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EFFECT OF NITROUS OXIDE ON PLANT CELL DIVISION: THE CYTOLOGICAL SIDE-EFFECT OF N_2O TREATMENT

ANNA SZELES

Department of Anthropology, Attila József University Szeged
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Abstract

Chromosome aberrations and disturbances of cell division in plant meristematic cells caused by long term nitrous oxide treatment are described. On the basis of detected aberrations, e.g. aneuploidy, polyploidy, irregularities of chromatin condensation, and micronucleus formation, the mutagenic activity of nitrous oxide is very probable. Since nitrous oxide is widely used in clinical practice the full-range mutagenicity test of nitrous oxide would be essential.
Key words: rye, nitrous oxide, chromosome aberrations, mitotic defects, micronuclei, mutagenicity.

Introduction

In a recent paper (SZELES, 1982) I have described the effect of nitrous oxide on mitosis of rye root-tip meristem cells. The metaphase blocking effect of N_2O was found to be reversible and following the termination of gas treatment the metaphase cells proceeded to more or less normal anaphases. Numerous aberrations of chromosomes and cell division were also observed which could be regarded as the cytological side-effects of N_2O treatment. Since, the nitrous oxide is widely used in clinical practice, the study of cytological disturbances caused by N_2O treatment seems to be necessary.

In the present paper chromosome aberrations and disturbances of cell division caused by long term nitrous oxide treatment are described. Instead of a complete registration and presentation of cytological findings only the most important groups of aberrations are reported: 1. micronucleus formation, 2. alterations in chromosome number, and 3. irregularity in condensation of chromosomes and nuclei.

Materials and Methods

Nitrous oxide (N_2O) treatment of rye (*Secale cereale*, $2n=14$) seedlings and cytological investigations were carried out as described previously (SZELES, 1982). Cytological examinations and microphotographs were made with a ZEISS NU-2 light microscope.

Results and Discussion

Cytological disturbances of plant meristematic cells treated with nitrous oxide were grouped into three categories: 1. micronucleus formations, 2. alterations in chromosome number, and 3. irregularities of chromatin condensation. These categories are in close connection in respect of their evolution and manifestation.

1) **Micronucleus formation**

The most characteristic cytological side-effect of nitrous oxide treatments was found to be the formation of micronuclei. The first appearance of micronuclei was detected after 4 a hour treatment at 6 atm pressure (see SZELES, 1982), and the number

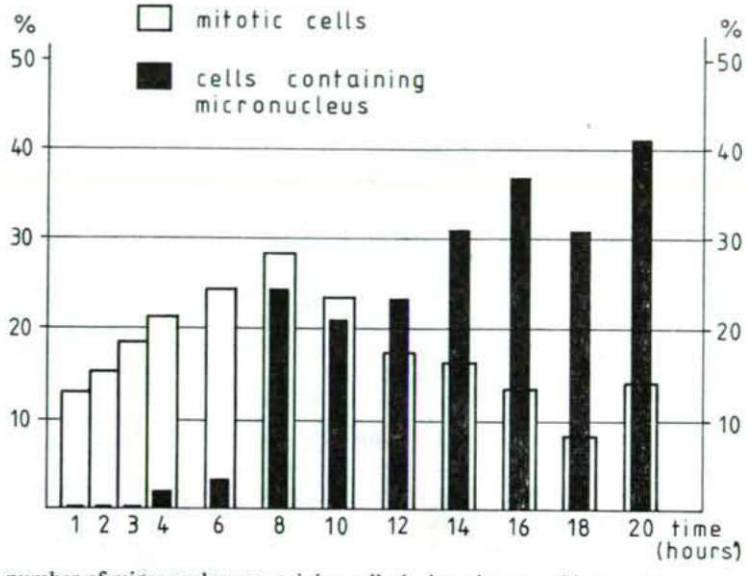

Fig. 1. The number of micronucleus containing cells during nitrous oxide treatment.

of micronuclei was dramatically increased at 6—8 h treatment. From 12 h treatment only a gradual increase of number of micronuclei was found (Fig. 1), showing that the disintegration of chromosomes starts approx. at the 10th hour of the gas treatment. Presence of micronuclei was also noticed after very short (2 hour) treatment. Obviously, these micronuclei were generated from the cells which were in mitosis at the beginning of the gas treatment.

