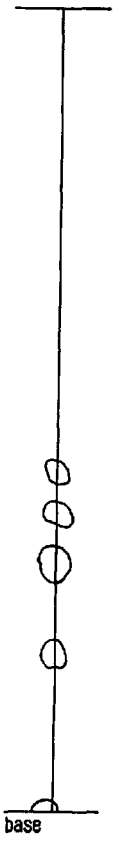
is probably a peptide and the other two have Rf values 0.30 and 0.20. At a much later stage (0.75 cm in length), the snails show precisely these two spots with enormously enhanced intensity. Adult or big snails (above 1 cm) show four spots with Rf values 0.42, 0.37, 0.31, 0.21 (0.20) (see Figure). (The solvent for all these was *n*-Butanol-acetic acid-water :: 4:1:1.) As the Rf-values alter considerably with temperature, they were redetermined in a room of constant temperature (about 25°C) and they turned out to be 0.34, 0.28, 0.22, 0.14.



Thus, the biochemical pattern of free amino acids is almost established at practically the hatching time and it is only accentuated up to a comparatively late stage. In this case, the position is quite different from that seen by KAVANAU[6], CHEN[7], and BRAHMACHARY[8] who detected a large number of amino acids in sea-urchin embryo, urodele embryo and germinating seeds of Mung bean. However, a second pattern of faint spots (detected only with chromatography) with higher Rf values is evident in snails above the size 1.45 cm. It is interesting to note that, even after starvation of two weeks, the pattern (including the second, fainter set) remains unaltered. This has been verified also with another species, namely *Viviparous Sp.* Unless the catabolic rate itself slows down in the starving snails, this might mean that the free amino acid pattern is maintained by breaking down some of the proteins. This would be further evidence for the 'stubborn' gene determined biochemical pattern of individuality[9].

Résumé. Les auteurs étudient l'état des acides-aminés libres aux différents stades de développement de l'escargot (Gastéropode) *Limnéa.* Cet état est constant de l'éclosion jusqu'à un stade avancé; ce n'est qu'après celui-ci que l'on trouve de nouveaux acides-aminés libres. Par contre, le modèle-type se maintient inchangé dans les animaux affamés.

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Research and Training School, Indian Statistical Institute, Calcutta, and Gerontological Research Unit, Indian Statistical Institute, Calcutta (India), July 11, 1962.

[8] Unpublished data.

[9] We are very thankful to Prof. E. HADORN and Prof. P. S. CHEN for their kind suggestions, and to Dr. P. R. PAL and Mr. S. BHATTACHARYA for extending to us the requisite laboratory facilities.

Influence of Cu^{++} and Zn^{++} Ions on the Effects of Ethyl Methanesulfonate (EMS) on Chromosomes

The chromosome-breaking activity of ethyl methanesulfonate (EMS) is still somewhat controversial, partly due to differences in experimental conditions. Some workers[1] have reported this monofunctional alkylating agent as causing chromosomal rearrangements, whereas others have not found such an activity[2].

In our own experiments, variations in the effects were observed on chromosomes as well as on seed germination and on plant growth. It was demonstrated that these effects may be (or are) dependent on experimental conditions such as temperature, duration of exposure and some water contaminations. In order to test this last possibility, distilled waters from several origins were investigated. Sharp differences were found to occur, which was an incitement to further investigations.

Those water fractions in which EMS exhibited an increased activity, were regularly found to be contaminated by copper or zinc or both together.

On the basis of these observations, the ions mentioned were tested systematically. To about 10^{-3} m*M* solutions of $CuSO_4$ or $ZnSO_4$ in bi-distilled water, EMS was added at several concentrations. When needed, pH was controlled by means of 0.1 molar Sørensen buffers. These solutions were used for treatments of resting seeds of *Vicia faba* for 3 h.

The following points could be clarified, using an anaphase scoring technique in *Vicia faba*: (1) Solutions containing neither ion under investigation were found to be inefficient in inducing chromosome aberrations. (2) Solutions containing either Cu^{++} or Zn^{++} were found to be efficient in this respect, the synergistic effect of the copper ions being greater than that of zinc (Figure 1). (3) Copper and zinc containing control solutions showed some activity (Figure 1), much higher for Zn^{++} than for Cu^{++}. These results are in agreement with VON ROSEN'S

[1] R. RIEGER and A. MICHAELIS, *Die Kulturpflanze* (Akademie-Verlag, Berlin 1960), vol. 8, p. 230.

[2] E. A. FAVRET, cited in Hereditas *46*, 622 (1960).

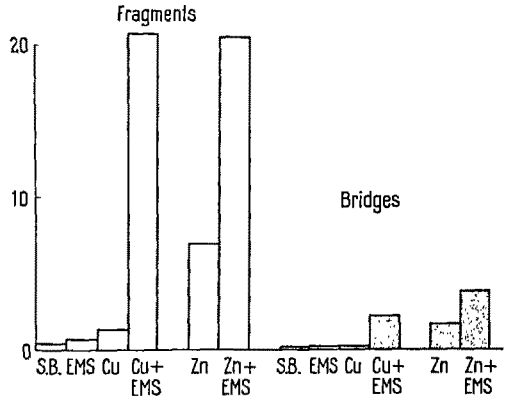

Fig. 1. Percentage of fragments and bridges with different solutions (S.B. = Sørensen buffer solution, see in the text).

