

9. Medical Physics. Part ACLASSIFICATION CANCELLED OR
CHANGED TO UnclassifiedI. Effects of Radiation on Meiotic Chromosomes BY AUTH. CG-DAR-1:2.2
of Plants
(Delphinium agacis L.)BY D. B. Anon DATE 3/15/74
ADCEffects of Radiation on Fragment Chromosome BehaviorBY W. B. Anon DATE 3/15/74
ADC

During the first meiotic division, the fragment chromosome may precede the rest of the chromosomes to the poles. This behavior is attributable to its unpaired or univalent condition, and such early movement may be termed precocity.

Definite standards have been established by which the precocity of the fragment may be judged so that differences between determinations of the percentage of precocious fragments made independently by two workers will be less than one percent.

None of the irradiated material showed a large change in the percentage precocity. The total percent precocity for all irradiated material was 35% while for the non-irradiated material it was 45%. This difference, as may be seen from Table I, is highly significant, even though the difference itself is not large.

The present workers believe that this reduction in precocity is a reflection simply of the clumping effects brought about by the irradiation; the tendency for the chromosomes to stick or clump together would presumably retard the free movement of the chromosomes to the poles.

It is not believed, however, that in these cases the irradiation has affected the chromosome division.

Subsequently, attempts have been made to find whether a sensitive period exists in the meiotic cycle. As indicated in Table II, there is no apparent evidence for such a period at present. Much more work needs to be done to establish the average percentages for each of the time periods. In addition, the results obtained for plant no. 620-2 may indicate a differential type of sensitivity to irradiation. Although such results may be attributable to chance, they might also be attributable to differences in nutrition or genetic constitution of the plant. The precocity percentages obtained on both control and irradiated material at the beginning of this work had upper limits at 50%, later 60-64%. Since the plants used in the subsequent work had been cut back several times to obtain buds at the right stage, it seems likely that the decreased vigor of the older plants may be responsible for the increase in precocity. This idea can be tested by the use of material grown on known nutrient levels, and by stricter limitation of the plant stage at which the collections are made.

In addition, information is being collected on the relationship between precocity, and the lagging and division of the fragment chromosome. The fragment chromosome may (1) proceed precociously to one pole (2) move to a pole in step with the other chromosomes (3) divide in step with the other chromosomes (4) lag behind the other chromosomes and divide or (5) be precocious

Table I

Plant No. Irradiated	Slide No.	Treatment ¹⁾	No. Cells with precocious fragment	No. cells without precocious fragment
Daj 619-21	126	20R - 4 hr.	15	35
Daj 619-21	118	20R -24 hr.	19	31
Daj 619-7	158	50R - 4 hr.	14	36
Daj 619-18	201	50R -24 hr.	18	32
Daj 619-18	208	50R -24 hr.	8	12
Daj 619-5	315	100R - 4 hr.	18	32
Daj 619-5	318	100R - 4 hr.	20	30
Daj 620-16	250	100R -24 hr.	18	32
Daj 620-16	257	100R -24 hr.	17	33
(35% precocious)			147	273
<u>Non-Radiated</u>				
Daj 619-21	127	control	21	29
Daj 619-21	131	control	21	29
Daj 619-21	132	control	22	28
Daj 619-18	206	control	25	25
Daj 619-18	209	control	18	17
Daj 619-18	210	control	24	26
Daj-619-18	212	control	20	30
(45% precocious)			151	184
irradiated			not irradiated	
precocious		147	151	χ^2 (with Yates' 298 correction): 7.43 df - 1 p < 0.01 457 755
not precocious		273	184	
		420	335	

1) In this, and subsequent tables the expression R - hrs., for example 50R-24 hrs. indicates material was collected 24 hours after an irradiation of 50 roentgens of x-rays.

