Studies on Polyploid Plants.

Irregularities in the Mitosis and Polyploidy Induced by Colchicine and Acenaphthene.

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THE effect of colchicine upon the behaviour of the nucleus was shown by Dustin,[1] Lits,[2] and others (*cf.* Chadkovski[3]). Blakeslee and Avery,[4] and Nebel[5] induced even polyploidy in various plants. I tested the effect of colchicine upon the plant tissues. Seeds from *Triticum vulgare*, *Tr. durum*, *Tr. timopheevi*, *Tr. monococcum*, *Tr. persicum*, *Secale cereale*, *Zea mays*, *Oryza sativa*, *Canabis sativus*, *Gossypium herbaceum*, *Vicia sativa*, *Vicia Faba*, *Nicotiana* species and species hybrids, *Beta vulgaris*, etc. were soaked in 0·5% aqueous solution of colchicine overnight and then transferred to Petri dishes for germination. Some were washed in running water after the treatment, others were not. Potato tubers were also included in the experiments.

Germination of the seeds was highly suppressed, the seedlings got enormously swollen, roots remained short but very thick. Those that were washed in running water after treatment, grew somewhat better than the others.

The effect of colchicine as revealed by the cytological studies was: (1) Enlargement of the cells and nuclei. (2) Abnormal nuclei as in plant galls [6–9] and in cancer. (3) Multinucleation (the nuclei were in some cells equal, in others unequal). (4) Increased number and occasionally the size of the nucleoli. (5) Abnormal mitosis. (6) Polyploidy. (7) Heteroploidy was rarely found.

The appearance of polyploid cells (tetraploid, octaploid and even of higher range) was the most striking phenomenon. In some cells 4, 5, 6 and occasionally even more nuclei were found. Sometimes micronuclei were also observed. These phenomena were found in all plants treated and studied cytologically.

Chromosome multiplication obviously proceeds as a result of chromosome division but failure of separation. The chromosomes appeared during the metaphase usually straight, somewhat mummified, probably highly dehydrated, sometimes even shortened and disorderly scattered in the centre of the cell, *i.e.*, the place usually occupied by the nucleus (or nuclei). They do not form a normal equatorial plate; regular spindle fails; this offers some difficulties in exact counts of the chromosomes when they are several times multiplied. It seems that colchicine suppresses the activity of the factors that regulate the process of chromosome arrangements in equatorial plate, the formation of spindle and of chromosome separation. Cytology and histology of plant tissues treated with colchicine is similar to that of animal cancer, plant galls and tumors.[6–9]

In connection with the work carried out with colchicine in our Institute, attempts were made to find agents that act in the way colchicine acts upon the plant cell. Prof. A. Schmuck who is in charge of the Biochemical Laboratory of the Institute of Genetics tested a series of chemicals. When wheat grains were covered with crystals of acenaphthene, the germinating seeds reacted morphologically in the way they reacted to colchicine. I started then cytogenetic work with this reagent.

Soaking seeds of *Triticum vulgare*, *Tr. monococcum* and *Secale cereale* in saturated aqueous solution of acenaphthene with crystals in excess during two days and then transferred to Petri-dishes and watered with the same solution and adding some crystals, we found that the seedlings react morphologically in the same way, as they react to colchicine solution. Embryos got enormously swollen and grew very slowly, the small roots became very thick, but grew very slowly (Fig. 1).

In studying cytologically the root tips and the embryos, the following peculiarities were noted.

1. *Cells.*—The majority of the cells of the seedlings treated with acenaphthene solution are much larger than in normal seedlings.

2. *Nuclei.*—A number of cells have two or more than two nuclei. Sometimes they are not equal in size. A very large number

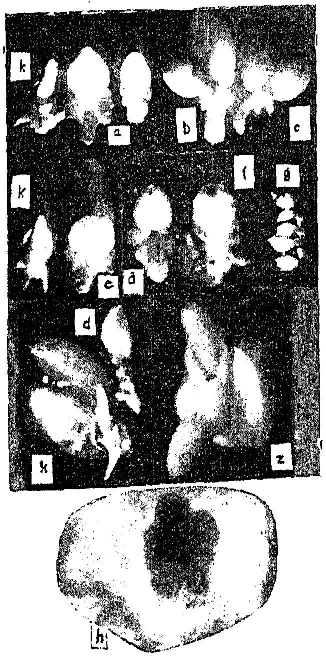

Fig. 1.

a, b, c, d, e and *f*—Seedlings from wheat treated: *c* and *f*—treated with acenaphthene; *a, b, d* and *e*—treated with colchicine; *g*—seedlings from tobacco treated with colchicine; *h*—one multinucleated cell from *Triticum timopheevi* treated with colchicine; *k*—control; *z*—Zea treated with colchicine.

of the cells have large and even giant nuclei. The nuclei are often deformed as in the cells of seedlings treated with colchicine.

