THE EFFECT OF X RAYS ON ROOT MERISTEM OF BROAD BEAN *(VICIA FABA)*

I. THE RELATIVE AMOUNT OF NUCLEAR DNA AFTER IRRADIATION

BY

CONSTANȚA SPÂRCHEZ, V. SORAN and Z. URAY

Broad bean *(Vicia faba)* root meristem of 5-day-old seedling was irradiated with X rays in the following doses: 50, 100, 150, 200, 300 and 500 R. The relative amount of DNA was measured by cytophotometrical techniques using the Feulgen reaction. The double wave-length method was used. The results show a slight increase of the DNA amount at 50 R, after 24 and 48 hours. At 100, 150 and. 200 R, the relative amount of DNA per nucleus decreased in exponential manner. At 300 and 500 R, the amount of nuclear DNA increased again. This increase may be related to the formation of new meristemic cells from the "quiescent zone" of the root.

The relationship between the DNA amount per nucleus, the synthesis of DNA and the changes of cellular cycle after ionizing radiation is known [1], [9], [13]. The research carried out so far has proved that ionizing radiations (X and gamma rays) inhibit the DNA synthesis within the nucleus and causes the retardation of cell division, thus changing the duration of cell cycle.

The researches on the DNA synthesis were usually made with tritiate thymidine. However, some old and new investigations [7], [9], [10] proved that Feulgen reaction may also be used for the nuclear DNA amount estimation after ionizing radiation.

Our investigations deal with the variations of nuclear DNA content within the root meristem of broad bean *(Vicia faba)* after X-ray irradiation. The amount of DNA was measured by cytophotometry of Feulgen reaction.

MATERIAL AND METHODS

The seeds of broad bean *(Vicia faba)* were soaked for 24 hours, in tap water. They were germinated on moistened filter paper, on Linhardt dishes. When the primary root was 2—3 cm in length, i.e. 5 day old, the root tops were irradiated with the following doses: 50, 100, 150, 200, 300 and 500 R. The irradiation was performed with the therapeutical apparatus TUR I, 180 kv, 10 mA, 1 Cu, CD, DFO-40 cm.

After 24 and 48 hours of irradiation, the meristemic zone of the root was fixed in Carnoy fluid for 24 hours. After fixation, the plant material was washed for 24 hours in continuous running water.

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The hydrolysis was made at room temperature (18—20°C) with 5 N HCl; the hydrochloric acid was removed by washing the plant material thrice with distilled water for 10—15 minutes each time. The staining lasted for $2^1/_2$ hours at dark in Schiff reagent. After staining, the root top was squashed on a microscopic slide of 0.6—0.8 mm thickness.

The relative amount of DNA per nucleus was expressed in arbitrary units. The measurements were performed at Leitz Ortholux Cytophotometer MPE, using the double-wave-length method according to Patau [15], Ornstein [14] and Mendelsohn [11]. We used 500 and 479 nm as pair wave-lengths.

The obtained data were statistically reckoned involving the arithmetical mean, standard deviation and significance of the difference between various data according to Student parameter.

In some cases, histograms analyses after various doses of irradiation were made.

RESULTS AND DISCUSSIONS

The results of our investigations are shown in Figs. 1—5. Figures 1 and 2 show the effect of X-ray irradiation in various doses on nuclear DNA amount within meristemic cells of broad bean root, after 24 and 48 hours of irradiation. The data are given separately for 3 kinds of nuclei : *a*) prophase nuclei, *b*) telophase nuclei (corresponding to the end of cell division) and *c*) interphase nuclei. It was proved that the smallest doses of irradiation, i.e. 50 R, caused a slight increase of the relative amount of DNA. This increase is sometimes significantly different from the control, and probably it is due to some perturbation induced by irradiation. We tried to calculate the probability of DNA synthesis inactivation using the formula : $W(D) = 1 - N/N_0$ in which W(D) is the probability of DNA synthesis inactivation depending on the applied doses, N is the relative amount of DNA after irradiation and N_0 is the relative amount of DNA in the control [8]. The result suggests that no inactivation of DNA synthesis occurred at 50 R, the probability of inactivation being close to zero.

