abnormal mitoses with unipolar spindles. This effect on mitosis appears with $1.85 \cdot 10^{-5}$ medazepam and $1.8 \cdot 10^{-4}$ M diazepam. This discrepancy could be linked to the stronger liposolubility of medazepam, as shown by the Log. P. (table 1). It is interesting to note that diazepam inhibits culture growth of *Dunaliella* in the same range of concentrations at which it prevents proliferation of several mammalian cells lines in culture[1,2,4,6]. In addition to that, Anderson et al.[2] have shown, on human fibroblasts in vitro, as we have on *Dunaliella,* that diazepam blocks centriole separation at metaphase. So it seems that the effect of diazepam on mitosis is relatively general, since it appears whatever the culture species, and that the target is the duplication and the separation of centrioles or basal bodies. Here, we have pointed out that not all the benzodiazepines have this effect, and therefore there is no direct relation between the activity of benzodiazepines on the CNS and their ability to inhibit the processing of mitosis. It is not possible at present to define the target of diazepam and medazepam in flagellate unicellular cells in relation to the formation of a unipolar spindle.

Recent reports show that 15 benzodiazepines, including diazepam and other newly synthesized ones, which inhibit the proliferation of mouse thymoma cells in culture, bind to the cells in a specific, saturable and reversible manner[4]. Scatchard analysis shows a single class of binding sites which appear as peripheral sites and are different from the specific sites of the central nervous system. The authors find a strong correlation between the binding constant of these compounds for the peripheral sites and their ED_{50} in inhibiting the incorporation of ^3H thymidine into the cells. They have concluded that these sites may be involved in the regulation of cell proliferation[4]. Our results on flagellate unicellular alga, which are situated far away from mammalian cells on the evolutionary scale, but which divide by the classical mitotic process[7], seem to indicate that diazepam and medazepam could interact with a very fundamental and widespread cellular mechanism.

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Septation and fragmentation in *Oedogonium* mitochondria as different and independent effects of dimethyl sulfoxide (DMSO) treatment[1]

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Summary. Oedogonium cardiacum Wittrock, a filamentous green alga, was treated with DMSO. The substance induced active swelling and fragmentation of mitochondria at both 5 and 7.5%. Septa were observed at 7.5% only and were not identical with the fragmentation sites. Thus it is concluded that internal partition by septa is not a prerequisite for mitochondrial fragmentation or division.

Key words. Dimethyl sulfoxide; mitochondria; mitochondrial septation; mitochondrial fragmentation; *Oedogonium cardiacum.*

The theory that new mitochondria arise by division of preexisting ones is generally accepted. Controversy exists about the possible involvement of septation, i.e. the partitioning of the matrix space by the inner membrane, in mitochondrial division or fragmentation (division under unphysiological conditions)[2]. In the present study, *Oedogonium cardiacum* was treated with DMSO, a substance that is widely used in human and veterinary medicine and serves as a solvent and as a cryo- and radioprotective agent in biological experiments[3]. Our experiments show that mitochondrial septation and mitochondrial fragmentation or division are independent from each other.

Material and methods. Oedogonium cardiacum Wittrock was obtained from the Algensammlung Göttingen (catalogue No. B 575-1a). The algae were grown vegetatively in a medium for Cyanophyceae[4]. Cytochemical localization of cytochrome oxidase was performed according to Smith[5].

For electron microscopy the cells were fixed with glutaraldehyde and OsO_4 according to the method of Kiermayer[6]. Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips 400 T and an AEI Corinth 500 electron microscope. O_2 uptake was measured in a 10 ml stirred cell, using an O_2 electrode (Yellow Spring Instruments, Biological Oxygen Monitor 53).

Chemicals were from Merck and Serva. DMSO and diamide were dissolved in the culture medium. The stock solution of 2×10^{-4} M antimycin A contained 2% DMSO.