3. *Nucleoli.*—In the nuclei of the treated seedlings one finds a large number of nucleoli. Large nuclei have many more and often larger nucleoli than the smaller ones.

4. *Chromosome numbers.*—(*a*) *Tr. vulgare* has $2n = 42$. In the root tips and in the young leaves cells with many more than 42 chromosomes were found. Exact count was impossible for two reasons: (1) because of the abnormal position of the chromosomes during the metaphase; they usually do not get arranged in metaphase plates, and (2) because of the large chromosome number.

(*b*) *Tr. monococcum* ($2n = 14$).—In some cells the normal chromosome number was counted, namely, 14. There were found very often cells with 28 chromosomes and with more than 28 (ca. 50, perhaps 56). The abnormal position of the chromosomes during metaphase offers great difficulties in counting the exact number of the chromosomes. Sometimes we have even difficulties in counting the chromosomes in cells with 14 chromosomes. One somatic plate with 12 chromosomes was found.

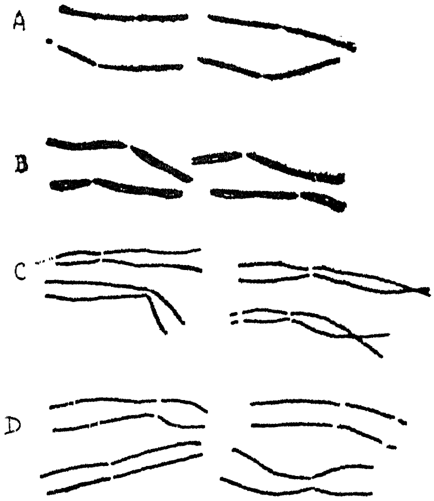

Fig. 2.

The mode of chromosome doubling in *Secale* treated with acenaphthene:

A—early metaphase, B—metaphase; C—late metaphase, D—like C, but a more advanced chromatid separation. The chromosomes are drawn from root tips, but somewhat semi-diagrammatically. Each chromosome is drawn separately in considering its position in respect to the others. The position of the chromatids is strictly preserved. In late metaphase the chromosomes and the centromeres completely divide, but do not separate toward the poles, remaining all together.

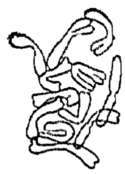

Fig. 3.

Cells with 12 chromosomes from *Tr. monococcum* treated with acenaphthene.

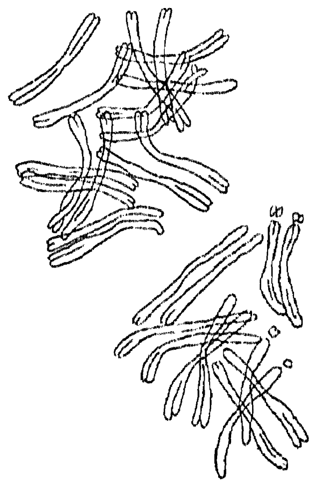

Fig. 4.

Tetraploid somatic metaphase with 28 chromosomes from *Secale cereale* treated with acenaphthene. The chromosomes are purposely separated into two groups because they are too much crowded.

(*c*) *Secale cereale* ($2n = 14$).—In the root tips of *Secale cereale* numerous metaphases were found. Cells with 14, 28 and ca. 56 chromosomes were found in one and the same section. In *Secale* as in *Triticum* the chromosomes do not get arranged in normal metaphase plate, but one gets the impression that they are scattered disorderly in the centre of the cell (*i.e.*, the position which is usually occupied by the nucleus) often without any tendency to equatorial arrangement. Regular spindles are not formed. The chromosomes divide, but they do not separate. Often they get arranged in parallel. In some plates one finds many chromosomes equal in size and shape lying side by side. This is also an evidence that the chromosomes divide but do not separate, consequently a duplication takes place. Groups of diploid cells were found surrounded by tetraploid and octaploid ones and *vice versa*.

Polyploid cells and polyploid sectors were found in roots, primordia and leaves. The chromosomes appeared morphologically very much like those found in the preparations made from material treated with colchicine. Histology and cytology of the tissues treated for a long time with acenaphthene is in all respects similar to that of plant galls and tumors (*cf.* Kostoff and Kendall, 1930).

It should be noted that colchicine affects more severely the plant tissues than acenaphthene. Seeds treated with the latter recover more rapidly. According to Prof. A. Schmuck acenaphthene is soluble to the extent of 0·0028% in water, and it sublimates. This makes it more convenient for producing polyploid plants. Colchicine is a strong poison. I injected 7 milligrams of colchicine into a rabbit; it felt sick, refusing food for two days; while ca. 25 milligrams of acenaphthene injected into another rabbit in the form of a suspension did not visibly affect the animal.

Taking all these points into consideration it seems to me that acenaphthene will be a better and more convenient agent for inducing polyploidy than colchicine. It should be also mentioned that the sources from which colchicine is obtained are highly limited. It is extracted from the various organs of *Colchicum autumnale*. The sources, however, from which acenaphthene is derived, are practically unlimited.

