MITOTIC ABERRATIONS INDUCED BY SUNFLOWER SEED OIL IN *ALLIUM CEPA* ROOT-TIP CELLS

by

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ABSTRACT

Abnormal mitotic divisions and chromosomal aberrations were observed in onion root-tip cells treated with various concentrations of sunflower seed oil and for varying times. All treatments induced similar chromosomal aberrations but the number and extent of the deviations were influenced by the concentration and duration of the exposure. Abnormalities included pycnosis, "sticky" anaphase and telophase bridges with or without fragments, c-mitosis and inhibition of cell division. Chromosome and chromatid breakage with erosion and fragmentation were maximum at metaphase and anaphase four hours after treatment with 0.1 ppm oil concentration. Binucleate cells, micro- and macronuclei and some polyploidy appeared following recovery in tap water. Spindle abnormalities were indicated by arrested metaphases, multipolar anaphases and misdivision at anaphase. It is suggested that these chromosomal and spindle aberrations were induced by the carbon-carbon double bonds present in the unsaturated acids of the sunflower seed oil which enabled them to function as electron donors and to undergo such reactions as the addition of hydrogen, of water and of acids.

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INTRODUCTION

D'Amato and Hoffman-Ostenhof (1956) have collected much data indicating that oil and aqueous extracts from the aged seeds of many plants produce mutations in other plants and extensive breakage in the chromosomes of onion root-tip cells. Because of the suspected correlation between mutagenesis and carcinogenesis an increasing number of chemicals and drugs are being tested for their ability to induce chromosome changes (Boyland, 1954; Levan, 1951). Swaminathan and Natarajan (1956), investigating the sensitivity of wheat and Vicia faba seeds to radiation found that immersion of seeds in various oils produces chromosome breakage. Further experiments (Swaminathan and Natarajan, 1959) showed that peanut, mustard and castor oils produce mitotic and meiotic aberrations in wheat. Morphological changes and viable mutations were found in bread wheat during the second generation. The purpose of the present investigation was to determine whether sunflower seed oil, commonly used in cooking, is able to induce structural changes in the chromosomes of onion root-tip cells.

LITERATURE SURVEY

R. J. Ludford (1953) has reviewed the pioneer work on the induction of mitotic aberrations. As early as 1887 the brothers Hertwig used weak solutions of nicotine, strychnine and morphine, or low temperatures to produce mitotic divisions with multipolar spindles in the eggs of invertebrates. Monasters resulting in monocentric mitoses were produced experimentally by strychnine, hypertonic sea water, ether, and mechanical agitation. In these mitoses there was no spindle formation and no cleavage. The chromosomes formed and doubled without cell division thus resulting in experimentally produced polyploid cells long before the discovery of colchicine (Hertwig, 1895; Morgan, 1896; Wilson, 1902; Boveri, 1903; cited by Ludford, 1953).

Meanwhile, the reports from pathologists on the incidence of abnormal mitoses in cancer cells stimulated interest in the action of chemicals on cell division. Solutions of potassium iodide, zinc sulphate, zinc chloride, cocaine and quinine were used seventy years ago to induce multipolar spindles, inhibition of spindle formation and various other mitotic abnormalities in epithelial cells of frog and epidermis of salamander (Schottlander, 1888; Galeotti, 1893; cited by Ludford, 1953). So it was accepted that the mitotic aberrations found in malignant cells could be duplicated in normal cells.

In 1928 Muller discovered that X-rays produce mutations in animals. At the same time Stadler found that X-rays have a similar effect on plants. This initiated the search for chemical agents capable of producing similar aberrations. Lits and Dustin observed the effect of colchicine on mitosis in 1934 and Blakeslee noted that colchicine produces polyploidy (1937). This led to an extensive study by Levan

and his workers (1938; 1943; 1945; 1948; 1949; 1951) of the so-called "c-mitotic" reaction connected with inactivation of the spindle apparatus.

The first permanent structural changes in the chromosomes due to chemicals were produced by Oehlkers in 1943. He observed translocations in the meiotic chromosomes of flowering plants after treatment with urethane and potassium chloride (cited by Oehlkers, 1952). At about the same time Auerbach and Robson (1946) reported that mustard gas caused the appearance of mutations in animals and Darlington and Koller (1947) induced breakage and rejoining in plant chromosomes by the same means.

After the appearance of these first publications, many chemicals were investigated and were found to have mutagenic activity. It would be impractical to review all the literature on the agents used to induce aberrations. Ludford (1953) has tabulated various mitotic abnormalities along with the chemical used and the organism tested. Koller (1954) has described chemically-induced breakage with reference to localization of breaks, molecular structure of agents and their mode of action. Boyland (1954) has considered some mutagens, their effect on chromosomes, and the relationship between mutagens and carcinogens and their effects on nucleic acid. Articles describing chromosome breakage induced by radiation, by chemicals, and arising spontaneously are included in "Symposium on Chromosome Breakage," June, 1952. [Papers]. London, Oliver and Boyd, 1953. (Heredity 6, Suppl. 1952). More recently, Swanson (1957) has reviewed the four most thoroughly investigated chemical mutagens; Biesele (1958) has published a comprehensive survey of

agents which disrupt mitosis; and Sharma and Sharma (1960) and Kihlman (1961) have reviewed the mechanism of chromosome breakage.

MATERIALS AND METHODS

Both dry bulbs and growing root-tips of the onion, Allium cepa ($2n = 16$) were used to test the effect of sunflower seed oil on mitosis. Depending on the time of the year, three different varieties of onion were used: pickling; cooking; and Dutch onion "sets." The variety made no difference in the effect of the oil treatments. The sunflower seed oil, SAFFLO, is sold locally for domestic purposes. It contains 21.3% oleic acid, 66.2% linoleic acid, less than 0.1% linolenic acid, and is recommended for cooking and baking. All bulbs were grown in the light at room temperature. Each treatment was done in duplicate with simultaneous controls. After treatment several roots were cut from each bulb and were fixed immediately in 1:3 acetic acid-ethyl alcohol. Roots were hydrolyzed for 10 min. in 1N HCl, then squashed in aceto-carmine stain (Belling, 1926). Temporary mounts were made. Slides were examined for cytological abnormalities in both dividing and non-dividing cells. Photomicrographs were taken of typical abnormalities found after each treatment. Data for tables were obtained from observations of a minimum of four root-tips from each treatment (two from each of the duplicates). If no mitotic divisions were apparent in four root-tips, additional root-tips were observed. No count was made of the total number of cells or root-tips used in the present investigation.

Four different procedures were followed:

1. Dry onion bulbs were placed in undiluted oil for 1 to 10 hours, wiped with an absorbent cloth and put on vials containing tap water. When roots had grown to a length of about $\frac{1}{2}$ inch, they were cut and fixed.

