CYTOGENETIC CHANGES AND ATYPICAL GROWTH INDUCED BY HEXACHLOROCYCLOHEXANE ($C_6H_6Cl_6$)

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SCIENTISTS occupied chiefly with applied science, especially such working in the fields of agronomy and medicine, often demanded with right, from the cytogeneticists, when they work on some theoretical problems to pay attention at the same time to the practical outcome of their studies giving sufficient theoretical background for elucidation of the methods adopted in the applied work. Having often heard such reproaches from various sides I have occasionally directed some of my studies to satisfy, as far as possible, some of the demands of the applied sciences. The results of such a recent study I have summarized and should like to report them. I shall mention here that the investigations are not yet completed in many respects but there are many points that deserve to be reported even at this stage of development.

The work of cytogeneticists and plant-breeders can often be methodically criticised when they treat material with certain insecticides or fungicides in order to protect their experimental material from parasites, but without considering the cytogenetic effect of chemicals applied in such cases.

The best insecticides and fungicides will be those which kill the plant parasites without affecting the plant organism. In fact they all affect more or less the host plant in various ways and degrees.[1,4] A series of fungicides and insecticides have similar effects on the plant organisms. Ethyl-mercury chloride (CH_3CH_2HgCl) for example, which is the active substance (2%) of the fungicide "Granosan", induces atypical growth, abnormal mitosis and polyploidy[3,4] reminding the effect of colchicine and acenaphthene.[5,6] Similar and at the same time very strong effect of this kind has hexachlorocyclohexane—another chlor-organic compound, recently recommended under various names. The insecticides "Agrocides", 7,3 etc., for example, the active substance of which is gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane, induce atypical growth suppressing the development of the roots, stems and coleoptyles of *Grammicious* plants, conditioning very striking thickening of these organs, especially of the growing points (roots, stems and coleoptyles).

Active insecticides, manufactured in various countries (hexachlorane, 666) and containing several isomers of hexachlorocyclohexane, including the gamma isomer, act in a similar, though not in an identical way. Hexachlorane, for example, stimulates the germination of certain *Cruciferæ* (*Brassica nigra*) and other plant seeds during the first two days of germination, so that they were even ahead in growth, when compared with the control samples and much ahead when compared with the germinating seeds treated with "Agrocide 7". But soon after this, the controls grow further, while the growth of the treated seedlings becomes strikingly suppressed. Some of the data of this material are given in Table I.

TABLE I

Suppression of the growth by hexachlorane and agrocide 7. Length in mm.

	Germinating seed of	Untreated control in mm.	Treated with hexachlorane in mm.	Treated with Agrocide 7 in mm.
1	*Zea mays* (roots) ..	22·03	12·07	8·90
2	*Panicum miliaceum* (coleoptyles)	10·00	4·76	3·32
3	*Pisum orvense* (roots) ..	23·21	9·62	7·73
4	*Cannabis sativus* (stem and cotyledon)	21·19	15·98	12·72
5	*Brassica nigra* (stem and cotyledon) ..	29·46	8·40	5·31
6	*Secale cereale* (roots) ..	20·46	2·65	1·33
7	„ „ (coleoptyles)	41·30	4·75	2·81

We have treated with insecticides containing hexachlorocyclohexane germinating seeds of the following plants: *Zea mays. Triticum vulgare, T. monococum, T. compactum, Secale cereale, Setaria italica, Panicum miliaceum, Helianthus annuus, Crepis capillaris, Vicia faba, V. sativa, Pisum sativum, Brassica nigra,* etc.

The cytological studies of the affected root, stem, and coleoptyle tissues show that the *agents act first of all upon the cytoplasm and interfere with the cytoplasmic processes* involved in the formation of achromatic figures. The chromosomes do not arrange in an equatorial (metaphasal) plate after prophase, but remain scattered approximately as they are during the prophase They appear less bent than usually. The "thickening" of the chromosomes and their reproduction and splitting proceeds although the process involved in the formation of achromatic figures are highly disturbed or even entirely inhibited. The chromosomes thus reproduce, but remain where they are, without moving toward the poles. In fact, poles are not formed After such a reproduction, the chromatolythic (nucleoproteolytic) processes proceed and nucleus is formed containing twice as many chro nosomes as before the beginning of the abnormal mitotic processes. The main trend of the processes conditioning chromosome doubling resemble those induced by colchicine, acenaphthene and other polyploidizing agents.

Since the insecticides continue to act further, the next abnormal Ab-mitosis ends with second chromosome doubling and so on. Thus tetraploid and octoploid cells are formed and even cells of a much higher degree of polyploidy. Along with these, certain diploid cells, that have not yet undergone Ab-mitosis can be still found.

Chromosome multiplication leads to the increase of the size and occasionally of the number of the nuclei and further to the increase of the size of the cells. Thus the cells grow instead of multiplying and differentiating resulting in the swelling of the roots, stems and coleoptyles.

