

Thus, since no other hydrated complex of the system is formed intermediately in the charge transfer reaction, it is justifiable to say that this step proceeds by way of the hydrated complex  $\text{Cd}(\text{CN})_2$ , though in all probability the ligands cannot be coordinated or split off simultaneously.

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## Chemically Induced Mutation and Sterility in Barley

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This paper gives some preliminary results from investigations concerning the induction, by chemical means, of mutations in higher plants. The experiments are complementary to treatments of the material with ionizing radiations<sup>1</sup>, which seem rather unspecific since they always cause mutation as well as sterility, the latter effect especially at higher ion densities. A variation within wide limits of the linear energy transfer and irradiation conditions gives only small changes as regards relative frequencies of mutation types, possibly except the erectoides case<sup>2</sup>. It was, therefore, our hope that an application of (a) chemically reactive compounds affecting biological materials differently, and (b) biochemically active compounds interfering with processes important to the synthesis of the gene material would, apart from an elucidation of the radiation induced mutation process, increase our possibilities to direct the mutation process.

In treatments of wet tissue with "radio-mimetic" chemical agents<sup>3</sup>, also when these are specifically nucleotoxic, it is extremely difficult to establish quantitative relationships based on a dosimetry. As compared to radiations, where active ions and radicals are formed anywhere in the tissue, diffusing molecules form gradients of change from outside towards inside of the tissue, cell, or cell nucleus. To some degree such difficulties might be overcome when dry resting seeds, which are in fact very insensitive to toxic agents, are treated with gaseous reactive chemicals. The imbibition of water and, in consequence, at least part of the final steps of the reactions will then follow afterwards. In the present study barley seeds containing 10–11 %  $\text{H}_2\text{O}$  were treated with a gas phase containing ethylene oxide, iodine, or chlorine. — Non-volatile compounds have to be applied in solution. Either resting seeds, or presoaked seeds, the germination process of which had been initiated by soaking in water for 24 hours, were immersed for different times in solutions of the agents studied.

All treatments were done at 20°C. Afterwards the seeds were sown in the field, and the mature plants obtained were analysed for survival, mutation, and sterility according to standard methods (cf. Ehrenberg and Nybom<sup>1</sup>). Below, "sterility" is defined as the frequency of spikes with more than 10 % empty spikelets.

*Tested compounds; results.* The following subdivision as to the expected mode of action can be made (cf. Table 1):

(a) *Alkylating agents*, which after radicalization or ionization react with nucleophilic groups, such as amino and ionized acid groups. Effects of *mustards* have been described earlier<sup>1,4</sup>. — *Ethylene oxide*, earlier found mutagenic in other organisms<sup>5,6</sup>, was in part given, for 6 days, in the gas phase with 20 % air; in this treatment seeds survive if they contain less than 10 %  $\text{H}_2\text{O}$ ; partly presoaked seeds were treated for 2 hours with solutions (0.5% kills the seeds). — *Formaldehyde*, which, attacking amino and other groups, induces methylene groups and bridges, gives according to Favret<sup>7</sup> mutations in barley. Under Favret's conditions, as well as in presence<sup>8</sup> of  $\text{H}_2\text{O}_2$  it was found inactive, however, although some sterility appeared.

(b) *Oxidizing agents.* *Chlorine* (24 h, 80 % being lethal) and *iodine* (saturated vapours, 48 hour's treatment lethal, 24 h used; washing in 10 % KI has to follow treatment) might oxidize (or substitute) almost everything.

Table 1.

