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# EXPERIENCE WITH THE HYDROXYQUINOLINE SMEAR METHOD

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Since several years LEVAN and co-workers studied the action of various chemicals exhibiting c-mitotic activity in chromosomes. Apart from their importance for the explanation of the mechanism of c-mitotic action, these experiments are significant from two other points of view. 1. Though various chemicals are known to possess an action on chromosomes resembling that of colchicine, colchicine is practically the only substance widely used to induce polyploidy. The superiority of colchicine is due to its low threshold of activity, combined with high water solubility and the absence of poison effect within a wide concentration range. However, it is not impossible that continued experiments may lead to the discovery of a substance with c-mitotic action as efficient as colchicine but cheaper in production (LEVAN and ÖSTERGREN, 1943). 2. Structures revealed after fixation of the chromosomes under definite conditions may contribute to the study of chromosome morphology. Thus, certain pretreatments may be found which facilitate the understanding of problems of chromosome morphology and physiology (LEVAN, 1949).

Though the first of these possibilities has not yet been realized, the second was so by the recent discovery of TJIO and LEVAN (1950 a and b) that pretreatment with 8-hydroxyquinoline (hitherto known as a bacteriostatic substance and a chemotherapeutic agent for vascular diseases of plants) yields chromosome pictures of exceptional clearness. According to TJIO and LEVAN (*l.c.*), it possesses a virtue above any of the chemicals studied by them before in that the metaphase chromosomes, though spread out due to the inactivation of the spindle, remain in their original position in the equatorial plate. The heterochromatine is clearly differentiated at all stages and the centromeric apparatus exhibits unusually clear conditions, four distinct centromeric bodies being visible in prophase-metaphase chromosomes.

In this laboratory, since the beginning of the year, we used hydroxyquinoline pretreatment to study the somatic chromosomes in species of the genera *Allium*, *Lactuca*, *Secale*, *Solanum* and *Vicia*. The treatment results in contraction (Fig. 1) and spreading of the chromosomes. Therefore, it is particularly useful in carrying out chromosome counts in species with small and crowded chromosomes as those of the genus *Solanum* (Fig. 2). The prophase chromosomes are usually very distinct, which facilitates a detailed study of their structure (Fig. 3). The characteristic features of the chromosomes, like primary and secondary constrictions are clearly visible. It soon became evident that the method can be used with advantage

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to determine the chromosome numbers in young leaves and we have employed it to locate the doubled plants in polyploidization experiments with *Forsythia ovata*, *Populus spec.*, *Spinacia oleracea*, *Lactuca sativa* and a number of species and species hybrids in the genus *Solanum*. The results of these experiments will be published separately. In two octoploid *Forsythia ovata* plants produced by colchicine treatment, there was not much difficulty in counting the 104 chromosomes in leaf smears prepared after hydroxyquinoline pretreatment, even at the prophase stage. However, during these studies we usually followed a procedure slightly different from that used by TJIO and LEVAN (1950, b) but analogous to the method suggested by GERSTEL (1949) for the preparation of smears after pretreatment with colchicine. GERSTEL's method comprises the use of hydrochloric acid as a combined fixing and macerating agent and acetic orcein staining, after a pretreatment with 0,2 to 0,3 % colchicine for the shortening and spreading of the chromosomes. No doubt, this method gives good results, but it is rather expensive in large scale work. Hence, we replaced colchicine by hydroxyquinoline ($C_9H_6NOH$) in that schedule.

Our method runs as follows:

1. Place the root tips or the basal portion of young leaves for 3 to 4 hours in a 0,002 M per litre solution of 8-hydroxyquinoline (0,29 gr/l in distilled water; gentle heating will be necessary for complete solution).

2. Transfer the material to warm ($\pm$ 60 °C) 1 : 10 aqueous dilution of concentrated hydrochloric acid; after 10 minutes rinse well in water to stop hydrolysis.

3. Place the material in a watch glass containing 1 % acetic orcein (2 % in the case of leaves) and warm it gently over the flame, taking care not to boil the solution. Leave the material in the stain for 5 minutes and then smear in a drop of the stain. For making the smear only the meristematic portion of a root tip or a small basal bit of a leaf is required. Subsequently the slides can be made permanent by any of the prevalent methods, our method being that of MCCLINTOCK.

An advantage of the procedure described is that a relatively slight, harmless pressure is sufficient to get a uniform spreading of the cells, while continued action of hydrochloric acid on the tissue is prevented. The method is particularly useful in cases in which a thorough maceration is essential to get the cells well spread, as in leaf smears of various plants and in root tip smears of *Gramineae*. It is a rapid method which can be adopted in extensive work using a suitable apparatus for the simultaneous treatment with hydrochloric acid of a large number of objects. We may, however, add that with this method the centromeric gap was usually narrow and consequently we could not observe the quadruple structure of the centromere.

As has been pointed out by TJIO and LEVAN (1950, b), the concentrations of 8-hydroxyquinoline applied exhibit radiomimetic and c-mitotic effects. SIMONET and IGOLEN (1944), studying the effect of a number of quinoline and isoquinoline derivatives on mitosis in *Hordeum distichum*, found that 5-methylquinoline, 5-isopropyl-8-methylquinoline and 6-butyl-8-methylquinoline possess distinct colchicine effects. Soon after starting our experiments with hydroxyquinoline pretreatment, we noted the presence of typical a-polar c-anaphases in a number of plates of *Lactuca sativa* and *Allium Cepa* (Figs. 4 and 5). In *Allium Cepa* roots, treated with a 0,002 M solution for 12 hours a few cells with 32 dividing chromosomes were noted (Fig. 6). Fragments, pseudo-chiasmata and anaphase bridges were observed now and then (Figs. 7 and 8).