Results. The mitochondria of *Oedogonium cardiacum* are up to several µm long and motile. Branches are frequently formed and retracted (fig. 1). Addition of culture medium containing 5% DMSO causes a transient plasmolysis[7], during which most mitochondria fragment into pieces of 1–2 µm length. Their motile behavior suggests that some fragments are linked to each other like a string of beads, parts of which are easily separated from each other during movement. The effects of 7.5% DMSO are similar. Many cells do not survive the deplasmolysis which immediately follows the plasmolysis. In the case of 5% DMSO about 26% and in 7.5% DMSO more than 50% of the cells die. Those which survive this phase are viable in DMSO for several days at least. The rate of fragmentation, i.e. the proportion of surviving cells in which all of the long mitochondria have divided into pieces of maximally 2 µm length, is about 75 (5% DMSO) and 88% (7.5% DMSO) respectively (64 and 96 cells examined). O_2 uptake is 78 and 65% of the control. Thus, it is almost unchanged when the corresponding mortality is taken into consideration.

DMSO up to 7.5% affects only the ultrastructure of mitochondria. Other parts of the cytoplasm appear normal. The fine structure of a control mitochondrion is shown in figure 1. Treatment with 5% DMSO for half an hour leads to a marked swelling of mitochondria which is statistically significant (data not shown, fig. 2). Constrictions are abundant. After 30 min treatment with 7.5% DMSO the matrix space is further enlarged. The cristae appear elongated and flattened with narrow intracristal spaces. Most conspicuous is the appearance of internal septa in about 80% of the mitochondria. The septa are independent of the constrictions which are also abundant (figs 3

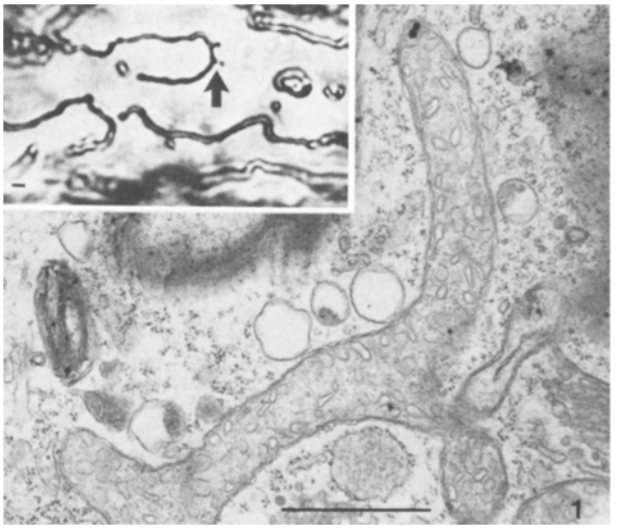

Figure 1. Elongated, branched control mitochondrion of *Oedogonium cardiacum*. Inset: Control mitochondria which were stained for cytochrome oxidase in the light microscope. The arrow indicates a constriction. Bar: 1 μm.

and 4). Electron dense structures at the origin of septa do not occur[8] and therefore septation probably takes place by fusion of parts of the inner membrane. Septa have never been observed in control mitochondria or in mitochondria treated with 5% DMSO.

Addition of 10^{-3} M cysteine to the DMSO solutions does not influence the results described above, i.e. the mitochondria are fragmented and swollen at 5% DMSO and fragmented, swollen and septated at 7.5%. Diamide up to 10^{-1}% does not affect the ultrastructure of mitochondria except for a slight swelling at the highest concentration. Antimycin A (7×10^{-4} M) prevents both swelling and septation.

Discussion. The most striking outcome of this work is the demonstration that mitochondrial septation occurs independently of mitochondrial fragmentation. This is shown clearly by the fact that fragmentation was observed both at 5 and 7.5% DMSO but septation only at 7.5% and that the fragmentation sites (constrictions) were not identical with the septation sites. These results and others concerning a rapid induction of mitochondrial septa in muscle or rhizodermal cells under pathological conditions and especially during swelling[8-11] indicate that septation merely reflects general cell damage[12] and is no prerequisite for mitochondrial division as suggested for mitochondria of mammalian tissues, protozoan or root tip cells[13-18]. It remains to be determined whether mitochondrial fragmentation during DMSO treatment is the same as division under normal conditions. The fact that cells with 'fragmented' mitochondria respire at a nearly normal rate and survive for several days or weeks means that fragmentation in this case is at least similar to mitochondrial division. Thus the *Oedogonium* mitochondria divide probably by constriction only, which is in accordance with the findings in control cells. Another effect of DMSO was a statistically significant swelling at both 5 and 7.5%. This is obviously not a specific effect because other authors have observed a condensation of the mitochondrial matrix[19,20]. The enlargement of the matrix space in *Oedogonium* probably has something to do with the increased membrane permeability caused by DMSO (for references see 3,21,22) although this is doubted by other authors (e.g. 23). The swelling is active because it can be prevented by antimycin. Swelling agents are probably released from other cell compartments[24]. The role of calcium uptake has still to be tested[8]. In any case, the swelling is not due to an oxidation of thiol groups within the membranes, as suggested for liver mitochondria[25], because DMSO acts as a reducing agent[3]. Accordingly, we did not observe any considerable swelling during treatment with the thiol oxidising agent diamide[25], and addition of cysteine to the DMSO solutions did not alter the results obtained with DMSO.

