small amounts of Hb A_1. Haptoglobin was absent in the blood.

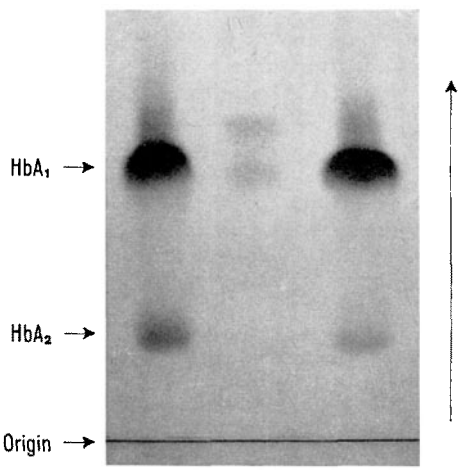
Considering the normal hemoglobin content of the red cells, we must conclude that the abnormal component in plasma and urine represents a secondary hemoglobin alteration. A similar fast-moving pseudoabnormal hemoglobin was found in 2 other cases of hemolytic anemias.

Case 2. By courtesy of Prof. DACIE, London, we received a serum sample from an adult male with hemolytic anemia following cardiac operation. The patient was operated three months previously and a valve prosthesis was placed in the aortic valve region. We found a secondary ahaptoglobinemia and in the starch block electrophoresis two benzidine positive fractions, one with the mobility of methemalbumin and the other migrating slightly faster than Hb A_1. No normal free Hb A_1 was present.

Case 3. In a blood sample deriving from an adult female with a hemolytic crisis of unknown origin, partially deteriorated during transport, a similar fraction was demonstrable in the hemolysate corresponding to 37% of the total hemoglobin. At pH 6.5 this fraction migrated more slowly towards the cathode than Hb A_1. After the hemolytic crisis no abnormal hemoglobin was detectable in erythrocytes and plasma.

A corresponding hemoglobin fraction can easily be produced in vitro. In a fresh artificial mixture of normal hemolysate with normal plasma no abnormal hemoglobin is demonstrable, but after storage of the mixture at + 4°C for 10 days, a fast-moving hemoglobin component appears with the mobility described here above.

In all these conditions the same fractions were obtained when the hemoglobin was converted into methemoglobin, methemoglobin cyanide or carboxyhemoglobin. We must assume that, after hemolysis with consumption of the whole plasma haptoglobin, a certain alteration or combination of the free hemoglobin is produced slowly in vitro and more rapidly in vivo. Methemalbumin and hemoglobin bound to haptoglobin can be excluded, and the complex of hemoglobin with glutathione formed normally in old hemolysates never represents a fraction with sharp separation in electrophoresis. Further investigations are in progress to clarify the nature of this pseudoabnormal hemoglobin[1].



Starch block electrophoresis pH 8.6. Left and right: normal hemolysate. Middle: plasma after hemolytic crisis in paroxysmal nocturnal hemoglobinuria (case 1). The benzidine reaction reveals free Hb A_1 and a fast-moving component. Methemalbumin is removed.

Zusammenfassung. Es wird ein pseudoanomales Hämoglobin beschrieben, das bei Zirkulation von freiem Hämoglobin im Plasma entsteht und im Urin ausgeschieden wird. Es handelt sich um eine sekundäre Veränderung des Blutfarbstoffes, die von der Haptoglobin- und Glutathionbindung verschieden ist.

H. R. MARTI

Medizinische Universitätspoliklinik, Basel (Switzerland), January 18, 1965.

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Influence of Thiol-Inhibiting Substances on the Effects of Ethyl Methane Sulphonate (EMS) on Chromosomes

Copper and zinc ions were demonstrated to enhance the aberration rate produced by EMS on chromosomes[1]. Other ions were also reported to modify the chromosome breaking ability of mutagenic compounds of the mesyloxy group. The modification was stronger for monofunctional than for difunctional compounds[2].

The problem arises of the possible mechanisms by which ions can modify the chromosome breaking ability of EMS.

