

demonstrates the log dose related alterations in both hypophysial and serum LH values. Intact animals showed a prominent depletion of pituitary LH with a concomitant rise in serum LH; in contrast, ME-lesioned recipients exhibited marked repletion of pituitary LH with simultaneous increments in serum LH.

These data provide strong evidence supporting the concept that a specific hypothalamic hormone can control the synthesis and release of pituitary LH. Initial substantiation was provided by the fact that ME lesions not only reduced pituitary LH stores, but also concomitantly lower-

ed serum levels of this gonadotropic hormone. Further substantiation was obtained from the effect of synthetic LRF employed as the stimulus. The present results agree with those reported by us²⁻⁴ concerning the effect of crude hypothalamic extracts on pituitary FSH, and support other reports¹⁰⁻¹³ pertaining to a dual effect of hypothalamic neurohormones on the synthesis and release of pituitary tropic hormones. Furthermore, the present data clearly demonstrate that both synthesis of LH and its release into the blood can occur simultaneously¹⁴.

Résumé. Des rats mâles intacts ou ayant une lésion du ME ont reçu une seule injection intrajugulaire de LRF synthétique. Les variations de taux de LH hypophysaire et sérique (en fonction du temps et de la dose réponse) montrent que le LRF contrôle la sécrétion (la décharge et la synthèse) du LH hypophysaire.

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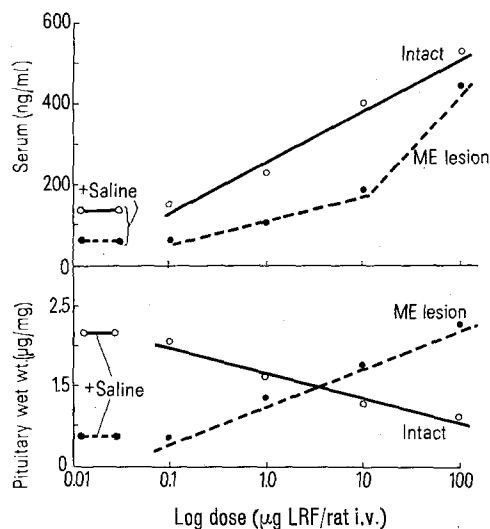


Fig. 2. Dose-response study: Effect of various doses of synthetic LRF on pituitary and serum LH of intact and ME-lesioned mature male rats.

¹⁰ Y. C. LIN, M. TAKAHASHI and Y. SUZUKI, *Endocrinologia jap.* 19, 145 (1972).

¹¹ W. C. WORTHINGTON JR., S. E. FOLSOM JR. and M. G. BUSE, *Endocrinology* 90, 1664 (1972).

¹² T. W. REDDING, A. V. SCHALLY, A. ARIMURA and H. MATSUO, *Endocrinology* 90, 764 (1972).

¹³ H. STEINER, M. MOTTA, F. PIVA, D. GILLESSEN and R. O. STUDER, *Acta endocrin. Copenh. Suppl.* 155, 5 (1971).

¹⁴ The technical assistance of K. KNUDSEN, J. BELL, C. CAVALCANTO, K. KOCH and J. TRACY is gratefully acknowledged.

Sensitivity to Mutagens of *Rumex acetosa* Chromosomes

Data on the radiosensitivity of several species of the genus *Rumex* have been reported¹. Some of these species were found to be highly sensitive due especially to their large nucleus and chromosomes. Few data on the effects of chemicals on such chromosomes have been reported so far. Only a difunctional alkylating agent, diepoxybutane, was tested².

On the other hand, several authors described the ability of some chemicals to break preferentially the sex

chromosomes of mammals. Since the occurrence of a $X Y_1 Y_2$ mechanism of sex determination has been demonstrated in *Rumex acetosa*³, it was worthwhile getting information on the sensitivity of such chromosomes to mutagens. For this reason, we selected two chemicals well known for their chromosome-breaking ability, methyl-methane-sulfonate and methyl-nitroso-urea. Their effects will be compared with those of ionizing radiations.

Material and methods. *Rumex acetosa* L. cultivar 'Large de Belleville' was chosen for the present investigation. Karyotype of this species, as well as some karyological particularities, were previously described⁴. Dry seeds were irradiated by ⁶⁰Co γ -rays (25°C, dose-rate 300 krad/h) at doses ranging from 100 to 3000 rads. Methyl-methane-sulfonate (MMS, Eastman Kodak) was used at concentrations ranging from 1×10^{-3} M to 1×10^{-2} M for 3 h and methyl-nitroso-urea (MNU, synthesized by the Biochemical Institute of Stockholm) at concentrations ranging from 5×10^{-4} M to 5×10^{-3} M/3 h. All the solutions were prepared extemporaneously. After this, seeds were abundantly washed, then sown on moistened filter paper in Petri dishes and incubated at 21°C. After 2 days, primary

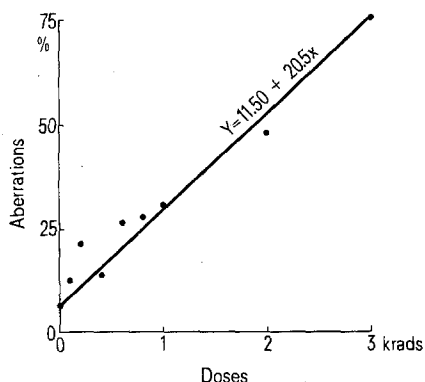


Fig. 1. Effects on ⁶⁰Co γ -rays on *Rumex* chromosomes (400 metaphases analyzed at each exposure).

¹ S. ICHIKAWA and A. SPARROW, *Genetics* 54, 341 (1966).

² J. ZUK, *Heredity* 24, 69 (1969).