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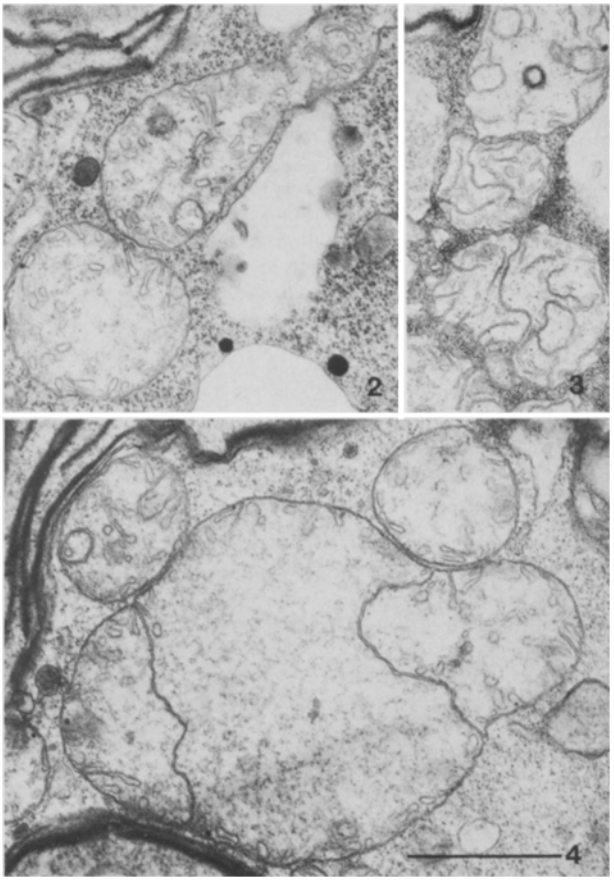

Figure 2. Cell treated with 5% DMSO for 30 min. The mitochondria appear swollen. Constrictions (upper right side) are visible.

Figures 3 and 4. Treatment with 7.5% DMSO for 30 min. The cristae are elongated but flattened. Septa are frequent and the matrix space is further enlarged. Bar: 1 μm.

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Karyotypic races of the common shrew (*Sorex araneus* L.) from northern Poland

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Summary. Karyotypic races of the common shrew which differed with respect to the combinations of chromosome arms in certain 2-armed autosomes were distinguished in Poland. Two eastern races with the arm combination *ik*, and one western race with the arm combination *hi* in the third pair of autosomes were established. In the contact area of these chromosomal forms, the fourth karyotypic race with the arm combination *hk* was found.
Key words. Shrew; *Sorex araneus;* karyotypic race.

The description of the G-band pattern for the chromosomes of *Sorex araneus* L. greatly facilitated differentiation of particular pairs of chromosomes and comparison of the karyotypes of shrews from different populations. It was found that out of 9 pairs of autosomes in *S. araneus*, 6 exhibit karyotypic polymorphism of the Robertsonian type. Up to the present time descriptions have been given of a large number of karyotypic races in different parts of the range of this species. They are known from England[2], from Sweden and Finland[3,4], from the FRG[5], from Czechoslovakia[6,7], from Switzerland[8] and from Western Siberia[9,10]. Two races have been described from Poland: one in the east and northeast[4,11–13] and the other in the north[13]. Further investigation should yield a more exact understanding of the problem of differentiation in the karyotype of the common shrew in Poland. The results of the first stage of these studies are presented below.