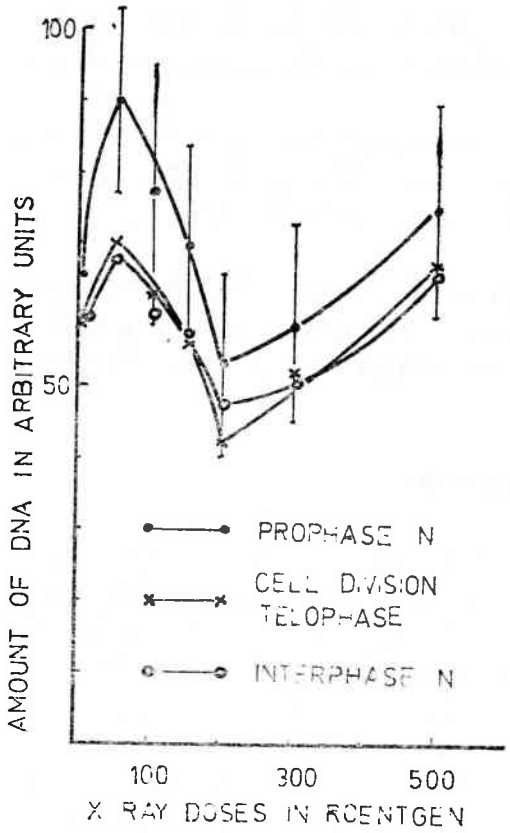

Fig. 1. — The action of various doses of X-ray on the nuclear amount of DNA within root meristemic cells of broad bean *(Vicia faba)* after 24-hour irradiation.

At 100, 150 and 200 R, the amount of DNA per nucleus decreased according to a dose-effect curve and the decrease of values may be related to a negative exponential curve according to the formula : $N = N_0 . e^{-\alpha . D}$ in which N_0 is the relative DNA amount in the

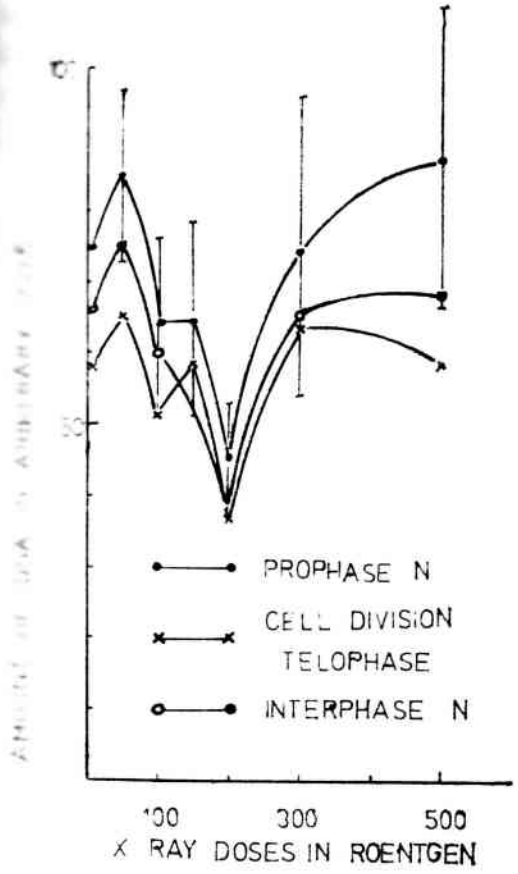

Fig. 2. — The action of various doses of X-ray on the nuclear amount of DNA within root meristemic cell of broad bean (*Vicia faba*) after 48-hour irradiation.

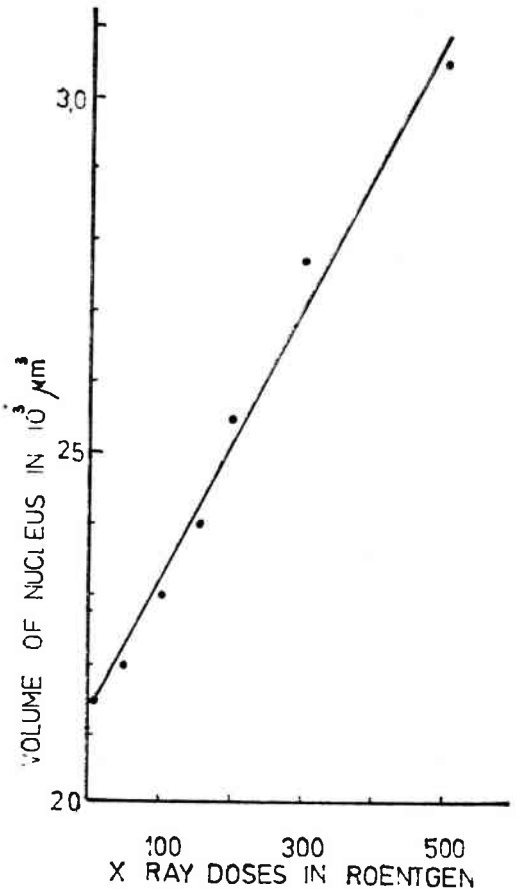

Fig. 3. — The relationship between applied doses of X-ray and the volume of nuclei.

control, N is the relative DNA amount in the irradiated material, D are the applied doses, $-\alpha$ is the probability of hitting the "target", and e is the base of natural logarithms (2.71828).