The first cytological mark of the micronucleus formation was the separation of individual chromosomes or groups of chromosomes in metaphase (Fig. 2, picture 1—4). These separated chromosomes preserving their integrity were gradually decondensed lacking the normal anaphase process and went to telophase (Fig. 2, pictures 5—12). In telophase, decondensation of chromosomes showed high degree of synchrony, regardless of the localization of chromosomes or chromosome groups within the cells (Fig. 3, pictures 1—6), and parallel with the formation of interphase nuclei the formation of micronuclei was proceeded (Fig. 3, pictures 7—12). During the N_2O treatment not only the number of cells containing micronucleus was increased (Fig. 1) but an increase in the number of micronuclei per cell was also found (Fig. 3, pictures 7 and 11). Micronuclei were very often associated (Fig. 3, picture 8) and overlapped on each other (Fig. 3, picture 11), therefore, the statistical analysis of the number of micronuclei per cells proved to be inadequate.

The mutagenic and micronucleus inducing activity of certain chemical agents shows direct correlation. In the last decade, using that correlations, the „micronuc-

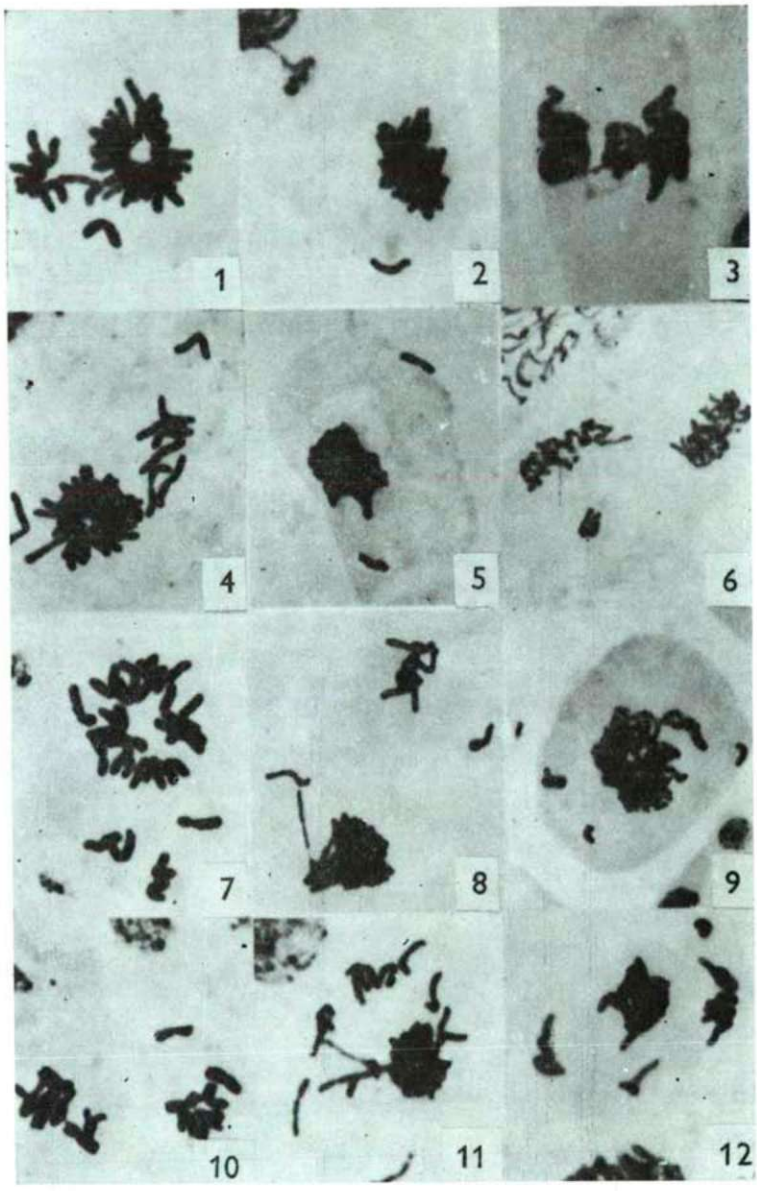

Fig. 2. Process of micronucleus formation (Magnification 650x)
1, 4, 6, 7, and 10 (6 atm 8 hours)
2, 3, 5 and 8 (6 atm 12 hours)
11 and 12 (6 atm 2 hours)
9 (6 atm 26 hours)

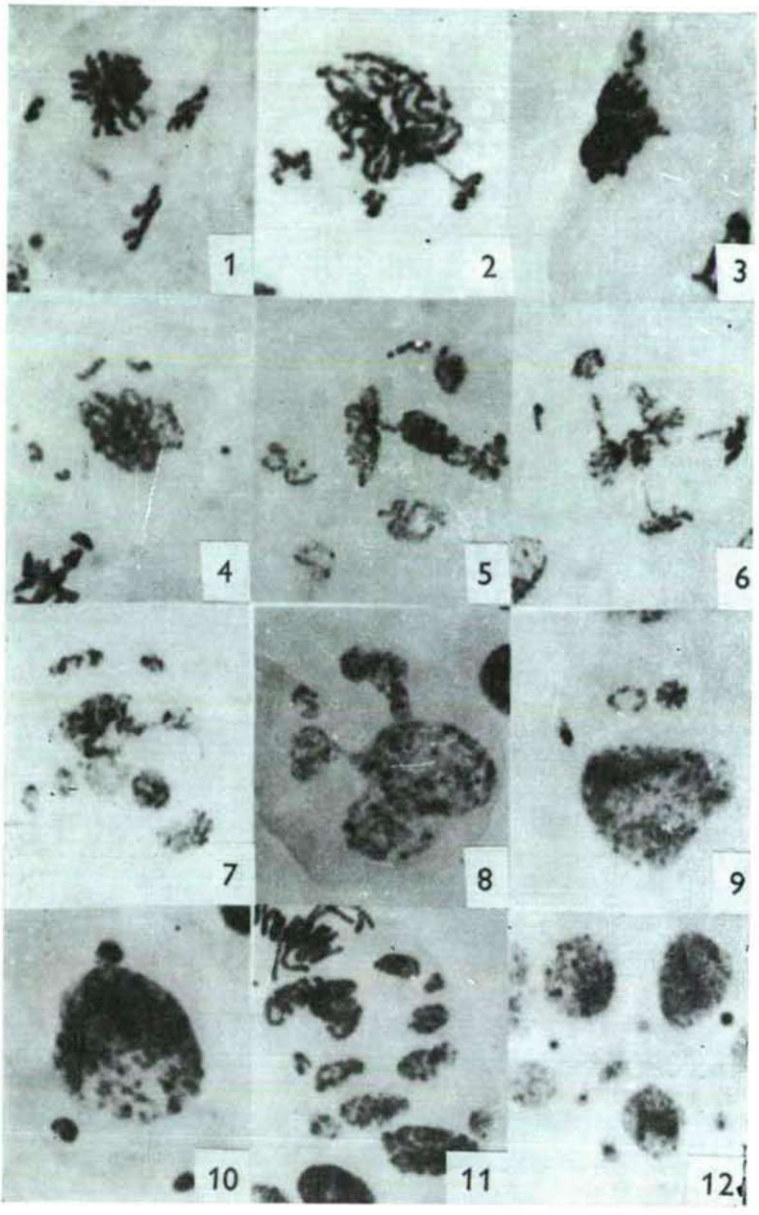

Fig. 3. process of micronucleus formation (650x)
1,9 and 12 (6 atm 8 hours + 30 min)
2,3 and 4 (6 atm 12 hours)
5 (11 atm 2 hours)
6 (12 atm 2 hours)
7 (7 atm 2 hours)
8 and 11 (9 atm 2 hours)