2. Bulbs with growing roots about $\frac{1}{2}$ inch long were put into undiluted oil for 4 to 60 minutes. Some roots were cut immediately after treatment, others were cut after recovery in tap water for 4 hours. Undiluted oil treatments were subsequently discontinued because of their lethal effect on the cells.

3. A homogenizer was used to mix sunflower seed oil and tap water. By using a dilution factor of 10, concentrations of oil and water were obtained which decreased from 10% to 0.000001% (0.01 ppm). Growing onion roots were treated with these concentrations for 4 hours, then were cut and fixed. It was observed that the weakest concentration, 0.01 ppm, produced very few abnormalities in onion chromosomes so it was discontinued. The root-tips placed in the 0.1 ppm concentration showed maximum chromosome breakage so this concentration was selected for the main body of the present investigation in which the duration of the treatment varied from 1 to 24 hours and the recovery time in tap water ranged from 0 to 7 days.

4. Dry bulbs of onion were put on vials containing 0.1 ppm oil and tap water emulsion. After 24 hours some of the bulbs were transferred to tap water for recovery periods of 24, 48, and 72 hours. Other bulbs were treated for 48 hours before recovery.

OBSERVATIONS AND RESULTS

Changes in the appearance of the nuclei and aberrations in the structure and distribution of the chromosomes were used as criteria to assess the effect of sunflower seed oil on the cells of onion root-tips. All treatments produced the same types of abnormalities but the number and the extent of the changes were related to the concentration of the oil, the duration of the treatment, and the recovery time. Results are summarized in Tables I-IV where the numbers indicate a gradation of response in terms of the extent of damage from none (0) to the maximum (4).

A. Mitotic frequency

The germination of Allium cepa bulbs was inhibited by undiluted sunflower seed oil. Some treated bulbs failed to produce any roots while others grew roots which reached a length of $\frac{1}{4}$ to $\frac{1}{2}$ inch after six days in tap water. Control roots reached this length in 24 to 48 hours.

All undiluted oil treatments of growing roots inhibited mitosis. There was a despiralization of coiled chromosomes and a reversion to interphase with complete cessation of cell division and disintegration of the chromosomes after 50 to 60 minutes exposure (Table I). Dilute oil treatments did not noticeably retard the development or growth of onion roots.

B. Chromosomes

Chromosome breakage was outstanding after dilute oil treatments. The damage ranged from the formation of a small gap to complete fragmentation (Figs. 1-3). Breakage was most extensive at metaphase and anaphase following treatment with 0.1 ppm concentration, reaching a maximum in roots grown from bulbs treated for 24 hours with 48 hours of recovery in tap water. Chromosomes in growing root-tips showed maximum

breakage 4 hours after treatment. Small gaps and breaks appeared first at metaphase (Fig. 4). These breaks extended across one chromatid or both, not necessarily at the same point. Fragments varied in size from small to entire chromosome arms. They were free or attached and almost always appeared double. There was frequently shattering and disintegration of all the chromosomes (Fig. 5). Along with breakage there was usually erosion which gave a "beaded" appearance if it extended along the entire length of the chromosome (Figs. 6, 7). There was slight evidence of reunion after breakage (Fig. 8). Chromosomes in one cell indicated possible translocations (Fig. 9).

"Stickiness" was induced in onion chromosomes by almost all concentrations of sunflower seed oil but was most pronounced after the 10% oil treatment (Table II). This stickiness varied in extent from fusion of all the chromosomes into a mass (Fig. 10) to the formation of anaphase and telophase bridges varying in thickness and in number, often with fragments (Figs. 11-14). Sometimes the entire chromosome complement was unable to separate (Fig. 15). Along with stickiness there was a despiralization of condensing chromosomes at all stages of mitosis (Fig. 19). The reversion of anaphase and telophase chromosomes to interphase without cell division was believed to be responsible for the appearance of the binucleate cells found in treated roots allowed to recover for at least 24 hours (Figs. 20, 21) and for the polyploidy observed in one root-tip 6 days after treatment (Figs. 22-24). Uncoiling at prophase resulted in the long, stringy chromosomes which were so numerous especially after undiluted oil treatments (Fig. 25). Relaxation of the spirals was noted in both the chromatids and half-chromatids (Fig. 26). Their multistranded structure showed plainly as the chromosomes uncoiled at telophase (Fig. 27).

Other cells contained excessively contracted chromosomes in all stages of division, often with chromosome breakage (Figs. 28-30). None of these abnormalities were observed in the control root-tips (Figs. 16-18).

C. Spindle mechanism

Along with overcontraction of the chromosomes caused by sunflower seed oil treatments there was a separation distally or along their entire length into sister chromatids and sometimes into half-chromatids (Figs. 31, 32). If the spindle mechanism had been impaired, the chromosomes were scattered throughout the cell indicating c-mitosis and resulting in a marked clarification of the karyotype (Figs. 33-36). Other spindle abnormalities were tripolar and multipolar anaphases usually with breakage and/or bridges (Figs. 37-39). A few somatic reductions were observed (Fig. 40) and some misdivision at anaphase (Fig. 41). Lagging chromosomes were present in some cells (Fig. 42) and micro- and macronuclei which might have been formed from laggards (Figs. 43-47).

D. Nucleus

Treatment with sunflower seed oil, especially in strong concentrations, caused interphase nuclei to contract into small heavily-stained lumps (Fig. 48). There was a leakage of nuclear material out of the cells (Figs. 49, 50). Nuclei appeared vacuolated and irregular in shape and size (Figs. 51, 52). Sometimes the chromatin was in droplets or reticulate (Figs. 53, 54). Large and small oil drops appeared in the cytoplasm, in the nucleus, or in both (Figs. 55-57). Abnormally large nucleoli were seen especially in reconstructed telophase nuclei (Fig. 58). Other anomalies detected were: unequal division into alternating small and large cells (Fig. 59); giant nuclei (Fig. 60); shattering and disintegration of pycnotic nuclei (Fig. 61).

DISCUSSION

A. Period of sensitivity

The breakage which sunflower seed oil produced in the contracted Allium cepa chromosomes of late prophase, metaphase and anaphase was most pronounced four hours after treatment. Kihlman (1955) calculated the duration of the entire mitotic cycle of Allium cepa to be about twenty-eight hours, of which twenty-five hours are spent in interphase, two hours in prophase-metaphase and three-quarters of an hour in anaphase-telophase. Hence, those chromosomes which showed damage at metaphase and anaphase four hours after treatment would have been in late interphase or early prophase at the time of treatment. It is also possible that the cells were already in metaphase and anaphase with arrest at these stages because of induced stickiness or spindle aberrations. No attempt was made to treat root-tip cells at specific stages in the mitotic cycle.