Quite unexpectedly, at 300 and 500 R, both after 24 hours and 48 hours of irradiation, the relative amount of DNA increased again. It was mainly at 500 R that the relative amount of DNA reached the values of the control. This result suggests that something had happend within the broad bean meristemic cells. We notice (unpublished data) that such an increase of the DNA amount per nucleus has never occurred when the dried seeds were irradiated. The only possible explanation for this increase is given by Clowes' data [2], [3], [4], [5], [6] concerning the role of the so-called "quiescent centre" in the renewing of the active cells of the root meristem, damaged or destroyed by irradiation. We think that after irradiation, at 300 and 500 R, most cells from our squash belonged to former quiescent zone cells. However, the nuclei of these cells are not normal because their volumes show continuous increase, depending on the applied doses (Fig. 3). The histograms in Figs 4 and 5 explain this increase of the nuclear volume by the increase of chromosome number and tetraploidy. Therefore, the cells from the quiescent zone have suffered

some damage or influence caused by irradiation. They did not show decrease of the relative DNA amount, but a significant lowering of the mitotic index, an increase of chromosomal aberrations or retardation of cell division. As a first reaction to irradiation, they increased the amount of DNA [5].

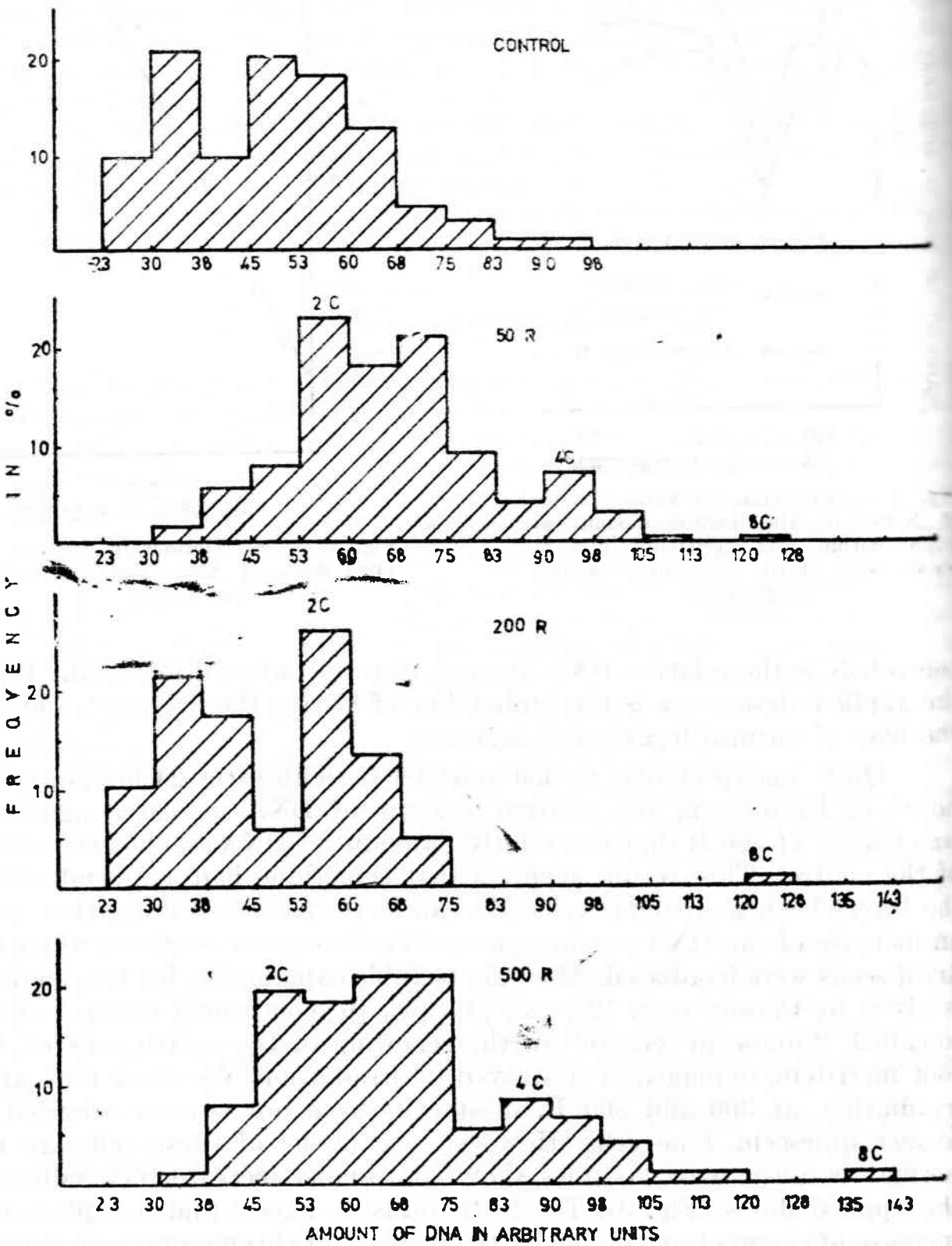

Fig. 4. — Histograms of DNA distribution at several doses of irradiation by X-ray, after 24 hour irradiation.

