



*The Interaction of Nucleic Acid and X-radiations
on the Chromatin of Rye and Pea**

LOUISE R. SIEBURTH
*Department of Agronomy
The State College of Washington*

HUSKINS (1948a) found chromosomes pairing and reduction divisions similar in essential respects to those of the germ cells in meristematic cells of *Allium* root tips treated with 8 per cent sodium ribose nucleate solution for 6 hours. Cells undergo regular reduction but by diverse processes: (1) chromosome pairing occurs with or without chiasmata formation; (2) anaphase separation is without a characteristic spindle; (3) segregation may occur at prophase without previous pairing. These varied features of meiosis found normally only in a wide range of organisms can be duplicated experimentally in a single plant. The spindle formation and kinomere orientation on it are abnormal, giving the appearance of a cell squashed in preparation in the first reduction division. The second division is commonly abortive (Huskins, 1948b).

The present study has two aims: (1) to duplicate the experiments of Huskins to determine if similar results could be obtained with rye and pea root tips; (2) to determine whether nucleate added before and after X-radiating would in any way modify the effects of the irradiation. As nucleate is part of the chromatin material in the nucleus, it was felt that there must be some interaction. Also, since X-radiations affect nucleic-acid-starved chromosomes, this seemed a plausible aim.

* This paper was submitted in partial fulfillment of the requirements for the degree of Bachelor of Science in Agriculture in Plant Breeding, Department of Agronomy, the University of British Columbia, April, 1949.

MATERIALS AND METHODS

The plant materials chosen were *Pisum sativum* and *Secale cereale*. Five treatments were set up as follows: (a) control; (b) X-radiation alone; (c) nucleate followed by X-radiation; (d) X-radiation followed by nucleate; (e) nucleate alone.

Rye and pea seeds were germinated on filter paper in petri plates until the primary root tips emerged. Peas took 3 days for primary root tip emergence, whereas rye root tips emerged in 2 days. Seeds given the two treatments, sodium ribose nucleate alone and nucleate followed by X-radiation, were immersed in an 8 per cent sodium ribose nucleate solution for a 16-hour pre-treatment. After this pre-treatment, seeds were X-radiated at 5000 r (140 KV) for the following treatments: (1) nucleate followed by X-radiation, (2) X-radiation followed by nucleate, and (3) X-radiation alone. In the treatment with nucleate following X-radiation a 14-hour immersion period was used. Nineteen hours after irradiation, rye and pea root tips were fixed in Carnoy's fluid and Randolph's Modified Navashin. Squash preparations were stained with aceto-carmin and paraffin sections with methyl violet.

Treatments using 200 r duplicated the above procedure except that the nucleate pre- and post-treatments were both for 21½ hours.

Since no cellular detail was observed in squash preparations of the 5000 r material, this step was omitted with the 200 r material.

OBSERVATIONS

Figure 1, rye cells 1, 2, and 3 show the characteristics of rye root tip cells which were X-rayed with 5000 r. Cell 1 shows two nucleoli and typical pycnosis. In cell 2, there were three nucleoli and pycnosis. Cell 3 shows a single nucleolus surrounded by pycnotic chromatin. Cells 4, 5, and 6 show the characteristics of rye root tip cells irradiated with 200 r. Cell 4 shows the type of cells which were found in the treatment, X-radiation alone. Chromatic "bands" were observed in the nucleolus and pycnosis. Cell 5 shows the effects of nucleate followed by X-radiation. The pycnotic chromatin is beginning to form chromosomes as in prophase of mitosis. Cell 6 shows the effects of X-radiation followed by nucleate. Some pycnosis is evident, but the chromatin is more evenly dispersed than in the treatment X-radiation alone, where the chromatin clumps together forming pycnosis. Cell 7 shows a cell from the control, which is in the energetic stage, in which the chromatic granules are

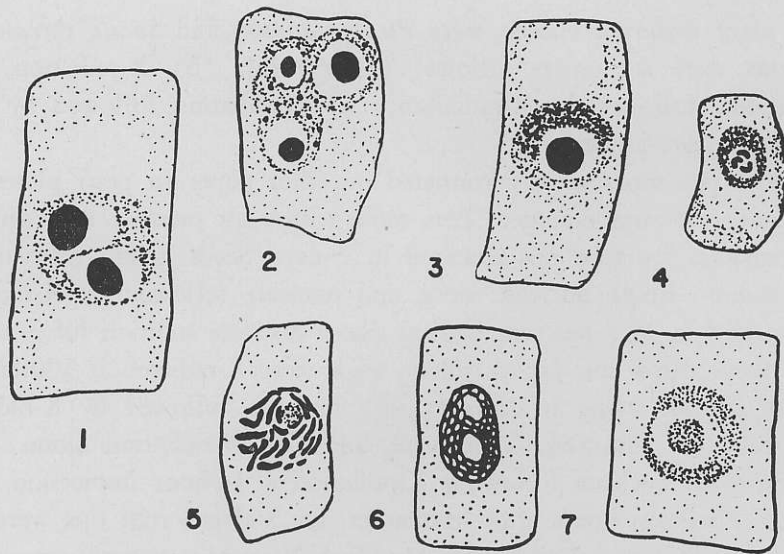


Fig. 1.—Rye cells 1-7 showing the effects of nucleic acid and X-radiations on the chromatin of rye root tip cells.