In this short paper I cannot discuss broadly the rôle of the polyploidy in evolution, which is well known to the cytogeneticists. I shall only stress here a few important points that should be taken into consideration when work is carried out with the above-mentioned agents and production of polyploid plants is aimed at for scientific or practical purposes.

Cytogenetic investigations carried out by numerous workers have shown that polyploid plants, produced by chromosome duplication in pure species or in hybrids of the flowering plants, represent an important source for new species. The chromosome number determined in about 2,500 species by various investigators might give us an idea as to the survival of the forms. The plants considered have from 3 to 100 gametic chromosomes. About 400 species out of 2,500 have 12 gametic chromosomes, about 350 species have 8 chromosomes, about 250 have 7 chromosomes and about 180 have 9 gametic chromosomes. Species that have 40 and more than 40 gametic chromosomes are about 50 out of 2,500 (*cf.* Fernandes,[10] Darlington[11]). Some of the plants having 12 and 8 chromosomes have been also considered as polyploids. The numbers given above show definitely that a large increase of the chromosome number affects the vitality of the plants. This regularity was also experimentally shown by the author.[12]

Hence the experiments in producing polyploid plants should be preferably performed with plants having relatively small chromosome number when practical problems are attempted. It should be also mentioned that a large number of cultivated plants are polyploids (usually allopolyploid), as for example, the best wheat varieties (*Tr. vulgare*, *Tr. durum*) and oats varieties (*Av. sativa*, *Av. byzantina*), sugarcane, New World cottons (*G. hirsutum*, *G. barbadense*), best potato varieties (*S. tuberosum*, *S. andigenum*), tobaccoes (*N. tabacum*, *N. rustica*), prunes, cherries, etc.

Methods and doses for treatment of various plants with the above-mentioned agents are and should be, in some respects different according to the nature of the plant and the stage and organ that is treated. Large concentrations and too long a treatment might lead to production of cells and sectors with too large chromosome numbers which might affect the viability of the cells and tissues. The effect produced by treatment of wheat with colchicine (0·5%) for several days without exosmosis is too drastic. It affects the vitality and deviates the normal trend of the development of the seedlings. Most of the seedlings develop a tumorous malformation instead of leaves, *i.e.*, the same agents that induce polyploidy induce also tumorous growth, when applied in relatively large doses. It should be mentioned that tetraploid shoots are rarely formed from the regions affected by plant galls, the cytology and histology of which is very similar to those of the tissues treated by large doses of colchicine and acenaphthene. The investigators should have in mind these circumstances when experimenting with these agents. My experiments show that washing off (exosmosis) of the agents after a certain period of treatment leads to a better recovery from the treatment.

In connection with this study, it seems to us worth-while to suggest a method that might lead to a successful attack of the problem of fighting some infectious diseases. A number of infectious diseases are prevented by previous immunization of the organisms with attenuated (less virulent) strains. If colchicine and acenaphthene induce changes in the cells of the micro-organisms (our preliminary observations on yeast cells indicate that this seems to be so) including bacteria, from which larger, less viable and less virulent strains for immunization can be obtained, parallel to that observed in the higher plants, the fighting of a series of infectious diseases can be considerably facilitated.

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Recent Researches on *Trochus*.

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Trochus niloticus has been commercially exploited for over a decade and a regular Trochus fishery was established in the Andamans in October 1929. In the initial season, the fishery yielded not less than 500 tons of shells within the first three months, but the yield, however, began to steadily decline and although the period of fishing has since then been doubled, the quantity of shells obtained at present has almost reached the low figure of 40 tons in one fishing season. The scarcity of full-sized specimens has also resulted in an indiscriminate fishing of shells of all sizes by both the licensed and unlicensed Japanese fishermen, so much so that, at the present day, the *Trochus* beds of the Andaman waters are getting rapidly depleted. Unless effective steps are taken by the Government to prevent overfishing, and to develop the fisheries on scientific lines, this important shell fisheries is threatened with almost complete extinction. The habits and habitats of this commercially important shell fish have also remained practically unknown for a long time. The Government of India appointed a special officer in 1930 to carry out a scientific study of the fisheries, but before the person appointed could gather any appreciable data, the contract was terminated as a result of the retrenchment policy of the Government. The work, however, was continued under the auspices of the Zoological Survey of India, and the recent investigations of H. Sreenivasa Rao have brought in much valuable information on the bionomics of *Trochus*. In a paper on the habits and habitats of *Trochus niloticus*,[1] he reports that the two common types of shells observed both in the Andaman and Nicobar Islands do not represent two distinct species as was supposed by some authors, but only plastic phases of one and the same species *T. niloticus* Linn., possibly brought about by differences in the rate of growth at various ages. The animals live chiefly on bottom deposits and fresh