality is commonly found in many plant and animal materials treated with various chemicals (DEYSSON 1968). These bridges induced by morphine sulphate cannot be attributed to the breakage and reunion of chromosomes because of the absence of any detactable chromosome breakage.

It was noticed that there is direct relationship between the prophase and metaphase stage. In the untreated roots, the frequency of prophase is normally higher than that of metaphase. On the other hand, there is an inverse relationship between metaphase and prophase frequency in the treated roots *i.e.* as metaphase increases prophase decrease. BRYANT (1969) has estimated the duration of the different stages in *Allium cepa* root tips using the autoradiographic technique. He found that the duration of metaphase and anaphase are very short (0.2 h and 0.1 h respectively), while the prophase and telophase lasted longer period (0.8 h and 1.2 h). These results could explain the occurrence of high frequency of prophase and the low frequency of metaphase in the untreated roots. The drop in prophase frequency in the treated roots may be either due to short duration of this phase or reduction in cell numbers entering mitosis. While the increase of metaphase percentage could be resulted from the lengthening of the metaphase duration that leads to its accumulation. Since no substantial differences in the MI with the increase of alkaloid concentration were noticed, when roots treated 4 h, it could be concluded that the variation in prophase and metaphase frequencies produced by the chemical were mainly due to the change of the relative duration of these stages. The chemical most likely reduce the prophase duration while increasing metaphase duration.

A drastic reduction in MI in the roots treated for long periods with various concentration was observed. This inhibitory effect of the chemical can result from either obstruction of the onset of prophase or to an arrest of one of the mitotic phases. In fact, the chemical, due to its action on the spindle, cause an arrest in metaphase, producing a high percentage of this stage on the expense of the prophase. But the mitotic phases numbers were very low. Thus the reduction in MI must be due mainly to the inhibitory action of the chemical on the onset of mitosis, in spite of the metaphase arrest. Therefore, morphine sulphate differ from colchicine action in that the latter cause an increase in MI due to the metaphase arrest as well as to its stimulatory action to mitosis (DAVIDSON and MACLEOD 1966 and WEBSTER and DAVIDSON 1969). This led us to conclude that morphine sulphate cause partial effect on spindle formation and also showed a mitodepressive affects particularly after long treatment. In this respect it is largely similar to other narcotic alkaloids (see DEYSSON 1968 for references).

Conclusions

The most recognisable effect of morphine sulphate in root tips of *Allium cepa* was its action on metaphase. C-metaphase, star metaphase, C-anaphase, anaphase with chromosome bridges were noticed.

A drastic reduction in mitotic index in the roots treated for long periods with various concentrations was observed. This inhibitory effect of the chemical can result from the obstruction of the onset of prophase. Morphine sulphate differs from colchicine action in that the latter cause an increase in MI due to the metaphase arrest as well as to its stimulatory action to mitosis.

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Figures at the end of the issue

A. KABARITY, A. EL-BAYOUMI, A. A. HABIB, Botanické oddělení Přírodovědecké fakulty University Ain Shams, Káhira: **Vliv síranu morfinu na mitosu v kořenových vrcholech *Allium cepa* L.** — Biol. Plant. **16** : 275—282, 1974.

Byl kvalitativně a kvantitativně sledován vlív síranu morfinu, jednoho z opiových alkaloidů, na kořenové vrcholy cibule *Allium cepa* L. Síran morfinu působil částečně na tvorbu vřetének a zejména po dlouhé aplikaci působil mitodepresivně.