The action of sunflower seed oil resembles that of X-rays (Swanson, 1957), 8-ethoxycaffeine (Kihlman, 1955), the phenols (Levan and Tjio, 1948), and some plant hormones (Sharma and Sharma, 1960), all of which produce maximum chromosome breakage during late prophase. This is contrary to the effect of most radiomimetic substances which damage the chromosomes during interphase when duplication occurs (Kihlman, 1961; Howard and Pelc, 1952). Swaminathan and Natarajan (1959) have suggested that the breakage induced in wheat by vegetable oils is spread over a period of time and occurs at the time of reduplication. Results of the present investigation indicate that chromosome breakage does not occur at this time in Allium cepa.

B. Chromosome breakage

The outstanding effect of dilute sunflower seed oil on onion chromosomes was the production of widespread erosion and breakage. It is suggested

that the rupture of "sticky" anaphase bridges could have caused the formation of some free fragments but mechanical difficulties in separation could not account for the complete shattering and disintegration of the chromosomes which occurred in some cells (Figs. 2, 3).

Chromosome erosion and breakage were observed by Sharma and Sharma (1960) after onions were treated with some plant hormones and vitamins. It was proposed that certain chromosome sites lose some of their quota of nucleic acid and so appear thinner than the rest of the chromosome segments. These thinner areas eventually break because of their changed constitution. Levan and Tjio (1948a, 1948b) produced chromosomal constrictions, gaps and fragmentation in onions with some of the phenols and compared their effect to that of x-rays. They noted that those phenols which are most readily oxidized produced the highest frequency of breakage and suggested that oxidation is in some way concerned with their activity. The phenols could affect the oxidation-reduction system of the cell directly or could result in the formation of some oxidation produce which affected the cell. If the phenols cause breakage by the addition of oxygen or the removal of hydrogen, they probably do have a mode of action similar to that of x-rays which have been shown to depend partly on the presence of oxygen for their chromosome-breaking ability (Swanson, 1957). 8-ethoxycaffeine has an effect on onion chromosomes comparable to that of x-rays and sunflower seed oil in the structural changes produced and also in the period of sensitivity (Kihlman, 1955).

C. "Stickiness"

"Stickiness" is the reaction of condensed mitotic chromosomes to many chemical agents and is one of the primary effects of irradiation.

Stickiness is indicated by the fusion or clumping of chromosomes and by the presence of sticky bridges caused by the adherence of the tips of separating anaphase chromosomes. Interphase nuclei show pycnosis or stickiness by rounding up into spherical, compact masses which stain intensely due to marked condensation (Brachet, 1957).

Swaminathan and Natarajan (1959) reported that vegetable oil treatments produce no stickiness in wheat seeds. However, treatment of onion cells with sunflower seed oil, especially with the 10% concentration, resulted in the formation of many sticky anaphase-telophase bridges and fused chromosomes. It is suggested that stickiness helps to prevent the completion of mitosis causing inhibition of cell division and lack of cell wall formation. This failure of cytokinesis is believed responsible for the appearance of many binucleate cells following oil treatment and recovery. Marsland (1958) found that the furrowing of a sea urchin egg can be delayed for only a limited time after which the cell is unable to divide and becomes binucleate.

Stickiness may be spontaneous and was found in untreated root-tips of Allium cepa in bulbs which had been stored at low temperatures (D'Amato, 1948). This condition was vital and did not prevent growth. Colchicine treatment of these roots caused full c-mitosis and also partial c-mitosis with fusion of chromosomes, inhibition of anaphase separation and lack of cell wall formation with consequent appearance of binucleate cells. D'Amato suggested that low temperature has a physiological effect which results in stickiness of the chromosomes. Sticky chromosomes are also found in tumors and result, perhaps, from the liberation of toxins by dying cells (Koller, 1949).

The actual cause of stickiness is not known. Darlington (1942) believed stickiness is due to the depolymerization of nucleic acid in the chromosomes. Levan (1949) stated that depolymerization of the nucleic acid and liquefaction of the matrix of the chromosomes result in stickiness, but Himes (1950) found no evidence of DNA depolymerization in genetically "sticky" corn. Biesele (1958) has listed many agents which cause stickiness and has suggested they do so by affecting the nucleic acids of the chromosomes. Among these agents are the mustards which react with phosphate and amino groups of nucleic acids causing depolymerization (Darlington and Koller, 1947). Kihlman (1955) suggested that x-rays and 8-ethoxycaffeine cause stickiness in onion chromosomes by interfering with the function of nucleic acids in the cells.

Kaufmann and Das (1954), observing stickiness, pycnosis, and chromosome bridges in growing root-tips of Allium cepa treated with ribonuclease, proposed that they result from the dissociation of the chromosomal nucleoprotein by the ribonuclease. Biesele (1958) also thought that protein denaturation is responsible for the stickiness induced in onion chromosomes by various phenols (Levan and Tjio, 1948a, 1948b).

D. C-mitosis

"C-mitosis" or colchicine-mitosis refers to the arrest of cell division at metaphase due to the inactivation of the mitotic spindle (Levan, 1938) and to excessive chromosome contraction (Ostergren, 1944). C-mitosis is induced by most organic substances (Levan, 1949).

Contraction is the most conspicuous feature of normal prophase chromosomes and is believed to be due to the progressive coiling of the

interphase chromonema (Swanson, 1957). This condensation process is not completed during prophase but continues through metaphase and into anaphase. If metaphase is delayed, the chromosomes continue to contract and can be much more condensed than normal as occurs after treatment with such agents as colchicine (Mazia, 1961). It is suggested that the supercontraction of the onion chromosomes observed after treatment with sunflower seed oil is caused by continued coiling during arrest at metaphase as a result of physical inability of the sticky chromatids to separate or to inactivation of the spindle.

The mechanism responsible for chromosome coiling is not known. Anderson (1956) proposed that coiling is due to configurational changes in DNA molecules and presented evidence that when the negative groups on the DNA are discharged with a polyvalent cation, condensation occurs. Such substances as spermine, cadaverine or smaller basic polypeptides have been found to condense isolated nuclei to resemble prophase condensation (Anderson, et al., 1960). Davidson and Anderson (1960) reported that the polyamines, putrescine and cadaverine, cause overcondensation of the chromosomes in meristematic Vicia faba cells at metaphase and anaphase-telophase. Sunflower seed oil appears to have a similar cationic effect on onion root-tip chromosomes.

Sharma (1956) stated contraction is due to dehydration of the chromosome arms and results mainly from viscosity changes in the cell which govern the swelling and contraction of chromosomes. Ludford (1953) argues that condensed chromosomes and spindle aberrations could be due to a change in the normal water relations in the dividing cell.