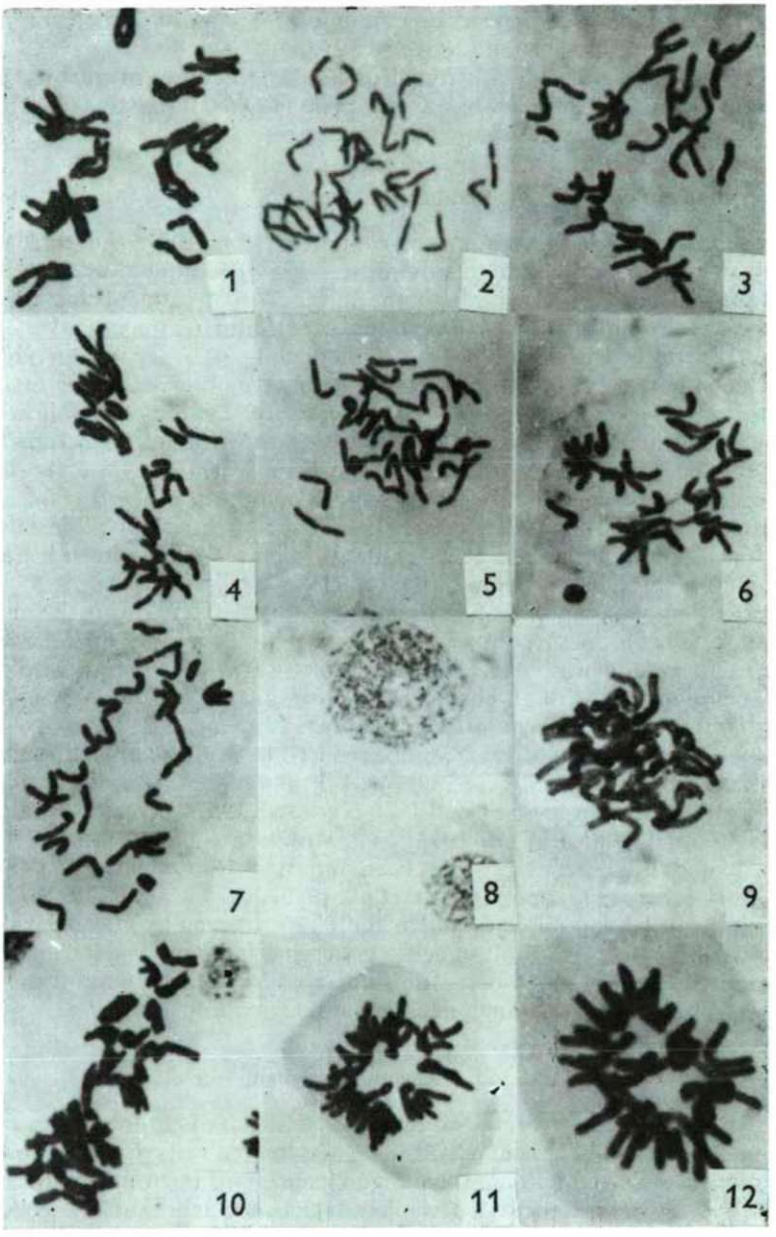

Fig. 4. Formation of polyploid cells by N_2O treatment (800x)
1 and 6 (6 atm 8 hours)
2 (12 atm 2 hours)
3, 4, 7, 10, 11 and 12 (6 atm 12 hours)
5 (11 atm 2 hours)
8 (6 atm 8 hours + 5 hours)
9 (6 atm 8 hours + 10 hours)

leus test" of the eukaryotic oragnism became one of the most important methods in the mutagenic tests (BOLLER and SCHMID, 1977).

Considering the significant micronucleus inducing activity of nitrous oxide, in respect to the common utilization of nitrous oxide in clinical practice, the full-range mutagenic test of nitrous oxide seems to be essential.