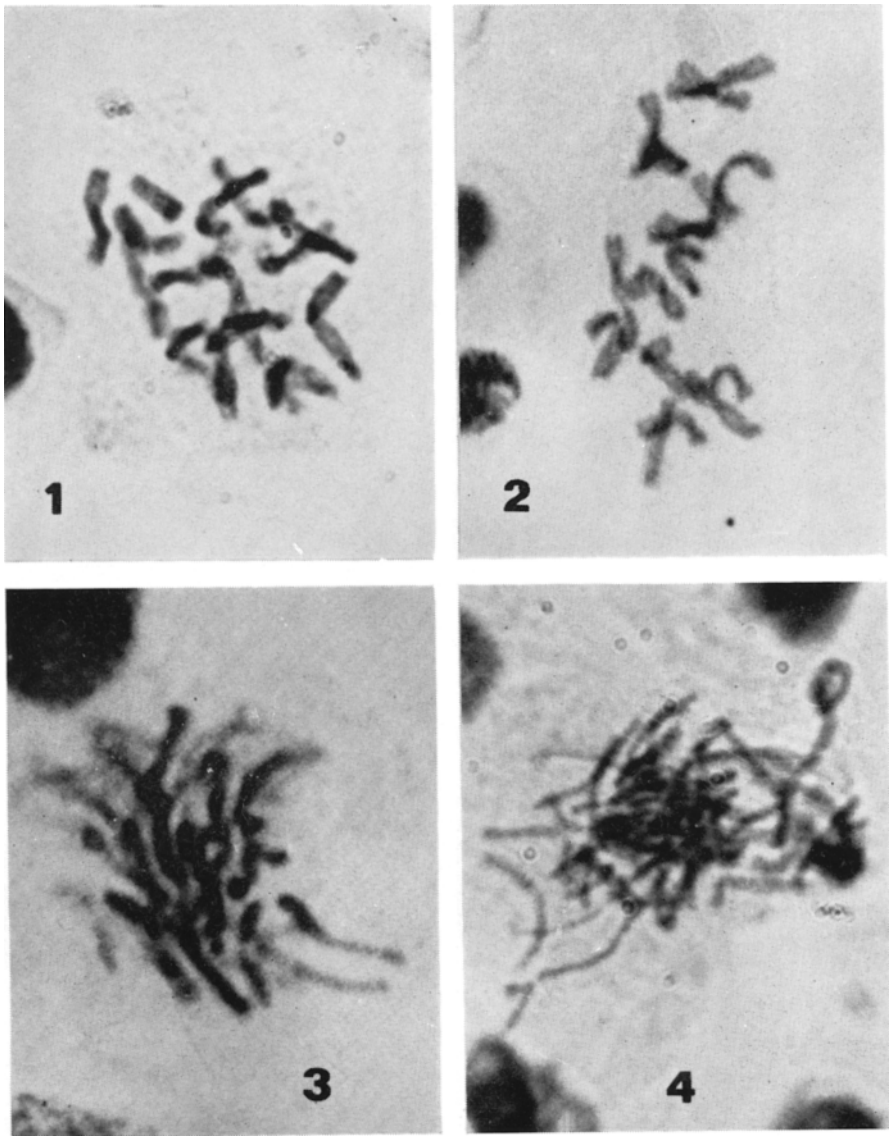
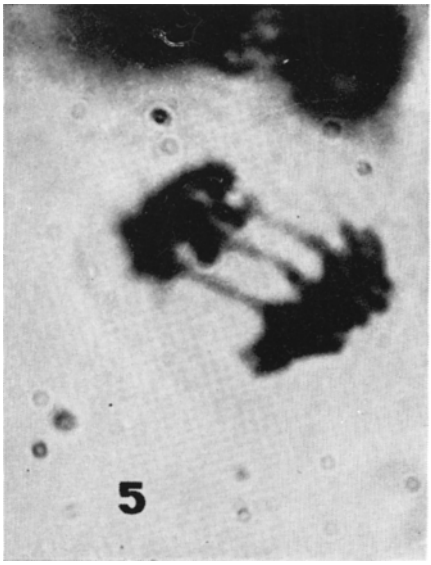
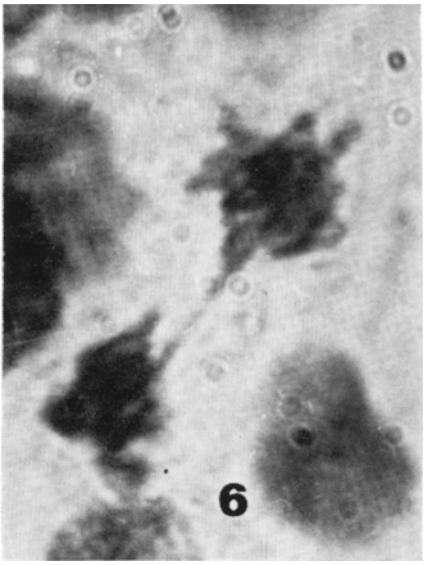


Fig. 1—2. C-metaphases after treating *Allium* cepa root tips with 2.63×10^{-5} mol ml^{-1} (Fig. 1). 2.30×10^{-5} mol ml^{-1} (Fig. 2) morphine sulphate for 4 h.
Fig. 3. C-anaphases after treating *Allium* cepa root tips with 1.97×10^{-5} mol ml^{-1} morphine sulphate for 4 h.
Fig. 4. Doubling of chromosome set of *Allium* cepa cells after the treatment with morphine sulphate (1.97×10^{-5} mol ml^{-1}).





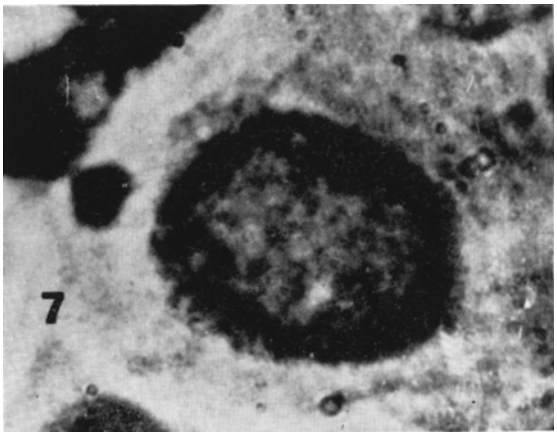


Fig. 5—6. Chromosome bridges in anaphases treated with 2.63×10^{-5} mol ml^{-1} morphine sulphate for 4 h.
Fig. 7. Micro-nucleus after treatment of 2.96×10^{-5} mol ml^{-1} morphine sulphate.