On the one hand, for copper and zinc, the possibility of a chemical reaction with EMS leading to a new substance with changed mutagenicity was ruled out[3]. The maximum synergistic effect was found when the ions were added before EMS treatment[3]. This effect was pH- and temperature-dependent.

On the other hand, it was supposed that some ions could interact with the activity of some enzymatic sys-

[1] J. and M. MOUTSCHEN-DAHMEN, Exper. *19*, 144 (1963).
[2] J. MOUTSCHEN, Thesis, Université Liège (1964).
[3] J. and M. MOUTSCHEN-DAHMEN, Rad. Bot. *3*, 297 (1963).

tems which would consequently modify the reactivity of the alkylating agent at the chromosome level, or the rejoining capacity of the chromosome fragments.

In order to discriminate between these possibilities, substances known to inhibit more specifically enzymatic reactions were tested.

Substances reacting with thiol groups are numerous (for a review see [4] and [5]). Sublimate ($HgCl_2$) and neoarsphenamine (sodium salt of *m*-diamino-*p*-dioxy-arsenobenzene methylene sulphoxylic acid) were chosen for the present investigation.

Dry barley seeds (caryopsis) of var. piroline and dry *Vicia faba* ssp. minor var. Åkerböna Weibull seeds were treated for 2 h at doses ranging from $^1/_{16} \cdot 10^{-5} M$ to $4 \cdot 10^{-5} M$ for $HgCl_2$ and $1 \cdot 10^{-4} M$ to $1 \cdot 10^{-2} M$ for neoarsphenamine.

After washing with running bidistilled water, the material was immersed in a solution containing 0.025 g to 0.8 g EMS per 100 ml bidistilled water. Seeds were grown as described in a previous paper[1]. Chromosome aberrations were observed and recorded during the first mitotic cycle after germination.

For metaphase observations in *Vicia*, seeds were treated successively with $HgCl_2$ $10^{-5} M$ for 3 h and EMS 0.4 g for 2–3 h, and root tips were afterwards treated with a colchicine solution (0.05 g per 100 ml for $1^1/_2$–2 h) before fixation (Carnoy for 2 h).

Preliminary experiments were designed to measure the toxicity of the two salts and to adjust concentrations which would not by themselves induce chromosome aberrations.

Concentrations of sublimate and neoarsphenamine respectively higher than $10^{-4} M$ and $10^{-2} M$ should be avoided owing to their toxicity.

In Figure 1a and 1b it can be seen that increased concentrations of $HgCl_2$ and neoarsphenamine can influence the amount of abnormal anaphases produced by a single dose of EMS (0.4 g%).

These curves reach a plateau. It shows that it is not necessary to use concentrations higher than $2 \cdot 10^{-5} M$ for $HgCl_2$ and $10^{-3} M$ for neoarsphenamine.

It could be proved that at the optimum concentration, the amount of damage is proportional to EMS concentration (Figure 2a and 2b).

This conclusion was also reached for Cu^{++} and Zn^{++}[1].

[4] Z. M. Bacq, Exper. *2*, 349 (1946).

[5] Z. M. Bacq, Actual. biochim., vol. 8 (1947).

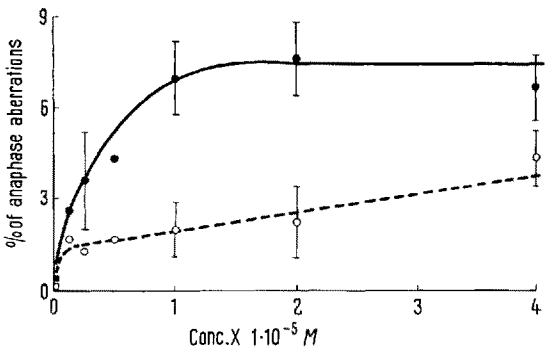

Fig. 1a. *Hordeum sativum* – Percentages of chromosome aberrations at anaphase for different $HgCl_2$ concentrations (300 anaphases). EMS concentration: 0.4 g/100 ml. White circles: salt alone. Squares: controls.