The chromosome reproduction and separation in *Zea mays* should be considered as a somewhat special case. We have observed in a series of cells that the chromatids of the somatic chromosomes are bent at the centromeres reciprocally to each other, each one, like V, thus both halves forming rather a X, the chromatids being attached at the centromere. These figures can be interpreted by postulating certain repulsion forces existing between the chromatids, the reproduction (rather the division) of the centromere, being somewhat delayed. This phenomenon occurs when the achromatic figure is highly or completely disturbed. In other words, it does not seem to be regulated to any extent by the forces evolved from the achromatic figure.

If we compare the polyploidizing potency of hexachlorocyclohexane with that of acenaphthene, we can with certainty affirm, that in *Gramineæ* the activity of both agents is approximately the same. Acenaphthene as polyiploidizing agent in leguminous plants, is not effective enough, while the activity of hexachlorocyclohexane is much more effective and we obtained in *Pisum* swellings resembling those induced by colchicine, as well as cells and tissues in *Vicia faba* having various degrees of polyploidy.

The solubility of hexachlorocyclohexane in water is very low, therefore, it is applied in the form of small solid particles. The particles act when they are in contact with the plant tissue. It has a specific smell, but the experiments failed, when it was tried to induce specific atypical growth from a distance. In this respect its effect differs from that of acenaphthene, the sublimating particles of which act from a distance even when the crystals are not in contact with the plant tissue. Floral buds of *Nicotiana tabacum* and raddish covered with hexachlorocyclohexane (mixture of various isomers), Agrocide 7, or hexachlorane showed none, or very few disturbances in the meiosis, as for example, occasional univalents and laggards or slight irregularities in the arrangement of the chromosomes in the equatorial plate, which probably may be due to a sequence of disturbances in the achromatic figure. Contrary to the very well expressed effect of acenaphthene upon meiosis in *Gramineæ* hexachlorocyclohexane does not affect, or its effect is quite insignificant upon meiosis in rye, when spikes were abundantly covered with Agrocide 7, Hexachlorane or mixture of isomers of hexachlorocyclohexane and inserted into test-tubes for 24 hours. Spikes of rye plunged into test-tubes with saturated solutions with some excesses of these substances, did not show significant effect, *i.e.*, significant disturbance in the meiotic processes. Similar experiments were carried out with tobacco and leguminous plants with similar results.

All these observations suggest that hexachlorocyclohexane acts upon the plant cells when its particles are in near contact with the tissue.

The pollen mother cells, being protected, first of all, by the tissue of anther and then by the corolla and callyx tissues (or by the glumes in rye) are not effectively affected by the agent.

Experiments were also carried out with yeast in the following way: Sterile grape juice was inoculated with pure culture of yeast to some of the flasks Agrocide 7 was added, to others Hexachlorane and to yet others hexachlorocyclohexane (mixture of isomers). After 24 and 96 hrs. no striking changes in the size of the yeast cells were observed and when compared with the controls the fermentation in the treated cultures being somewhat suppressed and later completely inhibited.

Preliminary experiments were conducted with caterpillars of *Limantria dispar*, to study whether the agent acts upon the caterpillars first as polyploidizer, and the death being as a subsequent effect of it. But the observations, hitherto carried out, do not seem to support such supposition. The studies in this line are now continuing.

The effect of hexachlorocyclohexane upon the somatic tissues of the plants is so striking that it can be used as polyploidizing agent, especially when one considers the fact that it is much cheaper than other polyploidizing agents. We are now carrying out further experiments in this direction.

In studying the disturbances in the mitotic processes induced by hexachlorocyclohexane we have found that in certain cases chromosome group or groups may move slightly in some direction or directions into cytoplasm. Such a slight separation may occasionally end in the formation of two or more than two aneuploid nuclei. Thus polynucleate cells or cells with monstrously deformed nuclei appear. In certain cases between such nuclei, a cell wall is formed. This leads to formation of cells with aneuploid chromosome numbers. Dead cells were occasionally found in the roots, stems and in the coleoptyles. They may have been aneuploid.

All these phenomena leading to polyploidy or aneuploidy are due to the activity of the agent upon the cytoplasm.

But the active agent, *i.e.*, hexachlorocyclohexane also induces certain changes in the nuclear elements, *i.e.*, in the chromosomes, no matter how rare they may occur Fragments of one chromatid or of chromosomes (both chromatids) were also observed, although very rarely.

Such insecticides or fungicides, when applied, may increase the hereditary changes in the cultivated "pure lines" leading thus to more rapid degeneration of the highly bred uniform varieties. This means that when one applies such insecticides or fungicides one should more frequently change the seeds of the varieties which he propagates, by using a fresh non-degenerated stocks.

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1. Kostoff, D., "Degeneration of the Pure Lines," Sofia, 1930 2. —, *Bull. Soc. Bot. de Bulgarie*, 1931, **14**, 87–92. 3. —, *Nature* London, *1939*, **144**, *334*. 4. —, *Phytopath. Zeitschr.*, 1940, **13**, 91–96. 5. —, *Compt. Rend. (Doklady), Acad. Sci. USSR.*, 1938, **19**, 189–92, 6. —, *Journ. Genet.*, 1940, **39**, 469–84.