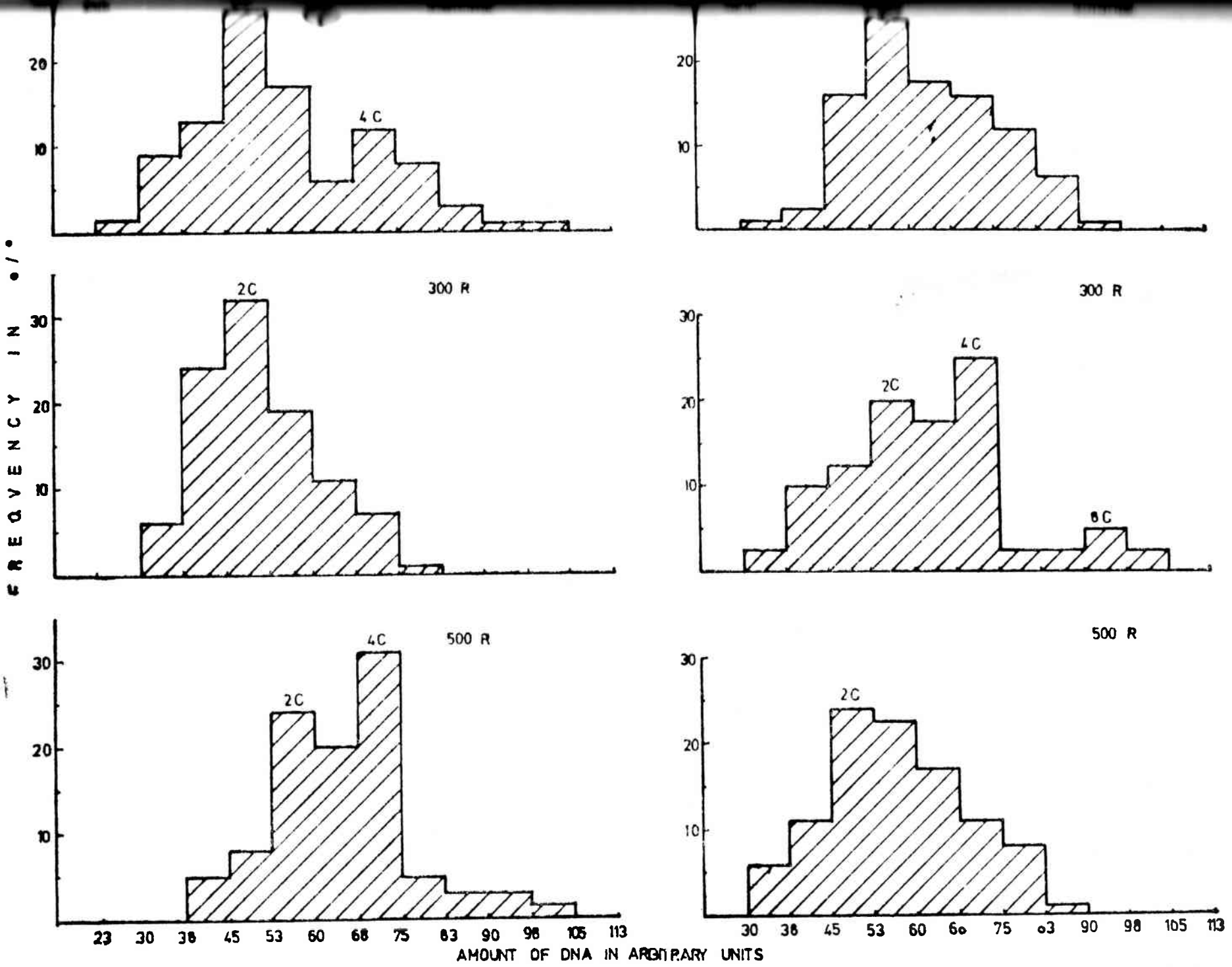

Fig. 5. — Histograms of the DNA distribution of several doses of irradiation by X-ray, after 24- and 48-hour irradiation.

CONCLUSIONS

1. The Feulgen reaction may be used as a measure for radiation on the nuclear DNA.

2. The variation of nuclear amount of DNA after irradiation in living plant material is very complex.

3. The smallest dose applied by us lead to a slight increase of the DNA amount, but this is probably not a real stimulation.

4. The doses between 100 and 200 R caused a decrease of the DNA amount per nucleus.

5. The increase of the DNA amount per nucleus at 300 and 500 R is probably due to the renewing of meristemic cells by the quiescent zone of the root.

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Centre for Biological Sciences
Str. Republicii 48
Cluj-Napoca,