Modification of the Radiomimetic Effects of Nitrosoguanidine by Cysteine in Plants

The number of chemicals giving protection against radiation damage both in animals and plants is large. That some of these compounds can also afford protection against radiomimetic chemicals has been shown in a few cases[1,2]. The aim of the present communication is to report a significant protection given by cysteine against the lethal and chromosome breaking effects of 1-methyl-3-nitro-1-nitrosoguanidine (NG), a known radiomimetic and mutagenic agent[3].

Resting seeds of barley (var. Volla) and secondary roots of *Vicia faba* (var. Hangdown) were used in the study. Solutions of NG were made in M/15 phosphate buffer adjusted to pH 6 and those of cysteine in double glass-distilled water. Seeds of barley (100 seeds in each case) were taken in a conical flask and 100 ml of test solution added to it. After a treatment of 16 h the solutions were drained away and seeds given a quick wash in running water (18 °C). Seeds were immediately put on thick moist filter papers for germination at 20 ± 2 °C. Seedlings were grown under day light hours and seedling height measured on the seventh day. In *Vicia* about 1 cm long secondary roots were given the treatment for 45 min. After the treatment the seedlings were allowed to recover in tap water. Fixation was made in 1:3 acetic acid alcohol following a pretreatment with 0.05% colchicine for 3 h. Feulgen squash preparations were made, and scoring was made at metaphase.

The concentrations of NG and the conditions of treatment used here have already been shown to have a strong radiomimetic ability in higher plants[3]. In the present experiments cysteine alone had no effect either on germination or seedling growth of barley seeds. Nitrosoguanidine (1 mM, 16 h) reduced germination by 30% and seedling height by 52%. In combination with cysteine ($6 \times 10^{-3} M$) the lethal effects of NG were fully neutralized and germination was again normal (Table I). Seedling injury calculated according to the method of CONGER et al.[4] was reduced from 51% to only 17.9%. As regards chromosome breakage in *Vicia* it can be seen (Table II) that following NG treatment about 30% cells were abnormal and up to 39.5 aberrations/100 cells were recorded. But when this treatment was carried out in the presence of cysteine, a significant reduction in damage was noted which was as high as 48% depending on the concentration of both NG and cysteine.

The mitotic index (Table II) however, remained unaffected. It may be pointed out that only combined treatments gave this protection and both pre- and post-treatments were largely ineffective.

Cysteine is known to protect against radiation-induced chromosome damage both in animals and plants[5-7] and the extent of protection achieved in present experiments is closely comparable to that given against irradiations[5-8]. The protective ability of cysteine was attributed mainly to its action as a reducing agent, i.e. by creating anoxic conditions during the treatment[9]. But how cysteine provides protection against NG damage cannot be said with certainty at present; but, by analogy with radioprotective work, it may be assumed that cysteine is operating by a lowering of the intracellular oxygen tension. In that case NG effects may be due to interference with respiratory chain directly or through some respiratory enzymes. Hitherto, NG was regarded to act only

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Table I. Barley (var. Volla) treated with nitrosoguanidine (1 mM, 16 h) alone or in combination with cysteine

Treatment	Germination % of control	Seedling height % of control	Seedling injury (%)
(1) Nitrosoguanidine, 1 mM	70.0	48.4	51.58
(2) Cysteine $3 \times 10^{-3} M$	100.0	97.9	2.15
(3) Cysteine $6 \times 10^{-3} M$	100.0	96.9	3.15
(4) 1 + 2	85.0	46.3	43.68
(5) 1 + 3	100.0	82.1	17.89

Data showing reduction in seed lethality and seedling injury by cysteine.

Table II. Data showing protection against NG induced chromosome breakage in secondary roots of *Vicia faba* by cysteine

Treatment		Abnormal cells (%)	Aberrations/100 cells		CHB + CHE	Protection (%)	Mitotic index
NG mM	Cysteine $\times 10^{-3} M$		Chromatid breaks (CHB)	Chromatid exchanges (CHE)			
–	–	–	–	–	–	–	6.8
0.75	–	29.5	26.4	12.8	39.2	–	6.1
0.75	3	22.5	23.6	9.0	32.5	17.10	6.3
0.75	6	16.0	14.7	8.1	22.8	41.84	6.7
0.50	–	28.5	17.1	18.3	35.4	–	6.4
0.50	3	18.5	16.4	10.3	26.7	24.58	5.8
0.50	6	15.6	10.0	8.3	18.3	48.33	6.0

200 metaphases analysed in each case.

as an alkylating agent[3]. Reduction in chromosome breaks is, however, due to a reduction in actual induction of NG-induced breaks rather than a higher proportion of restitution of broken ends, in view of the fact that frequency of chromatid exchanges and mitotic index did not show any increase with cysteine treatments and that only combined treatments were effective[10].

