

repeated twice on the same chloroplast, it suffered deformation and ceased responding to further changes in osmotic pressure.

The same result was also observed in spinach chloroplasts.

In our early experiments<sup>6,7</sup>, it was confirmed by spectrophotometry that the swollen chloroplasts never reverted to the initial state when the suspending medium was replaced by a hypertonic solution. However, the objects which were called 'swollen chloroplasts' in the early experiment did not become balloons, but appeared as granular flattened bodies which were produced by a weak solution of salts or sugars. In such weak solutions chloroplasts never form balloons. It can therefore be concluded from the present experiments that reversible swelling is observed only in the balloon-forming chloroplasts.

Photomicrographs of the grana particles suspended in distilled water are shown in Figure 3. Deformation to perfectly round particles (diameter about 0.2–1  $\mu$ ) occurs as a result of absorption of water. The large particles

probably originate in the stroma lamellae and small ones in the grana lamellae. From this experiment it is supposed that grana particles (grana and stroma lamellae), like chloroplasts, are enclosed by a semipermeable membrane.

*Zusammenfassung.* Isolierte Chloroplasten von Spinat und Nitella in Aqua dest. zeigen infolge Membranerweiterung 2 verschiedene Typen ballonförmiger Chloroplasten. Die Erscheinungen bei Grana sind ähnlich. Es wird die osmotische Natur des ballonförmigen Chloroplasten beschrieben.

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<sup>6</sup> K. NISHIDA, *Plant Cell Physiol.* 4, 247 (1963).

<sup>7</sup> K. NISHIDA and K. KOSHII, *Physiol. Plant.* 17, 846 (1964).

### Comparative Efficiency of Arsenicals in Modifying Effects of Ethyl Methane Sulphonate (EMS) on Barley Chromosomes

In a preliminary investigation on barley and broad bean, some thiol-inhibiting substances were demonstrated as enhancing the aberration rate produced by EMS on chromosomes<sup>1</sup>. Tested substances were mercuric chloride and neoarsphenamine (sodium salt of *m*-diamino-*p*-dioxo-arsenobenzene methylene sulphylic acid). Although it is known that arsenicals and other thiol-inhibiting substances are by themselves deprived of any mutagenic activity, it remains to utilize these substances as modifiers of the mutagenic action of the highly mutagenic alkylating agent EMS. It was evident from these preliminary experiments that some –SH bearing enzymes are involved in some way in the mechanisms by which EMS produces chromosome aberrations. Since only one arsenical was tested, the question remained whether some modifications of the arsenical molecules could lead to varied efficiency.

In the present experiment, the following arsenicals were used:

Pentavalent: (1) *p*-aminophenylarsinate of Na or Atoxyl (Hoechst), (2) 3-acetyl-amino-4-hydroxy-phenyl-arsonic acid or Spirocid (Hoechst) or Stovarsol, (3) *p*-hydroxyphenylarsinic acid or oxarsanilic acid (Hoechst);

Double bond: (1) Na salt of *m*-diamino-*p*-dioxo-arsenobenzenemethylenesulphylic acid or Neosalvarsan (Hoechst) or neoarsphenamine, (2) 2-Na propionate of di-( $\beta$ ,  $\gamma$ -dioxopropyl)-aminophenol-4'-arsino-5'- $\beta$ -(benzoxazolyl-[2]-mercaptol) or Spirotrypan (Hoechst);

Trivalent: 3-amino-4-hydroxy-phenyl-dichlorarsine hydrochloride or Clorarsen (Squibb).

The lay-out of the experiment has been modified with reference to the results of the previous experiment, in which the optimum effect was found to be strongly dependent on the concentrations of EMS and arsenical, duration of treatments, pH and temperature during and after treatment and the time of presoaking the seeds. Dry and 30 h presoaked barley seeds (caryopsis) of var. piroline were treated for 2 h with each arsenical at  $1 \cdot 10^{-3} M$ .