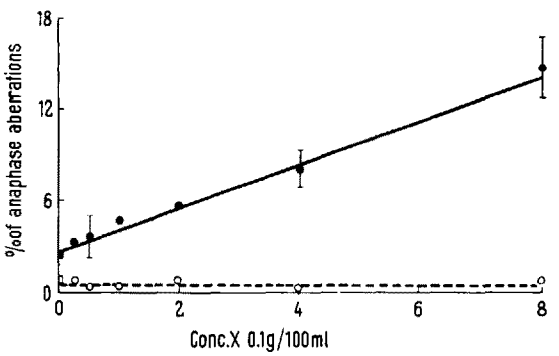

Fig. 2a. *Hordeum sativum* – Percentages of chromosome aberrations at anaphase for different EMS concentrations (300 anaphases). $HgCl_2$ concentration: $1 \cdot 10^{-5} M$. White circles: salt alone. Squares: controls.

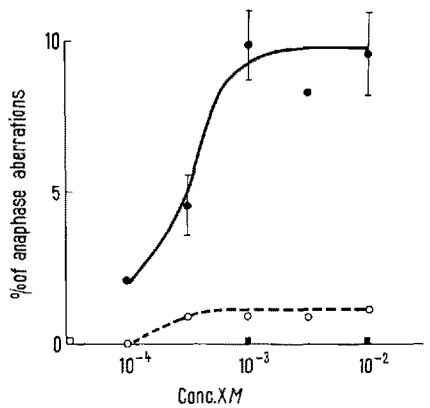

Fig. 1b. *Hordeum sativum* – Percentages of chromosome aberrations at anaphase for different neoarsphenamine concentrations (300 anaphases). EMS concentration: 0.4 g/100 ml. White circles: salt alone. Squares: controls.

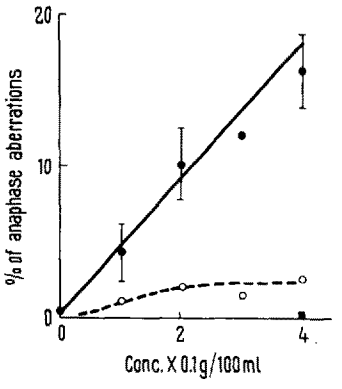

Fig. 2b. *Hordeum sativum* – Percentages of chromosome aberrations at anaphase for different EMS concentrations (200 anaphases). Neoarsphenamine concentration: $1 \cdot 10^{-2} M$. White circles: salt alone. Squares: controls.

As in the case of these two ions, it could be proved that the influence of the two investigated compounds was dependent on the pH of the solution (Figure 3a and b). For Hg^{++}, an optimum exists at pH 7.3, whereas for neoarsphenamine the activity seems to increase continuously at alkaline pH within the limits of the doses investigated.

The activity of these compounds is lower than for copper and zinc. A limiting factor for Hg^{++} is its toxicity. The results about the pH dependance could be fairly compared with those obtained with Cu^{++} and Zn^{++}, where the optimums were respectively pH 7.8 and 7.2.

The analysis of metaphase damage was carried out with *Vicia faba*. With $HgCl_2$, it was found that in this species the synergistic effect is still more evident than for barley.

In Figure 4, it can be seen that all classes of chromatid aberrations are involved in the effect. The increase is higher for two hit aberrations (quadriradial configurations and intrachanges). A sharper analysis showed that all classes of sister-unions, whether centric or acentric, are well represented. For breaks, increase is mainly due to isolocus, the proportion of chromatid breaks being rather low. In this investigation, no chromosome aberrations of the two hit type inter alia dicentrics or rings could be detected. It can be stated that this class of aberrations is not at all affected.