Zusammenfassung. Es wurden Gerstenkeimlinge und sekundäre Wurzeln von *Vicia faba* mit Nitrosoguanidin allein oder in Kombination mit L-Cystein behandelt. Im letztern Fall war der radiomimetische Effekt bis auf 50% reduziert. Es wird vermutet, dass diese Reduktion auf eine Änderung des Sauerstoffspiegels in der Zelle durch das L-Cystein zurückzuführen ist.

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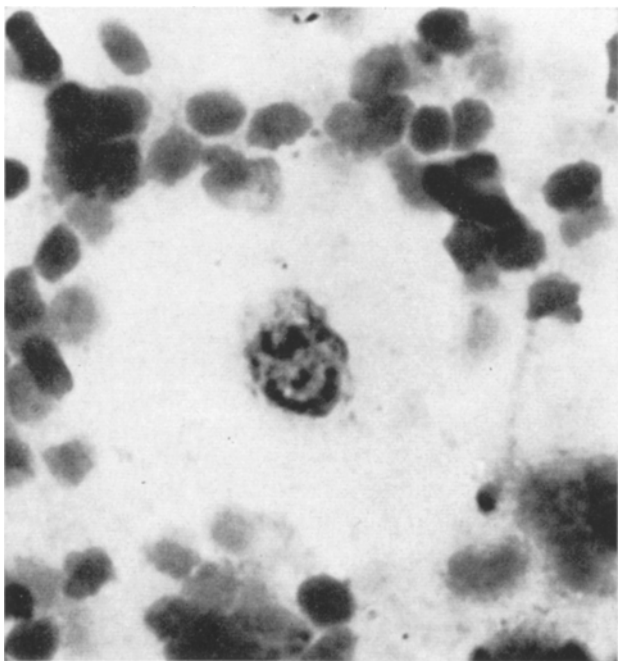
Evidence of Macrophages being 19 S-Antibody Producing Cells, as Shown by a Modification of the Plaque-Technique

The immunogenic function of macrophages is being investigated in many laboratories. The role of macrophages for antibody formation is supposed to consist of the following:

(1) The processing of the antigen after phagocytosis and the formation of a specific RNA in the macrophages. This RNA could be transferred to the antibody-forming cells and so induce the antibody synthesis[1,2].

(2) The uptake of the antigen by macrophages, synthesis of RNA and formation of an antigen-RNA-complex. This complex is highly immunogenic ('super-antigen') and is transferred to immunocompetent cells, inducing there the antibody synthesis[3,4].

(3) The differentiation of these macrophages into antibody-forming cells after uptake of the antigen.



A macrophage as a plaque-forming cell on the fifth day after immunization against SRBC. × 950.

We studied this differentiation by trying to find plaque-forming cells which have the morphological characteristics of macrophages. For this investigation we modified the plaque-technique of JERNE[5] by using a mixture of 0.2 ml of a 0.7% agarose-suspension, 0.05 ml of a 100% SRBC-suspension and 0.06 ml of a 20% mouse spleen cells sensitized in vivo against SRBC 5 days previously. A drop of this mixture was put on a glass slide and spread just as a blood smear. It resulted in a very thin layer, mostly a cell monolayer which was incubated and treated with complement as usual. We obtained very small plaques, visible at a low magnification. Each plaque-forming cell could be recognized and after staining with Giemsa cytologically identified.

4000 plaques have been observed microscopically. We clearly identified a few plaque-forming cells as macrophages (Figure). To be certain the that observed plaques were not artefactual, 2 control measures were used continuously: (1) incubation of the mixture without complement; (2) substitution of non-sensitized spleen cells for the sensitized cells. Our observations have shown that macrophages are able to carry antibodies. The experiment strongly indicates that macrophages can assume an antibody producing ability while still preserving their morphological characteristics.

Zusammenfassung. Milzzellen, die Antikörper gegen Schaferythrozyten bilden, wurden in einer modifizierten Plaque-Technik zytologisch untersucht. Dabei konnten etwa 3% der Antikörper bildenden Zellen als Makrophagen identifiziert werden.

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