| Group  | Compound                          | Concn. % | Sterility % | Mutations |                        | Relative frequencies of chlorophyll mutation types, % |                |               |        | Total no. of cases |
|--|-----------------------------------|----------|-------------|-----------|------------------------|---|----------------|---------------|--------|--------------------|
|  |                                   |          |             | %         | No. of spikes analysed | <i>albina</i>   | <i>viridis</i> | <i>xantha</i> | others |                    |
| a  | Control                           | —        | 4           | 0.054     | 15 861                 | 35  | 35             | 10            | 20     | 20                 |
|  | N-Mustard (Mac Key <sup>1</sup> ) | 0.7      | 24          | 0.25      | 729                    | 26  | 52             | 2             | 19     | 42                 |
|  | Ethylene oxide                    | 80 (gas) | 22.1        | 1.8       | 989                    | 50  | 39             | 3             | 8      | 36                 |
|  | Do (presoaked)                    | 0.12     | 9.5         | 0.75      | 1 872                  |   |                |               |        |                    |
|  | Do Do                             | 0.03     | 5.7         | 0.20      | 2 510                  |   |                |               |        |                    |
| b  | Chlorine (gas)                    | 20       | 18.4        | 0.3       | 696                    | 100   |                |               |        | 2                  |
|  | Iodine (gas)                      | sat.     | 6.1         | 0.6       | 908                    | 100   |                |               |        | 5                  |
|  | Dioxan                            | 1—5      | 6           | 0.24      | 5 500                  | 29  | 71             |               |        | 14                 |
| c  | Nebularine (presoaked)            | 0.025    | 1.4         | 0.8       | 1 293                  | 8   | 85             | 4             | 4      | 26                 |
|  | Do                                | 0.05     | 0           | 0.1       | 1 361                  |   |                |               |        |                    |
|  | Do (resting)                      | 0.025    | 1.6         | 0.2       | 1 373                  |   |                |               |        |                    |
|  | Do                                | 0.05     | 1.5         | 0.6       | 1 289                  |   |                |               |        |                    |
|  | Ethoxycaffeine                    | 0.2      | 4—57        | 0.1       | 2 930                  |   |                |               |        |                    |
| X-rays for comparison (1 000 r on resting seeds) |                                   | —        | 15          | 0.6       | ∞                      | 51  | 34             | 6             | 10     | ∞                  |

Sacharoff<sup>8</sup> found the latter compound mutagenic in *Drosophila*. — Due to the role ascribed to peroxides in radiation action, peroxide-containing *p-dioxan* was used (resting and presoaked seeds stand 24 h soaking in 5 % solution; 2—8 h used in experiments).

(c) *Purine derivatives* might be supposed to interfere specifically with nucleic acid metabolism. The *8-ethoxycaffeine* was found by Kihlman to break chromosomes in plant roots, the action being coupled to oxidative phosphorylation<sup>9</sup>. *Nebularine*, the tuberculostatic principle from *Clitocybe nebularis*, was used since it was found by Löfgren and Lünig<sup>10</sup> to be purine-9-D-riboside, *i. e.*, a nucleoside not occurring in nucleic acids of higher plants. — Resting or presoaked seeds were treated for 24 h with these compounds, the ethoxycaffeine at different oxygen tensions<sup>9</sup>.

(d) In a comprehensive experiment we tested the mutagenicity of *acridines* claimed by

D'Amato<sup>11</sup>. When applied as described by this author as well as under other conditions, *acridine orange* and *acriflavine* gave in 20 000 spike progenies only 4 mutation cases, *i. e.*, not different from the spontaneous rate.

When discussing the results (Table 1) it should be remembered that the third generation has not yet been studied. Since we are dealing with agents not tested before in barley, the induction of *Dauer*-modifications or plasmon mutations is not excluded offhand.

The experiments prove with full evidence that sterility (dependent on chromosomal rearrangements) and mutation are not induced by the same mechanism: Ethoxycaffeine gives rise to sterilities corresponding to those obtained by a treatment with up to 6 000 r, without any significant increase of the mutation frequency (at this sterility X-rays give about 4 %

chlorophyll mutations). With the reservations just expressed, the nebularine, on the other hand, gives a fairly high mutation rate without any simultaneous induction of sterility. In addition, this compound gives a distribution of mutation types (*virides* being dominating) quite different from that obtained in other treatments. It seems probable, therefore, that the application of agents disturbing the metabolism (incl. synthesis) of nucleic acids causes effects much more specific than those obtained with radiations.

The alkylating and oxidizing agents are, as expected, more unspecific, causing sterility as well as mutation. Ethylene oxide gives about the same distribution of mutation types as is obtained in X-ray experiments, whereas deviations from this distribution is indicated in other cases.

It should be added that optimum conditions for mutation induction by means of the compounds studied is not yet worked out. Therefore some series are omitted from the table. The fact that nebularine is most active at low concentrations (0.1 % which gives lower mutation frequencies, is omitted) indicates that the action of the compound is only manifested when meta-

bolism is fairly undisturbed. Ethylene oxide, on the other hand, acts proportionally to concentration.

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