In addition to inactivation of the spindle with arrest at metaphase, tripolar, multipolar and completely disorganized anaphases were

observed in cells of onion root-tips after sunflower seed oil treatment. Multipolarity in animal cells is apparently caused by abnormal division of the centrioles (Brachet, 1957). Although no centrioles have been seen in plant cells, it is assumed that equivalent organizing centers must be present with a similar function which were altered by sunflower seed oil treatments. Orientation of the spindle is related to centrioles and kinetochores about whose activity nothing is known (Mazia, 1960). It is believed that the kinetochore is not merely an anchorage for the chromosomal fibre but also is an active participant in movement. Impairment of its ability to function in this capacity could have been responsible for the laggards found after oil treatment.

The mitotic spindle seems to assembled from pre-existing macromolecules (Went, 1960; Mazia, 1957) consisting of a few proteins associated with RNA and lipids, enzymes and zinc associated into fibres bound together by S-S and protein-SH bonds, hydrogen bonds and other intermolecular bonds none of which is clearly understood (Mazia, 1957; Anderson, 1960). Since sunflower seed oil was observed to produce structural aberrations so rapidly in the onion chromosomes, it is suggested that the oil also destroyed the orientation, integrity and function of the mitotic apparatus by denaturing the nucleoproteins or dissolving the various bondings present.

E. Abnormal nuclei

It is suggested that the leakage of nuclear material out of the cells and the dispersal of chromatin in droplets throughout the cytoplasm which were observed in some onion root-tip cells after treatment with undiluted sunflower seed oil were due to death of the cells and destruction of the cell membranes.

F. Mode of action

The mode of action by which sunflower seed oil affects the cells of onion root-tips has not been demonstrated but can only be speculated upon. Many chemicals which produce chromosomal aberrations have very different properties and cannot be assumed to act in the same way. Most radiomimetic agents produce structural changes in chromosomes which appear between 8 and 12 hours after treatment. The breakage induced by these agents is connected with DNA synthesis which is completed in interphase (Howard and Pelc, 1952). A few agents, including sunflower seed oil, cause abnormalities which appear in metaphase within 2 hours after treatment. Sunflower seed oil seems to have a direct effect on the chromosomes, not on the chromosome precursors or on the processes involved in chromosome synthesis.

Sunflower seed oil contains 21-39 percent of oleic acid with one carbon-carbon double bond and 51-68 percent of linoleic acid with two carbon-carbon double bonds (Noller, 1957). Because of the presence of loosely-held electrons a carbon-carbon double bond serves as a source of electrons and acts as a base. It is suggested that sunflower seed oil produces chromosomal aberrations because of the basic properties of its unsaturated acids which can undergo typical reactions involving the addition of hydrogen, of water or of acids (Morrison and Boyd, 1959).

The exact structure of the chromosome is not known but it is postulated to be a linear, multistranded structure comprising long strands of DNA interlinked end-to-end with protein and cross-linked by hydrogen bonds (Anderson, et al., 1960; Allfrey and Mirsky, 1961; Crick, 1962). The spindle consists of macromolecular units of protein and RNA held together by protein-SH bonds and hydrogen bonds (Mazia, 1957). It is

suggested that the carbon-carbon double bonds of the sunflower seed oil undergo an addition reaction which breaks protein and hydrogen linkages of the chromosomes and spindle resulting in the disintegration of the chromosomes and the spindle fibres. Wolff (1960, cited by Kihlman, 1961) indicated that protein bonds are involved in chromosome breakage and showed that protein synthesis is necessary for rejoining to occur.

In addition to the direct degradation of protein and nucleic acids in the spindle and chromosomes, it is possible that sunflower seed oil causes structural deformation by interfering with normal metabolic processes in the cell. Ribonucleoproteins are believed to be essential for protein synthesis and an impairment of their ability to function in this capacity might produce chromosome breaks. Ribonuclease produces mitotic aberrations similar to those induced by sunflower seed oil including a marked enlargement of onion nucleoli (Kaufmann and Das, 1954, 1956). The abnormally large onion nucleoli observed by Brachet (1954) after treatment with ribonuclease and by Verema (1959) using cigarette-smoke extracts was related to probable disturbances in protein synthesis. Inability to produce the protein necessary to repair breaks would account for the lack of rejoining noted in this investigation and in that of Swaminathan and Natarajan (1959).

Another reaction which a double carbon-carbon bond is able to undergo is the addition of water. Changes in the distribution of water in the cell are characteristic of the various stages of mitosis. Ludford (1953) has shown that alteration in the tonicity of the medium resulted in chromosome clumping, stickiness and binucleate cells, all of which were seen in onion root-tips after oil treatments.

Anderson (1956) postulated that the chromosome exists as a charged DNA-protein gelwork where, in the extended state, the DNA phosphate groups are separated from the basic interconnecting protein groups. When polycations are introduced, there is a condensation of the chromosomes resulting in super-coiling seen after oil treatments. Excess of basic groups might tend to crosslink DNA strands in adjacent chromosomes which could account for the chromosomal "stickiness" observed.

Most tissue proteins have been shown to have a net acid charge indicating an excess of available carboxyl groups over amino groups. The entrance of sunflower seed oil would not only alter the gel-like properties of the chromosomes but would also change the predominant charge on the cell colloids causing degradation of the spindle materials with inhibition of the movement and distribution of the chromosomes. Localized alterations would affect restricted areas of the cell resulting in such anomalies as "reductional groupings", misdivison at anaphase, and lagging chromosomes.

It should be noted that this was a cytomorphological study of the effect of sunflower seed oil on meristematic cells of Allium cepa. The suggestions regarding the manner in which aberrations were produced in these onion cells are merely speculations. No specific biochemical tests could be introduced.

SUMMARY

Both dry bulbs and growing root-tips of the onion, Allium cepa, were treated with sunflower seed oil in various concentrations ranging from 100 percent to 0.01 ppm. Maximum structural changes were induced by 0.1 ppm which was used for the main body of this investigation.

Similar abnormalities were observed in the structure and distribution of the chromosomes after all treatments but the number and extent of the deviations were influenced by the concentration of the oil, the duration of the exposure, and recovery time. "Stickiness" with anaphase-telophase bridges, clumping, loss of pycnotic material from the cell and arrested mitosis with precocious reversion to interphase were most pronounced after concentrated oil treatments. Also observed were enlarged nucleoli, excessive chromosome contraction and spindle aberrations including tripolar and multipolar anaphases. Chromosome and chromatid breakage with attached or free double fragments were maximum at metaphase and anaphase four hours after treatment. Inhibition of cell wall formation resulted in the appearance of binucleate cells, micro- and macronuclei and some polyploidy after recovery in tap water.