After washing with running bidistilled water, seeds were immersed in a solution containing 0.3 g EMS per 100 ml bidistilled water for 3 h. During all the treatments, pH was adjusted at 8 with Sørensen buffer or citrate-carbonate for Clorarsen and the temperature was maintained at 22°C. Seeds were germinated as indicated in a previous paper<sup>2</sup>.

Chromosome aberrations were investigated in both metaphases and anaphases of the first mitotic cycle after germination. It seems that after all treatments, delayed effects on chromosomes do not occur.

The different aberrations induced by various treatments could be clearly distinguished in two groups.

The first group observed after treatment of both dry and presoaked seeds consists mainly in aberrations of the chromosome class both monotypic and ditopic. A low proportion of chromatid aberrations was observed.

The second class of aberrations consists of subchromatid types, i.e. breaks and reunions, appearing only with presoaked seed treatments. It has to be mentioned that minutes (smaller than 1  $\mu$ ) sometimes occurred after combined treatments but never in large amount, so we could discard them in the present study. Figures 1 and 2 compare the results obtained with the different compounds.

From the pooled data of all kinds of aberrations it can be seen that: (1) all the arsenicals are by themselves deprived of any significant activity on chromosomes. Arsenic ions are of low toxicity to plant cells, which corroborates previous findings by VON ROSEN<sup>3</sup>; (2) with combined treatments, there is a synergistic action between each arsenical and EMS. Previous findings with neoarsphenamine are thus confirmed<sup>1</sup>; (3) the amount of aberrations is generally lower at anaphase, probably due to aberration elimination. This elimination seems to work differently for each treatment; (4) in all cases but one (Spirotrypan) seeds are more sensitive when presoaked.

<sup>1</sup> J. MOUTSCHEN and N. DEGRAEVE, *Exper.* 21, 200 (1965).

<sup>2</sup> J. and M. MOUTSCHEN-DAHMEN, *Exper.* 19, 144 (1963).

<sup>3</sup> G. VON ROSEN, *Hereditas* 40, 258 (1954).

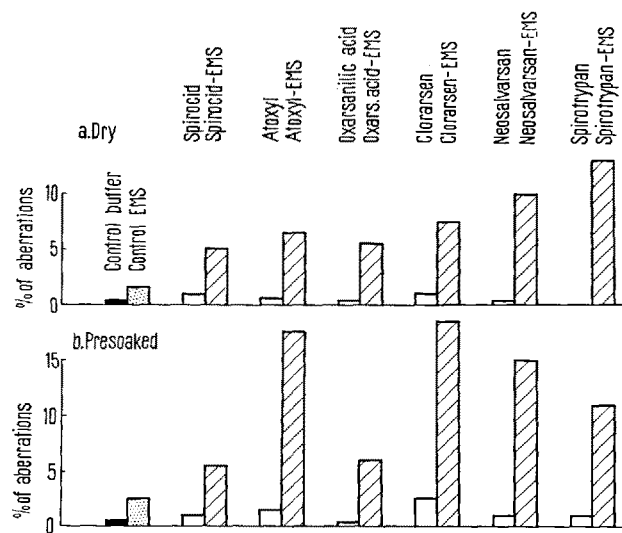


Fig. 1. % of aberrations recorded at metaphase (200 metaphases analysed in each case). Concentrations: arsenical  $1 \cdot 10^{-3} M$ , EMS 0.3 g/100 ml. a, Dry seeds treated. b, 30 h presoaked seeds. Striped: combined treatment.

