THE PHENOMENON OF NATURAL PROTECTION IN CHEMICAL MUTAGENESIS

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In the studies of mutagenicity of homogenates prepared from *Crepis capillaris* seedlings without seed coat, from seed coat, and from seedlings with seed coat, it has been found that 24 hr after the treatment of 26 hr old seedlings with EI there are no molecules of the mutagen itself or of its secondary products. Addition of EI to the same homogenates prepared from normal 50 hr seedlings has shown that the mixture of ground seed coat with EI causes sharp increase in the latter's mutagenic properties. At the same time, certain substances from cells of ground seedlings neutralize mutagenic molecules.

INTRODUCTION

Our knowledge of prolonged after-effects of alkylating agents is still inadequate. Investigations in this field are at present the major line of research in the mutation theory (Dubinin and Saprykina 1964; Romashov and Belyaeva 1966; Dubinin *et al.* 1968; Dubinin 1969). In an attempt to explain the finding that mutations sometimes arise after a long time following a cell was treated with a mutagen, a hypothesis of potential changes turning into genuine mutations only after certain time, is proposed (Swanson 1955); Dubinin and Dubinina 1968; Garina and Romanova 1970; Korytova *et al.* 1971). As an alternative or sometimes an extension of the explanation for the above observations, a possibility of prolonged existence of mutagenic molecules and their secondary products has been proposed (Froese-Gertzen 1964; Andreev *et al.* 1966).

There are several operational approaches permitting to throw some light on this problem. A thorough washing of seeds exposed to mutagen, is done to remove the molecules of the mutagenic chemical from the cells. This was done in *Crepis capillaris* seeds after single treatment with the alkylating agent ethyleneimine (EI) at a concentration 2.3×10^{-2}M. There was no decrease in the mutation rate due to this washing at the stages of cell cycle observed and analysed after washing (Dubinin and Chernikova 1972). With ethylmethanesulfonate, it was observed that its hydrolytic products in water had no mutagenic effect, and in the cells of germinating maize seeds mutagenic properties of its molecules rapidly disappeared (Ficsor and Beckman 1968).

Another approach is by studying mutagenic properties of homogenates obtained from the cells treated with mutagen (Andreev *et al.* 1966; Ficsor and Beckman 1968). If the homogenates prepared at different times after mutagenic treatment exhibit mutagenic properties, it can be presumed that the mutagen molecules or their secondary products were present in the cells, from which the homogenates were made. If the homogenates lose their mutagenic properties although the mutational process inside the cells is still under way, the previous hypothesis of realization of potential changes should be taken as a more probable explanation for the after-effects.

We tried to investigate the nature of mutagenic after-effects through extensive study of the effects of homogenates prepared from cells of *Crepis capillaris* seedlings treated with alkylating agents.

Materials and Methods

Experiments were conducted with *Crepis capillaris* seedlings. The experimental scheme is presented in Fig. 1. Seeds were soaked in water, seedlings after 26 hr growth were treated with EI at concentration $9.3.10^{-3}$M for 40 min. Washing was done in running water for 30 min and this was followed by another 24 hr of growth. Fresh homogenates were prepared from seedlings of 50 hr growth and used for treatment of normal 26 hr seedlings for 2 hr. Then they were rinsed in water and grown on colchicine solution (0.01 per cent). After 12 hr the normal seedlings treated with the homogenates were fixed in mixture of alcohol and glacial acetic acid (3:1). Homogenates were prepared by grinding in mortar with glass powder. One sample of homogenate was prepared from 150 seedlings and 20 mg glass powder. This extract was used for treatment of 15 rootlets with an average of 10 ground seedlings per 1 rootlet. Every test had a corresponding control. Chromosomal rearrangements in metaphases of first mitosis were analyzed.

Results and Discussion

Seedlings of 50 hr growth from which the homogenates were prepared consist of two biochemically different moieties, namely growing root meristem and seed coat. Seed coat of some plants contains certain substances lacking in their root meristem, like in horse beans (Dubinina and Dubinin 1967). It has been shown that under certain specific conditions seed coat of *Crepis capillaris* manifests mutagenic properties (Shevchenko *et al.* 1971). Analysis of different mutagenicity of seed coat