Results indicate that sunflower seed oil attacked the nuleoprotein of the chromosome itself and not the chromosome precursors. Since sunflower seed oil contains unsaturated acids with carbon-carbon double bonds, it is suggested that it acts as a base in the cell where it could rupture hydrogen and protein bonds in DNA and RNA, denature spindle and chromosome protein causing inhibition of the movement and normal distribution of the chromosomes, interfere with protein synthesis, and/or upset the water relations in the cell.

TABLE I. Effect of undiluted sunflower seed oil on mitosis and chromosomes in cells of onion bulbs and root-tips.

	Duration of treat.	Recovery time	Stickiness	Breakage	Over contraction	Spindle abnormalities	Remarks
Dry bulbs	1 hr.	6 days	-	-	-	-	no divisions
	4 "	6 "	1[a]	0	0	0	despiralization; pycnosis; oil drops in nuclei
	6 "	6 "	1	0	0	0	as above
	10 "	6 "	3	1	1	1	as above; oil in cytoplams
Growing roots	4 min.	4 hrs.	2	0	1	1	reversion; chromatin in drops
	5 "	4 "	2	0	1	1	as above
	10 "	4 "	2	0	0	0	cell wall bulging; reversion
	20 "	4 "	2	0	0	0	as above
	30 "	4 "	3	1	1	1	pycnotic chromatin leaving cells
	50 "	4 "	0	0	0	0	poor staining
	60 "	4 "	0	0	0	0	as above

a. Numbers indicate a gradation of response from 0 to the maximum at 4.

TABLE II. Effect of various concentrations of diluted sunflower seed oil on mitosis and chromosomes in cells of onion root-tips.

Conc. of oil	Duration of treat.	Recovery time	Stickiness	Breakage	Over contraction	Spindle abnormalities	Remarks
10%	4 hrs.	0 hr.	4[a]	1	1	1	despiralization; somatic reduction
	4 "	3 days	2	0	1	2	binucleate cells; abnormal daughter nuclei; disintegrating chromosomes
1%	1 "	0 hr.	2	0	1	1	
		4 "	1	0	1	1	despiralizing telo.
		24 "	1	0	0	1	
.1%	4 "	0 "	2	0	0	0	
.01%	4 "	0 "	1	2	1	1	
.001%	4 "	0 "	1	1	0	0	
.0001%	4 "	0 "	1	3	1	1	vacuolated nuclei
.00001%	4 "	0 "	1	4	2	2	

a. Numbers indicate a gradation of response from 0 to the maximum at 4.

TABLE III. Effect of 0.1 ppm sunflower seed oil on mitosis and chromosomes in cells of onion root-tips.

Duration of treat.	Recovery time	Stickiness	Breakage	Over contraction	Spindle abnormalities	Remarks
1 hr.	0 hr.	2[a]	0	0	0	telophase bridges
	4 "	1	4	1	1	huge telo. nucleoli; somatic reduction (7, 5)
	24 "	1	0	1	1	binucleate cells
	6 days	1	0	1	0	polyploidy; diplochromosomes
4 hrs.	0 hr.	1	4	2	2	vacuolated nuclei; large interphase nuclei
	4 "	1	1	1	1	abnormal telo. nuclei
24 "	0 "	2	0	2	2	quadripartite at meta.; somatic reduction
	4 "	2	4	2	2	
	24 "	1	3	1	1	
	3 days	2	1	0	0	binucleate cells
	6 "	1	1	1	1	uncoiled telo. bridge
	7 "	1	0	1	1	micro-, macronuclei

a. Numbers indicate a gradation of response from 0 to the maximum at 4.

TABLE IV. Effect of 0.1 ppm sunflower seed oil on mitosis and chromosomes in root-tip cells of onion bulbs germinated in the oil.

Duration of treat.	Recovery time	Stickiness	Breakage	Over contraction	Spindle abnormalities	Remarks
24 hr.	24 hr.	3[a]	0	0	0	many pycnotic nuclei
	48 "	1	4	3	3	micro- and macronuclei
	72 "	1	3	3	3	misdivision at anaphase
48 hr.	0 "	2	2	1	1	many giant nuclei
	24 "	1	1	1	1	
	48 "	1	1	1	1	
	72 "	1	1	1	1	somatic reduction; despiralization

a. Numbers indicate a gradation of response from 0 to the maximum at 4.

Effect of sunflower seed oil on the chromosomes of onion root-tip cells. Figs. 1-18.

Fig. 1. Late prophase with separation of chromatids and some breakage. (Dry bulb 24 hrs. in 0.1 ppm oil, 48 hrs. recovery). x1200

Figs. 2 and 3. Extensive fragmentation. (Treatment as in Fig. 1). x1500

Fig. 4. Erosion and breakage with double fragment at metaphase. (Treatment as in Fig. 1, except 72 hr. recovery). x1500

Fig. 5. Chromatid and chromosome breakage with free and attached fragments. (Treatment as in Fig. 4). x1500

Fig. 6. "Beaded" metaphase. (Treatment as in Fig. 1). x1500

Fig. 7. Eroded bridges and fragments at anaphase. (Dry bulb 8 days in 0.1 ppm oil, no recovery). x1500

Fig. 8. Anaphase bridge with large fragment indicating possible breakage and rejoining. (Treatment as in Fig. 1). x1500

Fig. 9. Possible breakage and translocations. (Root-tip, 1 hr. in 0.1 ppm oil, 4 hr. recovery). x1200

Figs. 10 and 11. Fused chromosomes. (Root-tip, 4 hrs. in 10% oil, no recovery). x1500