³ H. KIHARA and T. ONO, *Bot. Mag., Tokyo* 37, 84 (1923).

⁴ J. MOUTSCHEN, N. DEGRAEVE and B. MONFORT, *Cytologia* 37, 119 (1972).

Difference of sex response to the three mutagens (all data of Figures 1 and 2 pooled)

Treatment	No. of aberrations in			Probability ($df = 1$)	No. of aberrations in			Probability ($df = 1$)
	♂	♀	χ^2		Sex chromosomes	Autosomes	χ^2	
γ -Rays (100 to 3000 rads)	712	427	48.22	$P < 0.001$	149	440	1.19	$0.20 < P < 0.30$
MMS ($1 \times 10^{-3} M$ to $1 \times 10^{-2} M$)	496	410	2.29	$0.10 < P < 0.20$	263	489	22.32	$P < 0.001$
MNU ($5 \times 10^{-4} M$ to $5 \times 10^{-3} M$)	535	401	9.10	$0.001 < P < 0.01$	315	553	35.36	$P < 0.001$

root tips (about 5 mm long) were immersed in colchicine (0.05 g/100 ml) 2 h, fixed (Carnoy or Battaglia). Slides were prepared from Feulgen squashes overstained with Giemsa when needed, then mounted in DePeX.

Results. A relatively high frequency of spontaneous chromosomal aberrations was generally observed in *Rumex acetosa*. The dose-response curve for the total number of aberrations induced by γ -rays is almost linear between 100 and 3000 rads (Figure 1). Most of the aberrations belong to the chromosome class i.e. chromosome breaks, dicentric and rings. Occasionally, chromatid exchanges are observed (Figure 3).

The frequency of aberrations was significantly higher in the male plants, although differences with exposure could be observed, taking into consideration the relative length of male and female genomes (Table).

The dose-response curves to MMS and MNU (Figure 2) show a linear relationship within the limits of the concen-

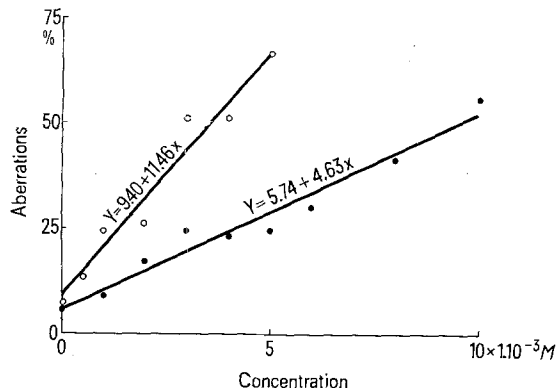


Fig. 2. Comparison of effects of MNU (○—○) and MMS (●—●) on *Rumex* chromosomes (400 metaphases analyzed at each concentration).

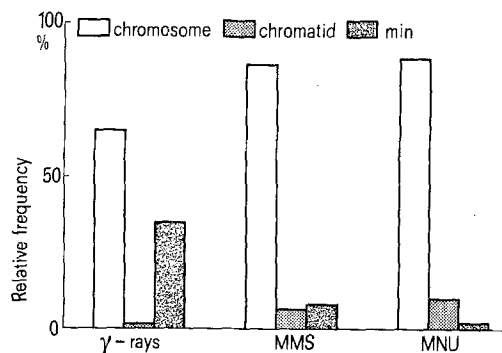


Fig. 3. Distribution of different types of aberrations after the 3 mutagens (data of all the treatments pooled).

trations investigated. The efficiency of MNU is greater than MMS, the D_{50} being respectively 1860 rads for γ -rays, $9.5 \times 10^{-3} M$ for MMS and $3.55 \times 10^{-3} M$ for MNU. The sensitivity of male cells, although varying with the concentration, was higher than the sensitivity of female cells after both treatments (Table).

The spectrum of different chromosomal aberrations was analyzed and found to be different after the use of each mutagen (Figure 3). The proportion of minutes is much higher after γ -rays. On the other hand, the proportion of chromatid aberrations is higher after the use of each chemical. The distribution of lesions along the genome, for both chemicals and γ -rays, is also different (Table). After γ -rays, the aberrations were distributed at random, whereas after chemicals they were preferentially localized in the sex chromosomes.

Discussion and conclusion. As concluded from a previous investigation⁵, *Rumex acetosa* can be considered as a sensitive species to both ionizing radiations and chemicals. The factors of sensitivity can be manifold. As stated by SPARROW et al.⁵, it is clear that nucleus and chromosome volumes can play a role. Other more physiological variables may also be at hand which would explain e.g. the differential sensitivity of both sexes.

It is likely that a larger nucleus volume of the male may be due to the occurrence of two Y chromosomes. It is also likely that sex chromosomes or specific parts of them show a higher sensitivity than autosomes. This picture could be somewhat complicated by the occurrence of some heterochromatic regions in the autosomes. Alternatively the differential heterochromatic balance in the male or female could evidently influence the sensitivity by way of metabolic modifications.

Résumé. Des graines de *Rumex acetosa* ont été soit irradiées par les rayons γ du ^{60}Co (100 à 3000 rads) soit traitées par deux agents mutagènes chimiques: le MMS et le MNU. Le MNU est plus efficace que le MMS comme inducteur de lésions chromosomiques. Les spectres des différents types d'aberrations chromosomiques produites par les 3 agents sont différents et les cellules mâles sont plus sensibles que les cellules femelles. Une sensibilité supérieure des chromosomes sexuels aux deux agents chimiques a été mise en évidence.

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8 February 1973.

⁵ A. SPARROW, R. SPARROW, K. THOMPSON and L. SCHAIER, Radiat. Bot. Suppl. 5, 101 (1965).

⁶ Recherche réalisée sous les auspices du Fonds de la Recherche Fondamentale Collective, Belgique.