2) Alterations in chromosome number

In the alterations of the chromosome numbers caused by the nitrous oxide the most pronounced was the polyploidy. Formation of tetraploid and higher polyploid cells showed correlation with both the duration of treatment and the applied pressure of gas. Tetraploid cells have been found at 6h 6 atm treatment and at 2 h 9 atm treatments. Disarray of chromosomes (Fig. 4, pictures 1—4) or separation of chromatids in the metaphase ring (Fig. 4, pictures 11—12) were observed as the first cytological marks of polyploidization. In the absence of cytokinesis, following the replication of chromosomes tetraploid cells were formed (Fig. 4, pictures 8—12). Increasing the time of the N_2O treatment (to 20 hours at 6 atm) the repetition of aforsaid process, higher polyploid (octoploid) cells could be formed (Fig. 5, pictures 1—4). Irregularity in this process might cause an assimetric polyploidization and results in formation of hexaploid cells. Figure 5 (picture 5) shows such a hexaploid cell that contains three independent diploid nuclei.

The formation of aneuploid cells by means of citokinesis following the micronucleus formation (Fig. 6, pictures 1—2) and (or that following polyploidization and micronucleus formation was detected (Fig. 6, pictures 3—4). Figure 6 (picture 5) shows an aneuploid cell with 16 chromosomes and picture 6 shows cell containing only 3 chromosomes and a chromosome fragment.

The alterations in chromosome number caused by N_2O treatment are in agreement with data reported by different authors. Nitrous oxide induced polyploidy has been described by ÖSTERGREN, 1954, 1957; NYGREN, 1955; KIHARA et al., 1960; TSUNEWAKI, 1962; DVORAK et al., 1973; SUBRAHMANYAM and KASHA, 1975. Also, nitrous oxide induced aneuploidy has been reported by ÖSTERGREN, 1954, 1957; DVORAK and HARVEY, 1973; and DVORAK et al., 1973.

It is noteworthy that polyploids and aneuploids produced by nitrous oxide treatment might serve as potential tools in cell genetics and in plant genetics.

The results of the present observations strongly suggest that the formation of aneuploid cells is carried aut through micronucleus formation.

3) Irregularities in condensation of chromosomes and nuclei

Different irregularities of organization (condensation) of chromosomes and nuclei were found to be characteristic to nitrous oxide treated cells. Figure 7 (picture 1) shows an interphase cell containing three highly condensed micronuclei. In the cells, in which the nucleus was fragmentated by micronucleus formation, different degree of condensation of micronuclei was often detected. Figure 7 (pictures 2—7) shows cells

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Fig. 6. Formation of aneuploid cells
1, 2, 3 and 6 (6 atm 20 hours)
4 (6 atm 8 hours)
5 (6 atm 8+15 hours)
Magnifications: 1, 2, 4 and 5=800x
3=600x
6=730x