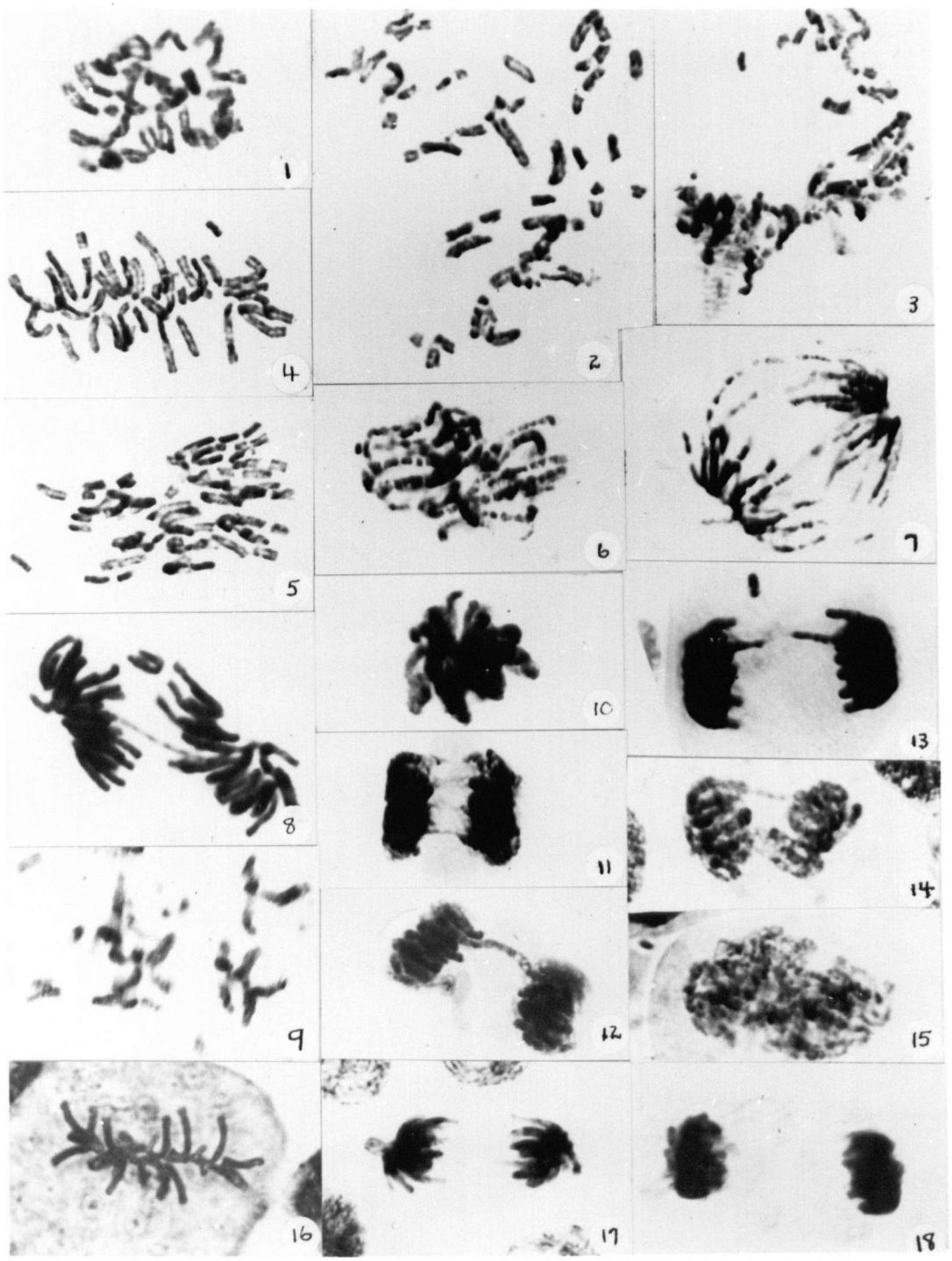
Figs. 12 and 13. Bridges with and without fragment at telophase. (Treatment as in Fig. 1). x1500

Figs. 14 and 15. "Stickiness" and uncoiling chromosomes at anaphase. (Treatment as in Figs. 10 and 11). x1500 and x2000

Figs. 16, 17 and 18. Normal metaphase, anaphase and telophase in control root-tips. x1200

I
EFFECT OF SUNFLOWER SEED OIL ON THE CHROMOSOMES OF ONION ROOT-TIP CELLS

(Figs. 1-18)



Effect of sunflower seed oil on the chromosomes of onion root-tip cells. Figs. 19-30.

Fig. 19. Despiralizing chromosomes at telophase. (Root-tip 4 hrs. in 10% oil, no recovery). x1500

Figs. 20 and 21. Binucleate cells. (Treatment as in Fig. 19, except 3 days recovery). x1500

Fig. 22. Anaphase in polyploid cell with 28 chromosomes going to one pole and 26 to the other. (Root-tip 1 hr. in 0.1 ppm oil, 6 days recovery). x2000

Fig. 23 and 24. Quadripartite chromosomes at prophase and metaphase in polyploid cells. (Treatment as in Fig. 22). x2000

Fig. 25. Long, stringy chromosomes. (Root-tip 4 min. in undiluted oil, 4 hr. recovery). x1500

Fig. 26. Uncoiling chromatids and chromosomes. (Dry bulb 24 hr. in 0.1 ppm oil, 48 hr. recovery). x1500

Fig. 27. Telophase bridge uncoiling showing multistranded nature of the chromosomes. (Root-tip 24 hr. in 0.1 ppm oil, 6 days recovery). x1500

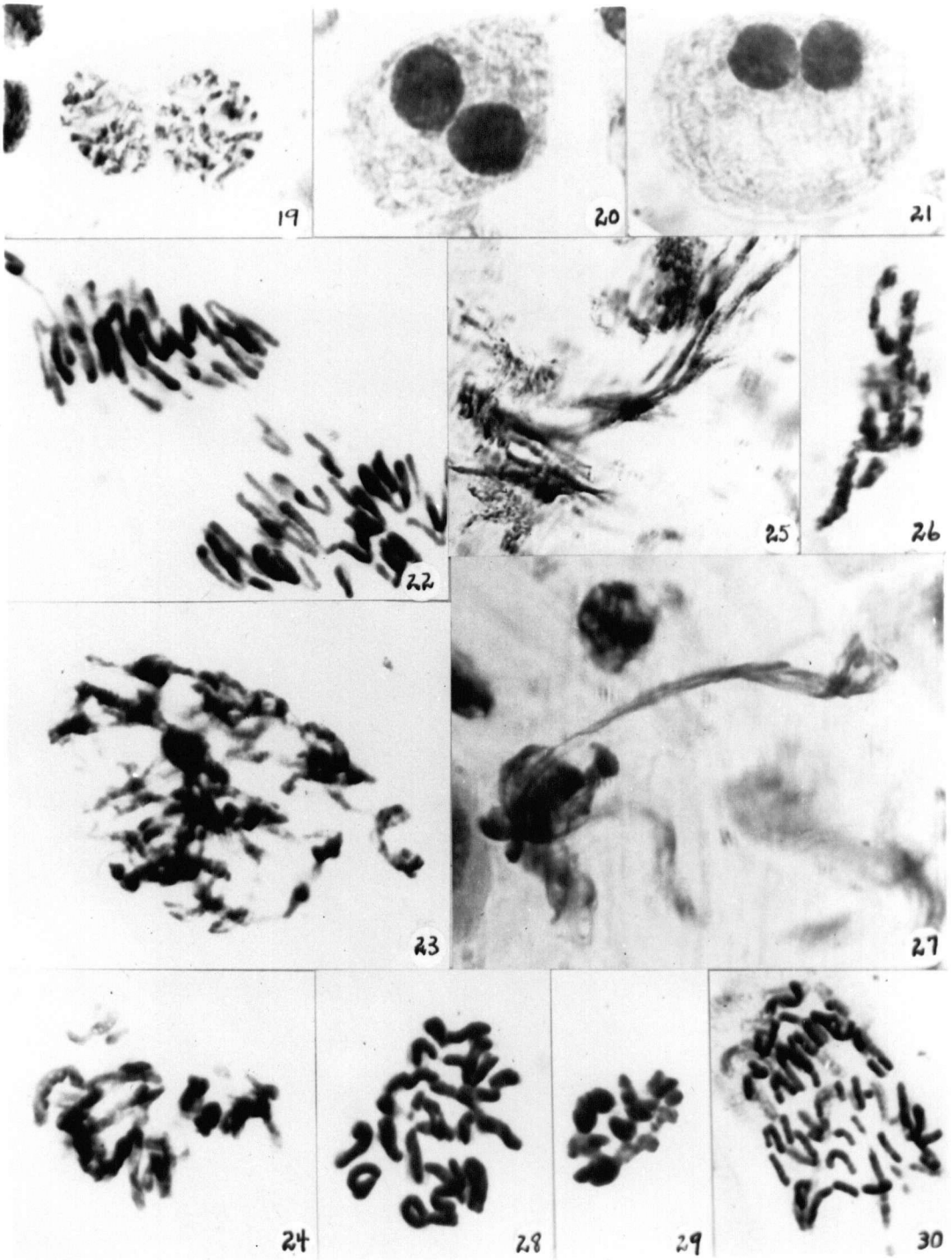
Fig. 28. Contracted chromosomes. (Treatment as in Fig. 27 except 48 hr. recovery). x1500