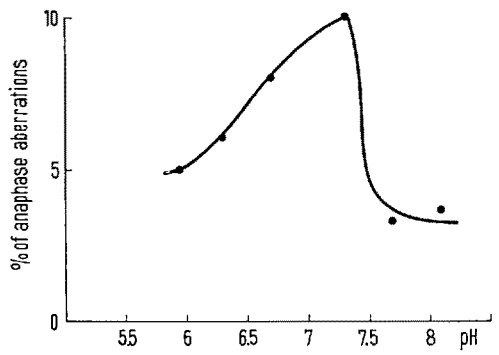

Fig. 3a. *Hordeum sativum* – Influence of pH (Sørensen buffer solutions) on the synergism (300 anaphases). $HgCl_2$ concentration: $1 \cdot 10^{-5} M$. EMS concentration: 0.3 g/100 ml.

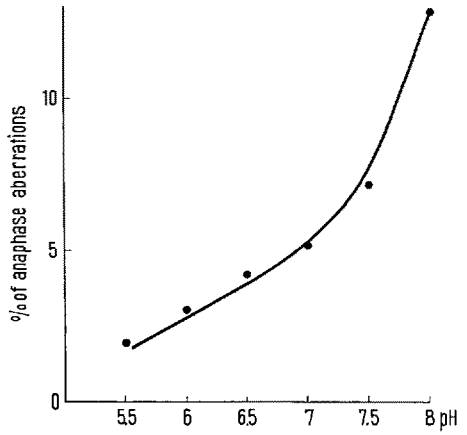

Fig. 3b. *Hordeum sativum* – Influence of pH (Sørensen buffer solutions) on the synergism (300 anaphases). Neoarsphenamine concentration: $1 \cdot 10^{-2} M$. EMS concentration: 0.4 g/100 ml.

From these observations it can be concluded that in *Hordeum* and in *Vicia*, thiol-inhibiting substances, sublimate and neoarsphenamine, interacted with the effects of EMS on chromosomes. A clear synergistic effect was obtained. For sublimate the interaction seemed to be more significant than for neoarsphenamine. As far as we can see, the synergistic effect dealt with the chromatid class of aberration. This last observation provided information on the period of the mitotic cycle at which the action is maximum.

In recent experiments, which are now extended, BAL (2,3-dimercaptopropanol) at $1 \cdot 10^{-4} M/3$ h was given to seeds after successive treatment with sublimate and EMS. In these experiments, the effects were completely suppressed. These results, reproduced in several conditions, reinforce the assumption that the thiol-inhibiting process is actually involved; this stimulates new research to detect which enzymatic systems might be responsible for the synergistic effect at the chromosome level[6].

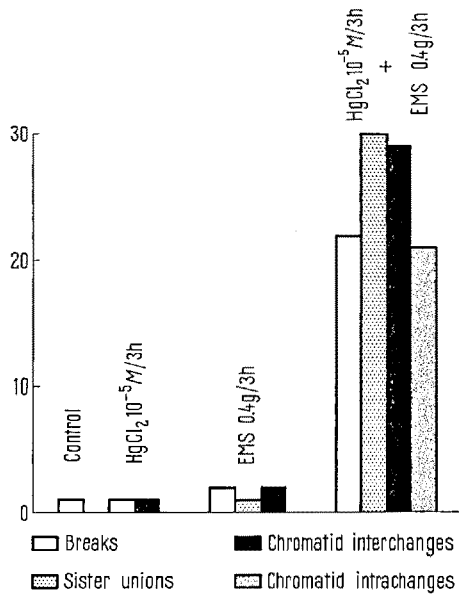

Fig. 4. *Vicia faba* – Distribution of metaphase aberrations (pH 7). $HgCl_2$ concentration: $1 \cdot 10^{-5} M$. EMS concentration: 0.4 g/100 ml.

Résumé. Des semences d'orge et des graines de fèverolle ont été traitées par des solutions de sublimé corrosif et d'un arsénoxyde avant traitement par du méthane sulfonate d'éthyl. Chacun des ions accroît considérablement les effets de l'EMS sur les chromosomes d'une manière dépendant du pH, de la concentration en ions et de la concentration en EMS.

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Laboratoire de Génétique, Université de Liège (Belgique), November 23, 1964.

[6] We wish to express our gratitude to Professor L. EHRENBERG for valuable suggestions. This work was subsidized by the Centre National d'Etude des Mutations (Belgique).