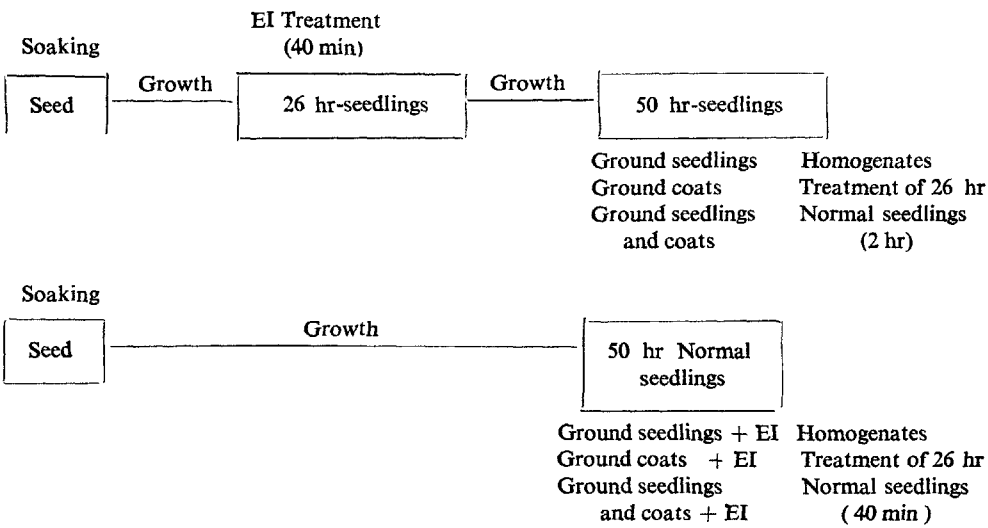

Fig. 1. The scheme of experiments.

and root meristem led us to prepare three types of homogenates: GS—homogenate prepared from seedlings after removal of seed coat; GC—homogenate from seed coat alone separated from seedlings, and GSC—homogenate from complete seedlings ground together with the seed coat. The cells of *Crepis capillaris* seedlings exposed to EI at 26 hr of growth, of which homogenates were prepared at 50 hr of growth, themselves manifested a high mutation rate (21.34±2.30 per cent). Tests for the action of the three types of homogenates (GS, GC, GSC) were repeated several times. None of the homogenates showed any significant mutagenic properties. Mutation frequency for each series of runs was 0.37 per cent for GS, 0.61 per cent for GC, 0.18 per cent for GSC (Table I).

TABLE I

Lack of mutagenic effect upon treatment of cells with homogenates from 50hr seedlings pre-treated with EI on the 26th hr of growth

Treatment	Mutability in seedlings		
	Number of examined metaphases	Number of rearrangements	Rearrangements (%)
Ground seedlings	1869	7	0.37 ± 0.14
Ground coats	2265	14	0.61 ± 0.16
Ground seedlings and coats	2134	4	0.18 ± 0.09
Total	6268	25	0.39 ± 0.08
Control	7420	22	0.30 ± 0.06
EI 9.3×10^{-3} M	1282	363	28.31 ± 1.48

Mean mutability for the three series of tests was 0.39±0.08 per cent with 0.30± 0.08 per cent in the control. Treatment with EI at a concentration 9.3×10^{-3} M under the same condition gave about 28.3 per cent mutation rate. The results obtained clearly indicate that the further 26 hr growth of seedlings after treatment with EI at 26 hr of growth led to disappearance of all mutagenic products in the seedling cells. Neither the seedlings as such, nor the coat of germinating seeds, nor the combination of both exhibited any mutagenic properties (Fig. 2). Comparison of these results with the evidence that exposure to EI at G_1 phase gave rise to both chromosome and chromatid rearrangements (Swanson 1955) and with the findings of the tests for storage of EI treated seeds (Dubinin and Dubinina 1968) suggests that the explanation for these should be sought in the concept of potential lesions.

Of direct importance to the mechanisms under discussion is the knowledge about the effect of the interaction observed between EI and GS, GC, and GSC homogenates. In these studies, equal amounts of EI were added to homogenates made from normal 50 hr seedlings and normal 26 hr seedlings were treated with mixture (Fig 1). EI concentration of the mixture was $0.5\times9.3\times10^{-3}$ M. Treatment of seedlings with this concentration of EI caused 9.6 per cent mutation rate of rootlets which were fixed

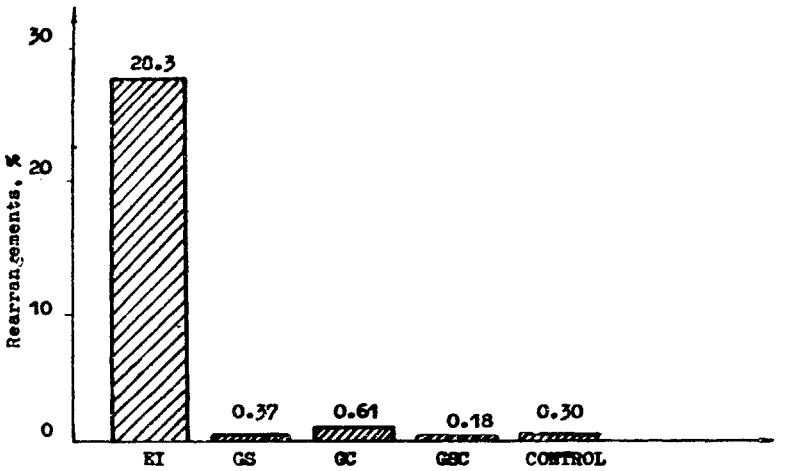

FIG. 2. Loss of the mutagenic effect of EI 24 hr after introduction into the cell.