Fig. 29. Abnormal chromosomal contraction. (Treatment as in Fig. 27 except no recovery). x1500

Fig. 30. Chromosome contraction and breakage at anaphase. (Dry bulb 48 hr. in 0.1 ppm oil, no recovery). x1500

II
EFFECT OF SUNFLOWER SEED OIL ON THE CHROMOSOMES OF ONION ROOT-TIP CELLS

(Figs. 19-30)



Effect of sunflower seed oil on the spindle mechanism of onion root-tip cells. Figs. 31-47.

Fig. 31. C-mitotic "x-configuration" and separation of chromatids. (Dry bulb 24 hr. in 0.1 ppm oil, 48 hr. recovery). x1500

Fig. 32. Separation of chromatids in contracted metaphase. (Root-tip 4 hr. in 0.1 ppm oil, no recovery). x1500

Fig. 33. Free and attached fragments, clarification of karyotype at late prophase. (Treatment as in Fig. 31 except 72 hr. recovery). x1500

Fig. 34. Excessive chromosome contraction at metaphase with breakage of one chromatid. (Root-tip 24 hr. in 0.1 ppm oil, no recovery). x1500

Fig. 35. Overcontracted anaphase. (Treatment as in Fig. 31). x1500

Fig. 36. Clarification of karyotype with contracted chromosomes. (Dry bulb 5 days in 0.1 ppm oil, no recovery). x2000

Fig. 37. Tripolar anaphase with breakage. (Root-tip 1 hr. in 0.1 ppm oil, 4 hr. recovery). x1200

Fig. 38. Multipolar anaphase with breakage. (Treatment as in Fig. 31). x1500

Fig. 39. Multipolar anaphase with bridges. (Treatment as in Fig. 31 except 24 hr. recovery). x1500

Fig. 40. Reduction division with uncoiling chromosomes. (Dry bulb 4 hr. in 10% oil, no recovery). x1200

Fig. 41. Misdivision at anaphase. (Treatment as in Fig. 33). x1500

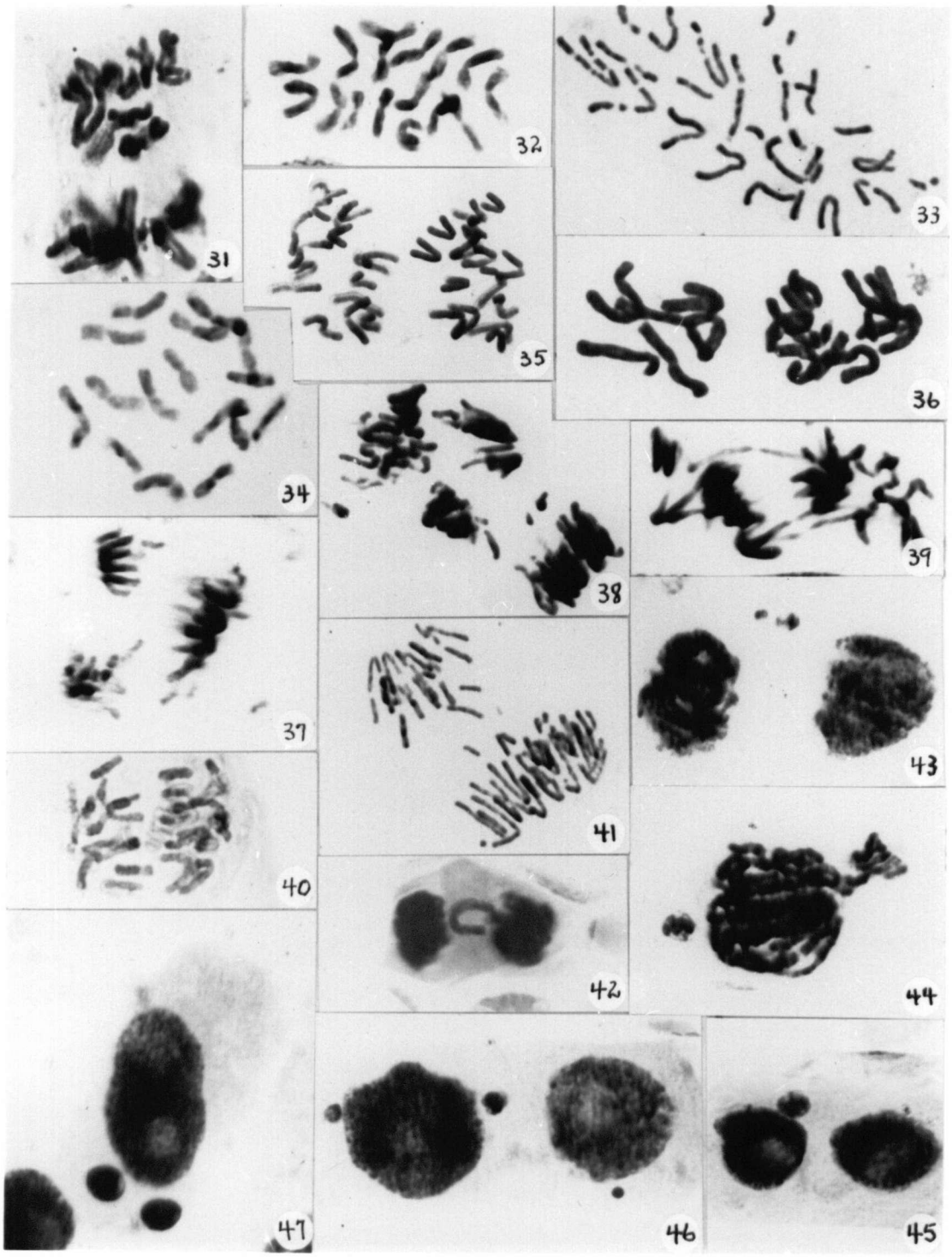
Fig. 42. Laggard. (Treatment as in Fig. 31). x1500

Fig. 43, 44, 45 and 46. Micro- and macronuclei. (Treatment as in Fig. 31). x1800

Fig. 47. Macronuclei. (Treatment as in Fig. 31 except 7 days recovery). x1800

III
EFFECT OF SUNFLOWER SEED OIL ON THE CHROMOSOMES OF ONION ROOT-TIP CELLS

(Figs. 31-47)



Effect of sunflower seed oil on the nucleus in onion root-tip cells Figs. 48-61.