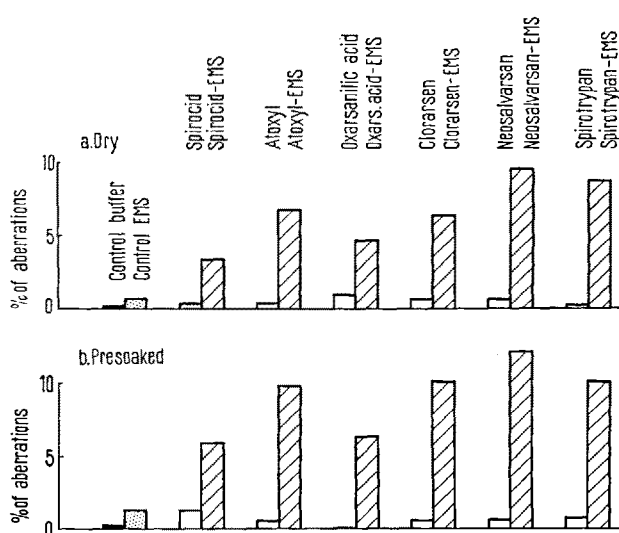


Fig. 2. % of aberrations recorded at anaphase (600 anaphases analysed in each case). Concentrations: arsenical  $1 \cdot 10^{-3} M$ , EMS 0.3 g/100 ml. a, Dry seeds treated. b, 30 h presoaked seeds. Striped: combined treatment.

The exception can probably be imputed to sampling errors. The increased effect can be attributed to the appearance of aberrations of the subchromatid class.

From the comparative efficiency of the arsenicals, and considering all the criteria together, viz. metaphase and anaphase effects and increased effects after presoaking, the following conclusions can be drawn: (a) the pentavalent compounds (Spirocid, Atoxyl and Oxarsarnilic acid) are less efficient; (b) the efficiency of the double bond compounds (Neoarsphenamine and Spirotrypan) is higher and also seems to be more regular than the effects of pentavalents; (c) the efficiency of the trivalent compound (Clorarsen) can in some respects be compared with the double bond compounds.

These findings give strong indications that the behaviour of the arsenicals in plant tissues is in many respects similar to that in higher animal tissues<sup>4</sup>. However, all the substances tested are definitely less toxic for plant tissues.

The extension of previous experiments with thiol-inhibiting substances has proved the implication of specific -SH enzymes in chromosome rejoining processes.

The possible implication of other enzymatic systems is under investigation<sup>6</sup>.

**Résumé.** Des semences d'orge ont été traitées par 6 arsén oxydes (3 pentavalents, 1 trivalent, 2 molécules à doubles liaisons) avant d'être traitées par une solution de méthane sulfonate d'éthyl (EMS) à 0,3 g par 100 ml/3 h. Ces substances accroissent considérablement les taux d'aberrations des chromosomes normalement produits par l'EMS. Il s'agit principalement d'aberrations de types chromosomique et subchromatidique. Les dérivés trivalent et à double liaison sont les plus actifs. Le problème de l'inhibition d'enzymes à fonction -SH intervenant dans la genèse de ces aberrations est discuté.

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<sup>4</sup> J. F. DANIELLI, *Cell Physiology and Pharmacology* (Elsevier Publ. Co., New York 1950).

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### Effect of Angiotensin on the Cardiac Arrhythmias Induced by g-Strophanthin

Recently a number of papers have been published concerning the antiarrhythmic activity of some biologically active polypeptides. PANISSET and BEAULNES<sup>1</sup>, and

BRODEUR and BEAULNES<sup>2</sup> have shown that oxytocin and lysyl<sup>8</sup>-vasopressin prevent cardiac arrhythmias in anaesthetized dog, induced by chloroform and adrenalin. BEAULNES et al.<sup>3,4</sup> have observed the same effect with angiotensin. These authors have shown that in vitro

<sup>1</sup> J.-C. PANISSET and A. BEAULNES, *Rev. Canad. Biol.* 20, 47 (1961).

<sup>2</sup> J. BRODEUR and A. BEAULNES, *Rev. Canad. Biol.* 23, 37 (1964).

<sup>3</sup> A. BEAULNES, G. GARIEPY, J. BRODEUR, and E. BELTRAMI, *Fed. Proc. (Abstr.)* 23, 121 (1964).

<sup>4</sup> A. BEAULNES, J.-C. PANISSET, J. BRODEUR, E. BELTRAMI, and G. GARIEPY, *Circulation Res.* 14, 11 (1964).