TABLE II

Mutagenic properties induced by a mixture of homogenates from normal 50 hr seedlings and ethylene imine

Treatment	Mutability in seedlings		
	Number of examined metaphases	Number of rearrangements	Rearrangements, %
Ground seedlings + EI	5071	28	0.55 ± 0.10
Ground coats + EI	1184	487	41.13 ± 1.86
Ground seedlings and coats + EI	7902	83	1.05 ± 0.11
Control	12095	26	0.22 ± 0.04
EI $0.5\times9.3\times10^{-3}$M	1599	155	9.69 ± 0.77

12 hr after the treatment. It might be expected that the effect of EI in such treatment would be the maximum as compared to those exerted by homogenates from seedlings growing for a certain time after EI exposure.

The results were quite unexpected. Addition of EI to each of the homogenates caused different modifications in the mutagenic effect (Table II). After treatment of 26 hr seedlings with the mixture GC+EI, the mutation frequency was 41.13± 1.86 per cent, with the mitotic cycle heavily blocked (poor mitotic index). As was mentioned above, seed coat of some plants contains substances displaying pro-mutagenic or mutagenic properties. It is expected that interaction of EI with ground seed coat would give rise to highly mutagenic products (Dubinin and Scherbakov, 1965) causing sensitizing effect of EI, expressed in the sharp increase of mutation frequency.

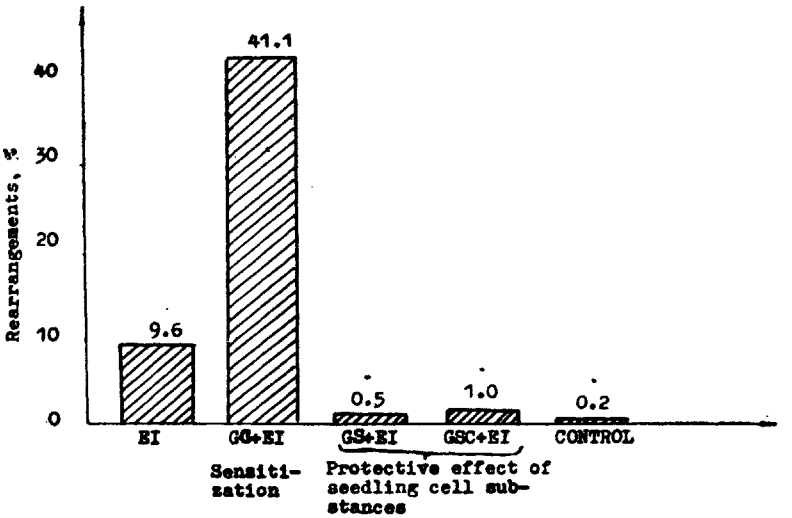

FIG. 3. Protective and sensitizing effects of ethyleneimine in combination with homogenate substances.

The results of interaction between EI and ground seedlings after removal of the seed coat were most impressive. Upon introduction into GS homogenate EI completely lost its mutagenic properties. While under the action of the mixture GC+EI the mutation rate was 41.13 per cent, in case of the GS+EI action it was only 0.55 per cent. Consequently, cellular protoplasm of ground seedlings contains substances which almost immediately neutralize all mutagenic molecules.

Tests for the action of the mixture GSC+EI presented much interest. This mixture must, presumably, contain both activated protective substances (GS), and activated sensitizing substances (GC). What will be the action of this mixture on normal seedlings? In these tests, the mixture GSC homogenate+EI induced very few, if any, mutations—1.05 ± 0.11 per cent (Table II). It suggests that the tremendous potential sensitized mutagenicity resulting from mixing GC and EI was neutralized by the reaction with the protective substances released from the ruptured cells of seedlings.

Our studies demonstrate the existence of a peculiar natural protection in the cell against genetic effect of alkylating agents (Fig. 3). It has been shown by special tests that such substances are formed at a certain metabolic stage in germinating seeds. Studies into the biochemical nature of these substances, modes of action of this formerly unknown natural protective system and its implications on the intact cell are very important in view of both the problem of protection and getting insight into the nature of mutations.

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