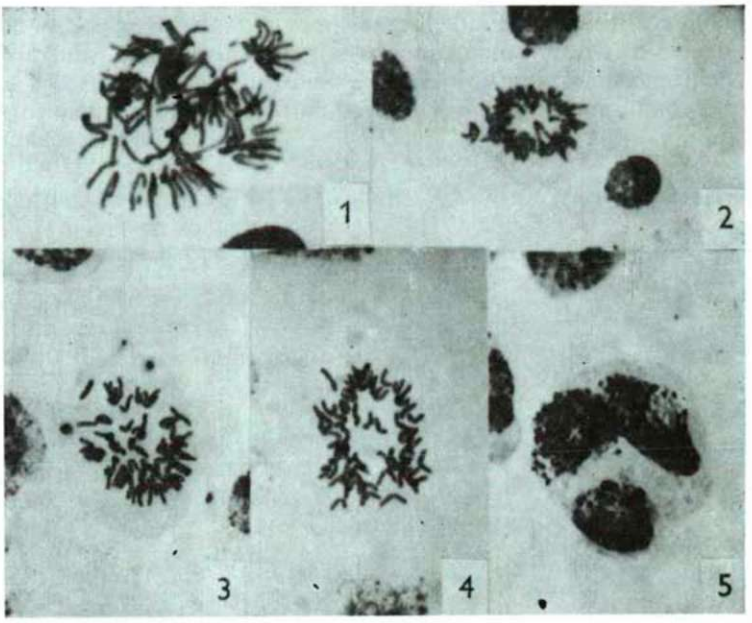
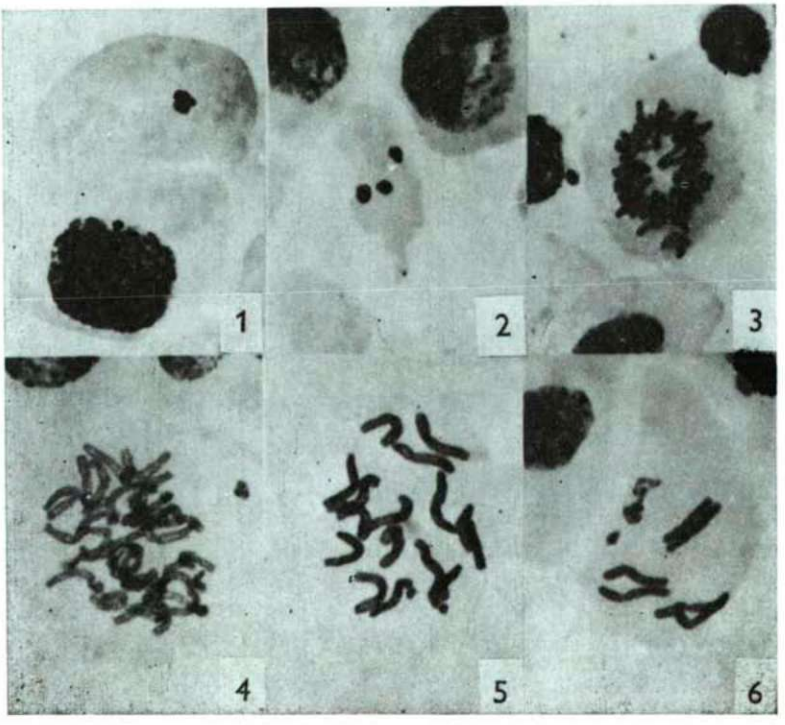


Fig. 5. Poliploid cells produced by N_2O treatment (730x)
1 (6 atm 8+15 hours)
2 and 3 (6 atm 20 hours)
4 (6 atm 26 hours)
5 (6 atm 24 hours)



containing more or less condensed chromosomes and interphase micronuclei as well. Different degree of chromosome condensation was also found, and prophase and metaphase chromosomes were visible in the same cell (Fig. 7, picture 7—9) or among metaphase chromosomes highly despiralized chromosomes were also found (Fig. 7,

