On the Balloon-Formation of Isolated Chloroplasts and Grana

Since the study of KNUDSON[1] on isolated chloroplasts, it has been found that when isolated chloroplasts are suspended in distilled water or a dilute solution of sugar they exhibit bubble-like protrusions (blebs) developing from their surface[2,3]; sometimes each chloroplast becomes a round bleb (balloon) as a result of the extension of its membrane[2–5].

In our studies on the swelling of isolated chloroplasts the balloon-formation described above was observed in two different types with a microscope, and it was clearly shown that the balloons thus formed quickly reverted to their initial state when the suspending medium was replaced by a hypertonic solution. In addition to this, grana particles isolated by centrifugation assumed the shape of balloons as did chloroplasts. The present paper reports the result of these experiments.

Spinach leaves were homogenized for 15 sec in a mixer with a 0.5 *M* sucrose solution buffered with 0.04 *M* Tris (pH 7.4). The brei was then filtered through cloth and the chloroplasts were separated from the filtrate by centrifugation at 1000 *g* for 7 min. The grana were sedimented from the supernatant by centrifugation (2000 to 5000 *g*). All the processes described above were carried out at 0–3°C.

The balloons were formed by the addition of a small number of chloroplasts or grana to distilled water placed on a glass slide. They were stirred for a few seconds with a glass rod, and then observed microscopically (× 1500).

In experiments with nitella cells, the chloroplasts were isolated in an apparently undamaged condition by excision of one end of an internodal cell followed by gentle scraping of the cell wall to expel the cell contents into distilled water.

Figure 1 shows the balloon-formation of spinach chloroplasts. The vast majority of them show that the grana and stroma are spread over the inside of the balloon, and in a few cases the interior is empty as a result of diffusing out of the contents into the suspending medium. Figure 2 shows the chloroplasts of nitella cell which have turned into balloons. As a result of absorption of water, the membrane has become distended, and both the grana and stroma have moved to a peripheral part of the balloon. The type of balloon-formation observed in spinach was scarcely found in nitella.

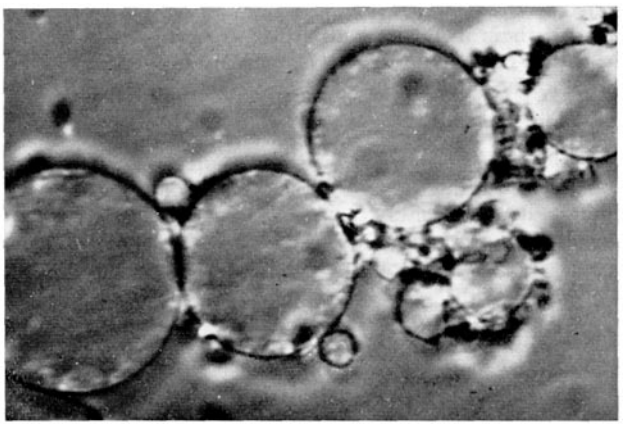

Fig. 1. Balloon-formation of isolated chloroplasts of spinach. The small balloons probably originate from grana particles. × 1500. Phase-contrast microscope.

Balloon-formation of nitella chloroplasts seems to be conditioned by many factors. In some cases the chloroplasts isolated from the upper internodal cell form balloons with less difficulty than those from the lower internodal cell. The chloroplasts obtained from nitella cells which have been kept in the dark for 1–2 days form a balloon quite easily, while the cells collected from ponds in the daytime and not kept in the dark show extreme difficulty in the formation of balloons.

When the suspending medium was replaced with 0.5 *M* sucrose, the balloons at once reverted to the state of the original chloroplasts. When such reversible changes caused by distilled water and hypertonic solution were

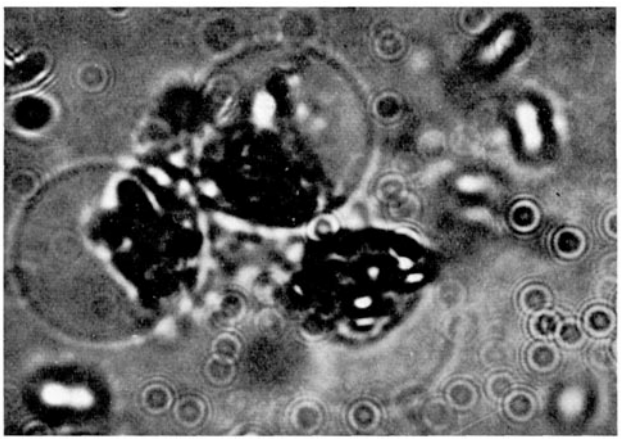

Fig. 2. Balloon-formation of isolated chloroplasts from a nitella cell. × 1500. The contents of the chloroplasts (probably grana, stroma, and starch grains) seem to have been pressed to the sides of the balloons.

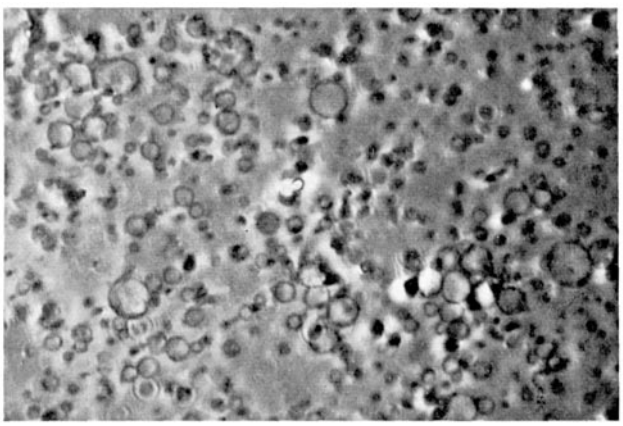

Fig. 3. Balloon-formation of isolated grana particles of spinach. × 1500.

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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[6,7], 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 μ) 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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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[1]. Tested substances were mercuric chloride and neoarsphenamine (sodium salt of *m*-diamino-*p*-dioxy-arsenobenzene methylene sulphoxylic 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-acetylamino-4-hydroxy-phenylarsonic acid or Spirocid (Hoechst) or Stovarsol, (3) *p*-hydroxyphenylarsinic acid or oxarsanilic acid (Hoechst);

Double bond: (1) Na salt of *m*-diamino-*p*-dioxy-arsenobenzenemethylenesulphoxylic acid or Neosalvarsan (Hoechst) or neoarsphenamine, (2) 2-Na propionate of di-(β,γ-dioxypropyl)-aminophenol-'4-arsino-5'-β-(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[2].

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 monotopic 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 μ) 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[3]; (2) with combined treatments, there is a synergistic action between each arsenical and EMS. Previous findings with neoarsphenamine are thus confirmed[1]; (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.

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