Fig. 48. Pycnotic nuclei. (Root-tip 5 min. in undiluted oil, 4 hr. recovery). x1200

Fig. 49. Nuclear material leaving the cells. (Root-tip 30 min. in undiluted oil, no recovery). x2000

Fig. 50. Nucleus leaving cell. (Treatment as in Fig. 48). x1500

Fig. 51. Vacuolated telophase nuclei. (Root-tip 4 hr. in 1 ppm oil, no recovery). x2000

Fig. 52. Abnormally-shaped nuclei. (Dry bulb 4 hr. in undiluted oil, 6 days recovery). x1000

Fig. 53. Chromatin in droplets throughout the cytoplasm. (Treatment as in Fig. 48). x1200

Fig. 54. Chromatin in strings giving a net-like appearance. (Treatment as in Fig. 48 except 24 hr. recovery). x2000

Fig. 55. Oil drops in cytoplasm. (Root-tip 4 hr. in 10 ppm oil, no recovery). x2500

Fig. 56. Oil drops in both nucleus and cytoplasm. (Dry bulb 10 hr. in undiluted oil, 6 days recovery). x1200

Fig. 57. Oil drops in nuclei. (Dry bulb 6 hr. in undiluted oil, 6 days recovery). x1200

Fig. 58. Abnormally large nucleoli at telophase. (Root-tip 1 hr. in 0.1 ppm oil, 4 hr. recovery). x1500

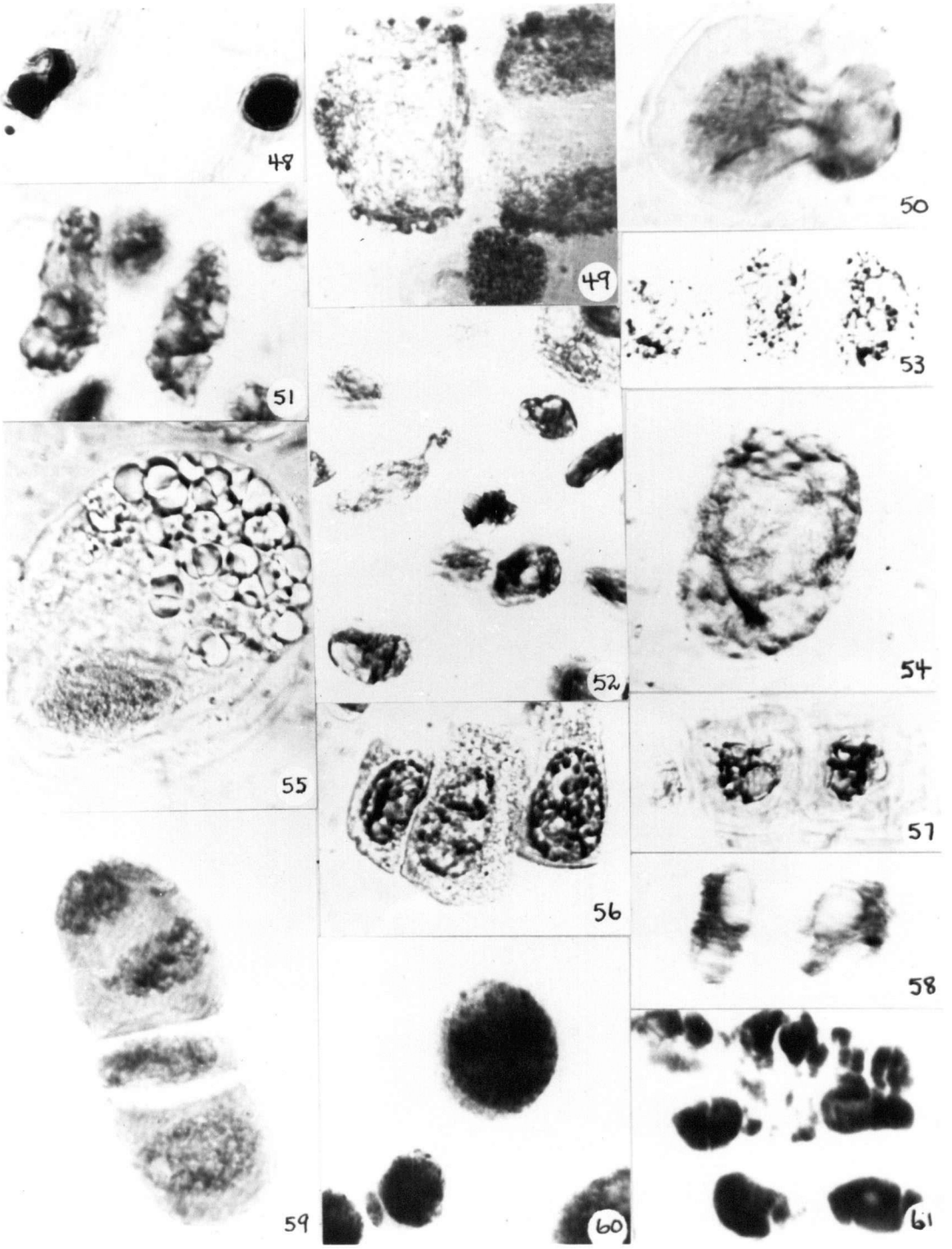
Fig. 59. Division into small and large cells. (Treatment as in Fig. 56). x1500

Fig. 60. Giant nuclei. (Root-tip 1 hr. in 1% oil, 4 hr. recovery). x1200

Fig. 61. Shattered pycnotic nuclei. (Root-tip 24 hr. in 0.1 ppm oil, 4 hr. recovery). x1200

IV
EFECT OF SUNFLOWER SEED OIL ON THE NUCLEUS IN ONION ROOT-TIP CELLS

(Figs. 48-61)



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Date November, 1962

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