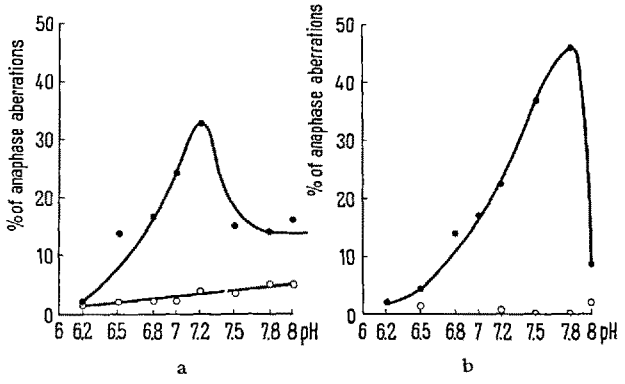

Fig. 2a. Percentage of anaphase aberrations at different pH. • Zn + EMS, ○ Zn (control) (± 300 anaphases).
Fig. 2b. Percentage of anaphase aberrations at different pH. • Cu + EMS, ∘ Cu (control) (± 300 anaphases).

data on ion effects on root tips[3]. (4) The effect of the pH on this combined action was investigated and an optimum was found to occur around pH 7.8 for copper and pH 7.2 for zinc (Figure 2, a and b). In these experiments Sørensen buffer solutions alone were found to be practically inactive (Figure 1). (5) The chromosome-breaking activities of Cu^{++} and Zn^{++} depend upon the concentration of EMS (Figure 3, a and b). At doses as high as 4 g EMS per 100 ml water, the germination was so affected that no cytological observation could be made.

The present results seem to eliminate the possibility of action of a reaction product between the cation and the product of hydrolysis of EMS, i.e. methanesulfonic acid (although it is not excluded, of course, that EMS or methanesulfonate anion may influence, by complex formation, the uptake of the ions). The fact that the biological effect is obtained at low concentrations of the cation,

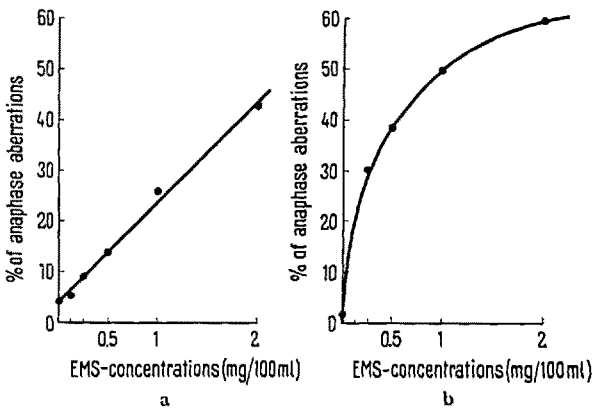

Fig. 3a. Percentage of anaphase aberrations with Zn at increased EMS-concentrations (mg/100 ml) (± 300 anaphases).
Fig. 3b. Percentage of anaphase aberrations with Cu at increased EMS-concentrations (mg/100 ml) (± 300 anaphases).

that it is pH-dependent, and that it increases with the EMS concentration, seems to favour an explanation of the effect as some kind of enzyme inhibition. Cu^{++} and Zn^{++} are in fact well known to interfere with enzymatic reactions. The present investigations also show that the ions by themselves cannot explain the chromosome-breaking activity in which it is clear that EMS itself is playing a role.

Other independent experiments to be reported elsewhere lead to the same conclusions. The chromosome-breaking mechanisms are being investigated.

The data presented here demonstrate that it would be worth using well standardized conditions in mutagenesis with EMS[4].

Résumé. La capacité de l'EMS de casser les chromosomes n'existe que lorsque des ions ont été ajoutés pendant les traitements. A ce point de vue, Cu^{++} et Zn^{++} se révèlent très efficaces. Cette réaction dépend du pH et varie avec la concentration ionique et la concentration d'EMS. On donne une explication probable de ces faits.

J. and M. Moutschen-Dahmen

Genetics Department of the Royal Forestry College, Stockholm (Sweden); Institute of Radiobiology of the University of Stockholm; and Genetics Department of the University of Liège (Belgium), October 17, 1962.

[3] G. von Rosen, Hereditas *40*, 258 (1954).

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Un cas nouveau de polymorphisme chromosomique intraspécifique chez un Mammifère (*Leggada minutoides* Smith. Rodentia-Muridae)

Le seul exemple certain de polymorphisme chromosomique intraspécifique chez un Mammifère est actuellement connu, celui de l'Insectivore *Sorex araneus* L.[1] où il se manifeste sous la forme d'une variation robertsonienne affectant plusieurs paires d'autosomes, d'où des formules diploïdes allant de 21 à 31 (♂), de 20 à 30 (♀).

[1] G. B. Sharman, Nature *177*, 941 (1956). - C. E. Ford, J. L. Hamerton et G. B. Sharman, Nature *180*, 392 (1957). - A. Meylan, Rev. suisse Zool. *68*, 258 (1960). - R. Matthey et A. Meylan, Rev. suisse Zool. *67*, 223 (1961).