In order to find out whether it is possible to induce polyploidy with 8-hydroxyquinoline, we treated seeds and young seedlings of *Lactuca sativa* and *Spinacia oleracea* with different doses; the results were negative. Similar experiments carried out by the present authors and also by Ir A. F. M. BROEKMANS at this Laboratory with *Allium*, showed that the range between the threshold of activity and the lethal effect is very narrow which will probably render this substance unsuitable to induce polyploidy.

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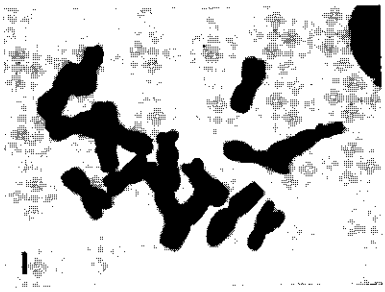
## EXPLANATION OF FIGURES

Figures 1 – 8. Root tip chromosomes treated with 8-hydroxyquinoline, fixed in hydrochloric acid and stained with acetic orcein (× 1100).

Fig. 1. *Allium Cepa* (2n = 16). Note the extreme contraction of the chromosomes.
Fig. 2. *Solanum tuberosum* (2n = 48). The spreading effect is clearly seen.
Fig. 3. *Allium Cepa.* Prophase.
Fig. 4. *Lactuca sativa.* (2n = 18).
Fig. 5. *Allium Cepa.* In both figures 4 and 5, the centromeres in the left cell are still undivided, those in the right cell have just divided.
Fig. 6. *Allium Cepa.* A tetraploid cell in a smear prepared after treating live roots with 0,002 M solution for 12 hours.
Fig. 7. *Allium Cepa.* Terminal sticking at anaphase resulting in a false bridge.
Fig. 8. *Allium Cepa.* Pseudo-chiasmata, giving the appearance of rod and ring bivalents.
The preparations in figures 7 and 8 were made from roots treated with 0,002 M solution for 6 hours.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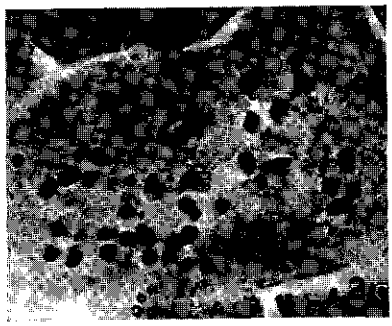
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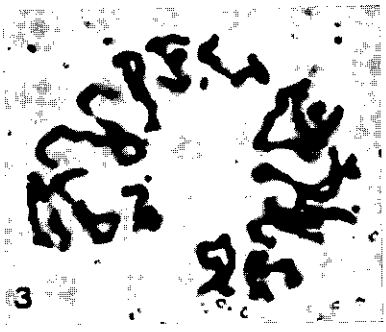
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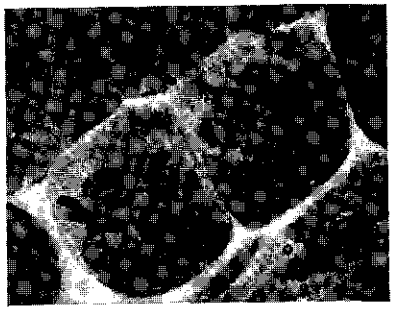
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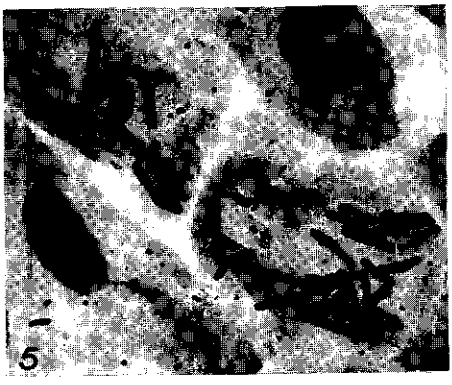
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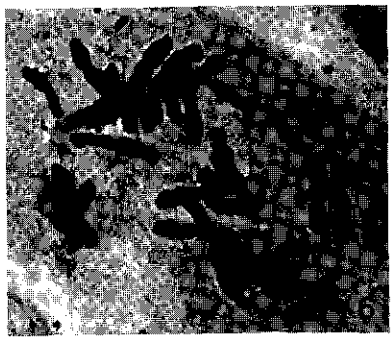
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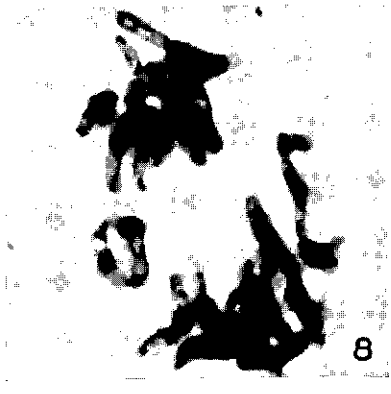
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