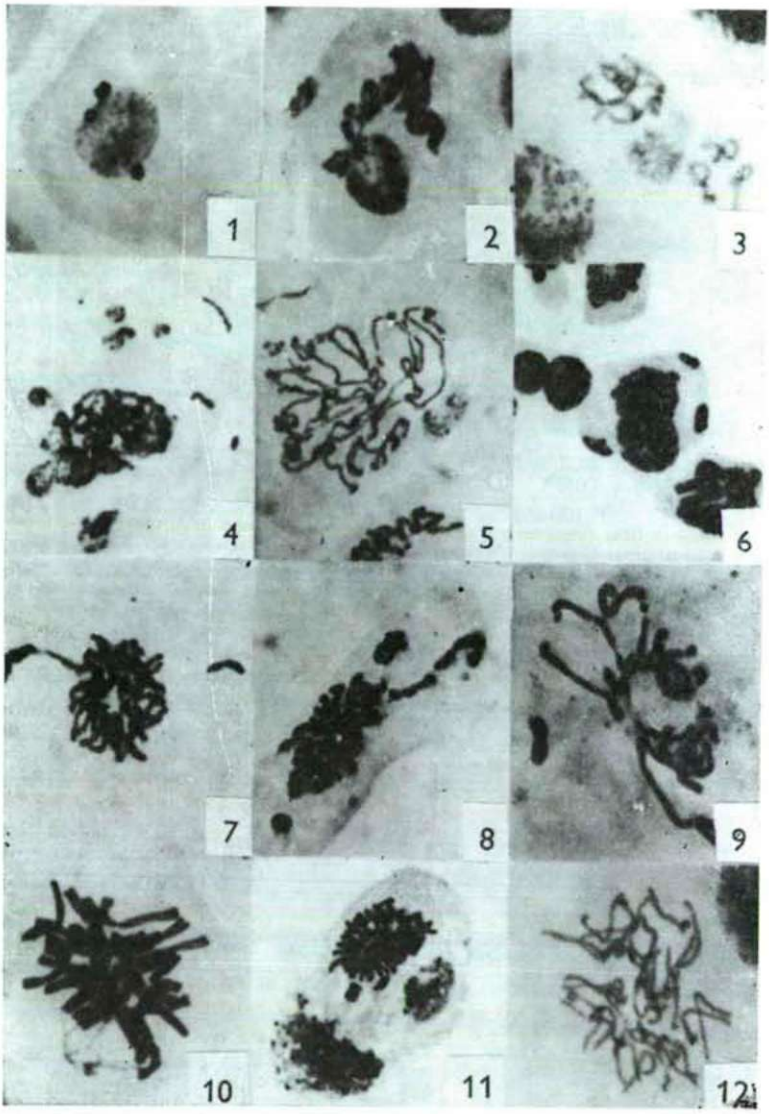


Fig. 7. Irregularities of chromatin condensation (800x)
1 and 4 (6 atm 20 hours)
2, 5, 8 and 9 (6 atm 8 hours)
3 (6 atm 8+5 hours
6 (6 atm 26 hours)
7, 10 and 11 (6 atm 12 hours)
12 (6 atm 8 hours+30 min)

picture 10). Figure 7 (picture 11) represents an assimetric polyploid cell containing both interphase and mitotic nuclei. In some cases irregular centromere division and chromatid separation was detected. On figure 7 (picture 12) a cell is shown in which

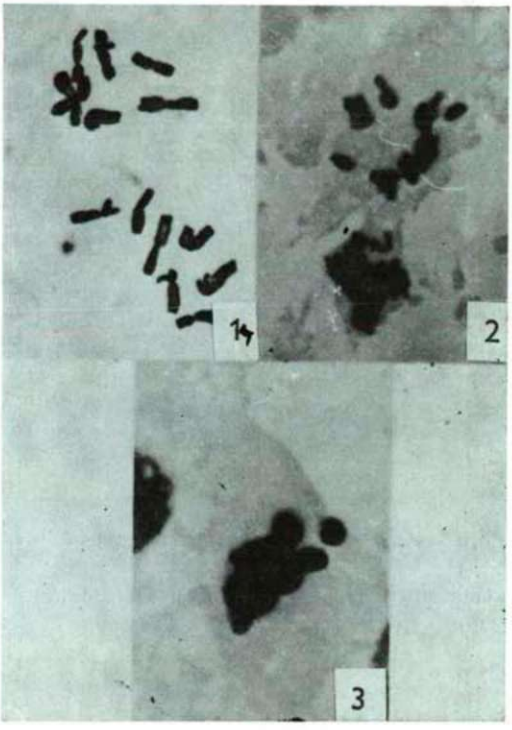


Fig. 8. Chromosome breakage (picture 1, arrow) and disintegrated nuclei or chromosomes after 6 atm 8 hours treatment (pictures 2 and 3)

the separation of chromatids and the division of centromeres are accomplished at prophase.

There is an open question whether the irregularity of chromatin condensation is a specific effect of the nitrous oxide or simply the physical consequence of the hight pressure.