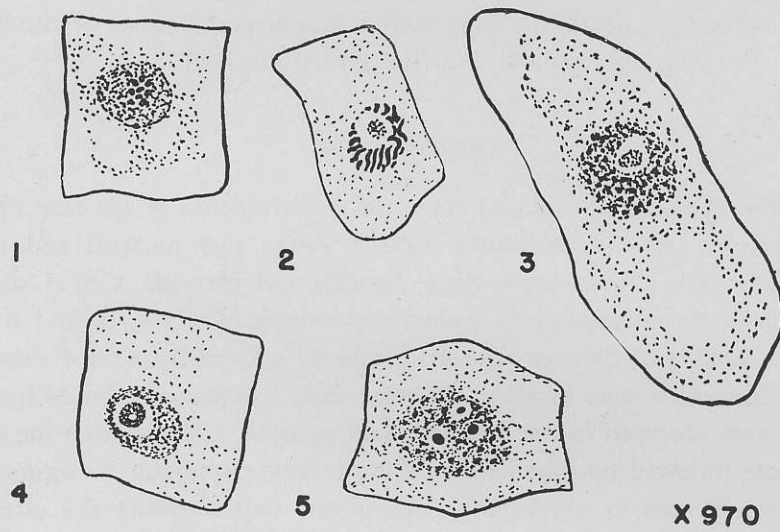


Fig. 2.—Pea cells 1-5 showing the effects of nucleic acid and X-radiations on the chromatin of pea root tip cells.

evenly and finely dispersed both in the nucleolus and in the rest of the nucleus.

Figure 2, pea cells 1, 2, and 3 show the effects on the pea root tip cells of 200 r. Cell 1 shows the effects when root tips were treated with X-radiation followed by nucleate. The nucleolus shows large, distinct, even clumping of the chromatin. Cell 2 shows normal prophase, where the chromosome strands are first forming, which is typical of cells in the treatment nucleate followed by X-radiation. Cell 3 shows typical pycnosis found in cells receiving X-radiation alone. Cell 4 shows a cell used as a control. Cell 5 shows the effects of cells treated with 5000 r, in which the nucleus shows dense pycnosis and three nucleoli that appear solid in outline due to the extreme dense clumping. This was also typical of the rye cells receiving the same treatment.

The above observations on the cells of peas indicate, as they did with the rye-cell observations, that the cells were in the energetic stage, or early prophase when treated. Root tips treated with nucleate alone apparently were in the energetic stage.

DISCUSSION

The cytological observations from the rye and pea material show the following characteristics: rye and pea cells treated with 5000 r show pycnosis and multi-nucleoli. The material shows that the nucleolus has such dense clumping of the chromatin that the nucleolus is solid in outline. The nucleolus of the rye cells is much larger than in the pea cells. The nature of the pycnosis of the rye and pea cells was observed to be the same. The material treated with 200 r was similar in its effects except that the nucleolus in rye cells showed the chromatin in distinct "bands," whereas, pea cells were normal. Normal prophase of mitosis was observed in rye and pea cells treated with nucleate and followed by 200 r. The cells of rye and pea treated with 200 r and followed by nucleate show different characteristics. In the rye cells, the chromatin is evenly dispersed but is somewhat pycnotic in nature. No nucleolus differentiation was observed. This observation seems to point to the conclusion that nucleate may exercise a "healing" effect, after the cells have been treated with X-radiation. The pea cells on the other hand show normal granular chromatin, except that the nucleolus shows distinct even clumping of the chromatin. Both rye and pea cells used as control were in the energetic stage, with the chromatin in the granular form. The rye and pea material

treated with nucleate alone showed energetic nuclei, and prophase in the rye material. The material, therefore, did not prove or disprove the findings of Huskins (1948*a*, 1948*b*).

In future experiments radiation of the material should be followed by the addition of nucleate at different times. In this way, the length of time that the nucleate might function after irradiation, and at what mitotic stages, could be determined. Further investigations might also indicate whether or not nucleate could then be used to influence the mutations induced by X-radiations.

SUMMARY

Seeds of both rye and pea treated with 5000 r units of X-radiations showed nuclear pycnosis. These cells must have been killed for there were no cells found actively dividing. All cells apparently were in the energetic stage.

The material from rye and pea root tips treated with 200 r also showed pycnosis, but evidently the cells could continue to divide since some divisions were observed.

The rye and pea material treated with sodium ribose nucleate and followed with 200 r showed cells in normal prophase. This would indicate that the chromosomes may not have been "nucleic-acid-starved" and that the X-radiation may not have affected materially the nucleus and its contents. Thus the nucleate added to the root tips previous to irradiation may tend to counteract the destructive effects of X-rays on the nucleus. On the other hand, the cells of irradiated rye and pea root tips treated with nucleate subsequently show less pycnosis. The chromatin is evenly clumped. The adding of the nucleate following X-radiation may have, additionally, a "healing" effect.

As mentioned previously, since all the cells were in the energetic stage, Huskins (1948*a*, 1948*b*) work could not be proved or disproved.

ACKNOWLEDGMENT:—The author wishes to express her gratitude to Dr. V. C. Brink for his supervision of this paper.

LITERATURE CITED

- Huskins, Leonard C. 1948*a*. The experimental induction of reduction division in somatic tissue. Royal Society of Canada, Section 5, Biological Sciences. Mimeo. 1-page. June.
- . 1948*b*. Chromosome multiplication and reduction in somatic tissue; their possible relation to differentiation, "reversion," and sex. *Nature* 161:80.