Material and methods. Examination was made of the karyotypes of 21 common shrews caught in six localities in northern Poland in 1984 (table 1, fig. 6). Mitotic chromosome preparations were immediately made on the spot from the spleen by the conventional air-drying technique, and stained with Giemsa. G-bands were obtained by digesting the preparations in 0.25% trypsin solution, using the method of Seabright[14], with some modifications. The chromosomes of all the animals used in this study were examined by means of G-bands. The particular arms of 2-armed chromosomes and 1-armed chromosomes were given letters *a–u*, after Fredga and Nawrin[4].

Results. The karyotypes of the shrews examined differed with respect to the number of autosomes (2- and 1-armed), the occurrence of heterozygous pairs of autosomes and the arm combinations in 2-armed autosomes.

Metacentric pairs of autosomes formed of the following arms: *af, bc, jl, tu* and sex chromosomes were unvarying in the karyotypes of the shrews examined. Chromosome arms indicated by the letters *g-r* (with the exception of the elements *j* and *l*) were subject to variation. Various combinations of these arms were found in the metacentric autosomes of shrews from northern Poland.

Table 1. Number of animals from localities in northern Poland used for the chromosome studies

No. of locality	Locality	Geographic coordinates of localities	No. of animals studied
1	Popielno	N 53°45′, E 21°37′	6
2	Łęgucki Młyn	N 53°47′, E 20°08′	1
3	Krzewsk	N 54°05′, E 19°28′	1
4	Laska	N 53°55′, E 17°34′	1
5	Słupsk	N 54°29′, E 17°02′	5
6	Stobnica	N 52°42′, E 16°36′	7

Shrews from Popielno were characterized by metacentric autosomes with a combination of chromosome arms *ik, gr, hq* and *mn* (polymorphic pair). The *hq* combination occurred in 2 members of this sample, but in 4 individuals the elements *h* and *q* were not joint and occurred in the form of acrocentrics. Elements *o* and *p*, on the other hand, always occurred as acrocentrics (table 2, fig. 1).

The karyotype of the female caught at Łęgucki Młyn, unlike the preceding, was characterized by the presence of metacentrics *hk* and *io*. In this case also metacentrics were found with the combinations of *gr* (heterozygous pair) and *mn* arms, while only elements *p* and *q* occurred as acrocentrics (fig. 2).

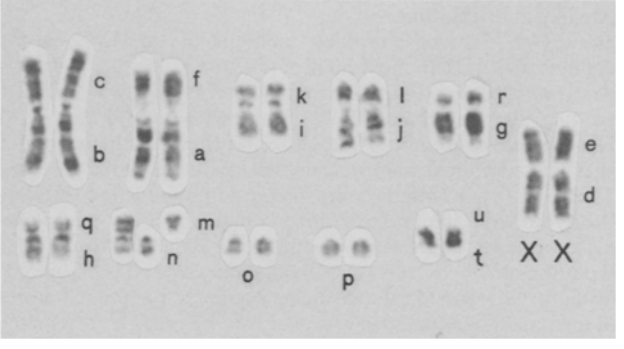

Figure 1. Karyotype of a heterozygous female (2n = 23) from Popielno.

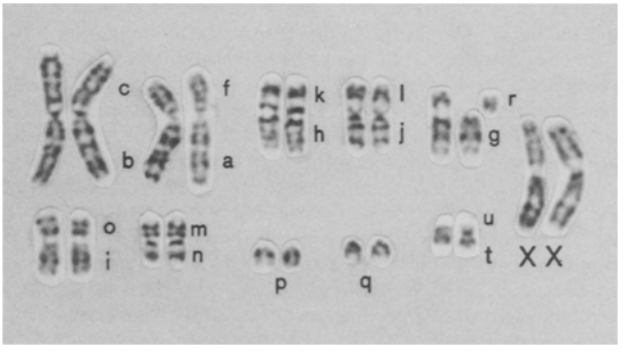

Figure 2. Karyotype of a heterozygous female (2n = 23) from Łęgucki Młyn.