It should be noted that in the early hours of the treatment, at high nitrous oxide concentrations, breakage of chromosomes (Fig. 8, picture 1) disintegrated presumably-mitotic cells (Fig. 8, pictures 2—3) were observed too. After termination of gas treatment the most frequent anaphase abnormalities were the unequal chromosome distributions (Fig. 9, pictures 1—4), anaphase bridges (Fig. 9, pictures 4—5), and chromosome separation (Fig. 9, pictures 3—6). These irregularities might also play an important role in the aneuploid formation.

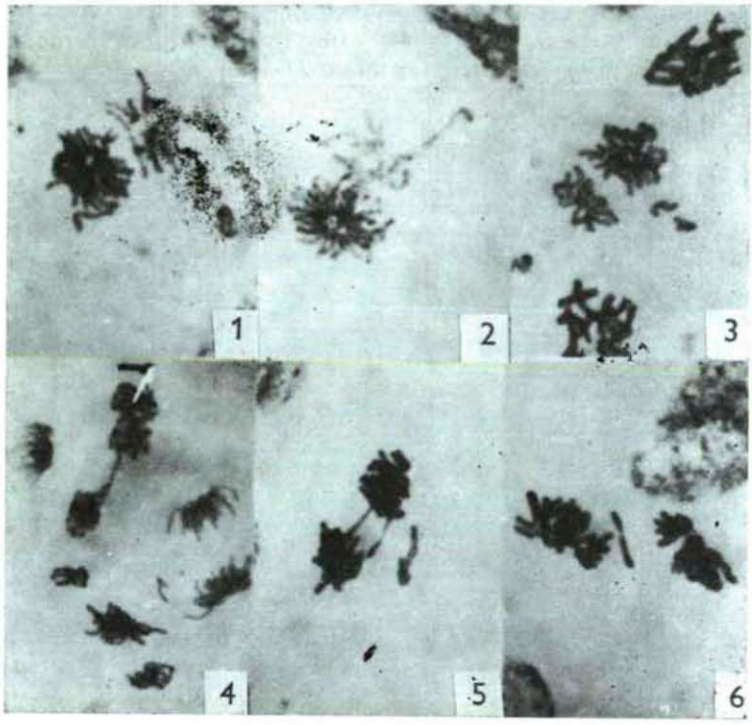


Fig. 9. Anaphase abnormalities after terminating a 6 atm nitrous oxide treatment. Magnification: 730x

References

BOLLER, K. and SCHMID, W. (1977): Chemische Mutagenese beim Sanger, Das Knochemark des Chineschen Hamsters als In-vivo-Testsystem. — Humangenetik *11*, 35—54.

DVORAK, J. HARVEY, B. L. and COULMAN, B. E. (1973): The use of nitrous oxide for producing eupolyploids and aneuploids in wheat and barley. — Can. J. Genet. Cytol. *15*, 205—215.

KIHARA, H. and TSUNEWAKI, K. (1960): Production of polyploid wheat by nitrous oxide. — Proc. Japan. Acad. Sci. *36*, 658—663.

NYGREN, A. (1955): Polyploids in *Melandrium* produced by nitrous oxide. — Hereditäs *41*, 287—290.

ÖSTERGEN, G. (1954): Polyploids and aneuploids of Crepis capillaris produced by treatment with nitrous oxide. Genetica *27*, 54—64.

ÖSTERGREN, G. (1957): Production of polyploids and aneuploids of Phalaris by means of nitrous oxide. — Hereditäs *43*, 512—516.

SUBRAHMANYAM, N. C. and KASHA, K. J. (1973): Selective chromosomal elimination during haploid formation in barley following interspecific hybridization. — Chromosoma (Berl.) *42*, 111—125.

SZELES, A. (1982): Effect of nitrous oxide on the mitosis of plant cells. — Acta Biol. Szeged. *28*, 167—179.

TSUNEWAKI, K. (1962): Production of polyploid wheat by nitrous oxide; II. N_2O-treatment during fertilization with reference to culture media. — Seken Ziho *14*, 14—20.

Address of the author:
DR. ANNA SZELES
Department of Anthropology,
Attila József University,
H-6701 Szeged, P.O. Box 660
Hungary