Table II

Precocity of fragment chromosome of various periods after irradiation at 50R. (Each figure based on a count of 50 cells)

	Plant No.	619-7	620-8	619-18	620-2	621-2	Average
Time							
% Actual precocity of control		34	60 56 (58)	36 48 40 (42)	38	44	
% of control precocity % control		(100)	(100)	(100)	(100)	(100)	(100)
4 hours		82					82
7-1/2 hours			103 110				106.5
24 hours				78			78
49 hours			69		100	86	85
55 hours			83		137	68 105	98
75 1/2 hours			69 52		142	82	86

at metaphase and then lag and divide at anaphase. If the non-precocious chromosomes are the ones which will divide, then

- (A) % precocious at metaphase
 + % divided or lagging at anaphase = 100%

If the non-precocious chromosomes frequently move to a pole in step with the other chromosomes, then

- (B) % precocious at metaphase
 + % divided or lagging at anaphase < 100%

If some of the precocious chromosomes later begin to divide and become laggards, then

- (C) % precocious at metaphase
 + % divided or lagging at anaphase > 100%

Anaphase counts were obtained from a number of different materials on which precocity counts were made. Table III indicates a relatively high correlation between percentage precocity and percentage dividing and lagging will be obtained if further work yields such close agreement. In addition the data indicate that the fragment chromosomes rarely move in pace with the bivalents. The facts that no values under 100% were obtained, and that the average is above 100% indicates also that some of the precocious fragment chromosomes later lag and divide. Such behavior has been observed directly, with both halves of the completely divided and separated fragment near one of the telophase I nuclei.

Quantitative Analyses of the Effects of Radiation on Chromosomes

In addition to the work outlined in above, attempts are being made to set up quantitative expressions for other effects of radiation on chromosomes. The purpose of this work is twofold. First is the practical aspect of obtaining an easily used measure of the effects of radiation on chromosomes. Second is the goal of obtaining more information about the general physiological effects of radiation in their relationship to specific genetic effects.

Although several other characteristics are being studied, only two, metaphase clumping, and diakinesis stranding will be discussed here.

Metaphase clumping.

It has been known for many years that nuclei injured by irradiation or other agents become condensed and pycnotic. Likewise, the chromosomes of dividing cells so treated tend to aggregate on the metaphase plate.

By utilizing a plant with a few, large chromosomes, it is possible to set up an index for variation in clumping. The index employed is $8-n$ where n is the total number of visibly separate chromosomes. The index values are zero for no clumping and 8 for complete clumping. The values obtained for the two examinations made are:

Table III

Relationship of Precocity to Lagging and Dividing in Fragment Chromosomes

Plant No.	Treatment	At Anaphase I		At Metaphase I		Obs. %	Exp. %	
		No. Cells	% Lagging & Dividing	No. Cells	% Precocious			
620-8	Control	50	64	100	58	122	100	
"	7-1/2 hr	42	45	100	62	107	100	
"	55 hr	50	58	50	48	106	100	
"	75 1/2 hr	24	68	100	35	103	100	
621-2	49 hr	50	62	50	38	100	100	
"	55 hr	50	76	50	44	120	100	
"	75 1/2 hr	50	64	50	36	100	100	
							Av. =	108.3

% Lag & Divide,
+ % Precocious

Plant no.	Slide No.	Treatment	No. of cells	Average clumping index	Diff.	df t	p
Daj 620-16	260	control	50	0.84		98	
Daj 620-16	267	100R-24 hr.	50	4.30	3.46	7.52	<0.001

Clumping index thus seems to give very definite differences between irradiated and non-irradiated material. Further work is necessary to determine how accurate a measure of dosage it may be. In addition, the physiological reasons for clumping are still an almost complete mystery.

Diakinesis strands

At diakinesis, strands are apparent running from one chromosome to another. The nature of this material is not known, but it seems to be typically associated with regions of the chromosomes expected to be hetero chromatic. By using the fragment chromosome as a marker, it is possible to obtain a measure of the amount of stranding per nucleus without counting more than a small fraction of such strands in each cell. The counts are made simply of the number of strands connecting the fragment chromosome with any or all neighboring chromosomes. Radiation seems to increase the amount of stranding, but since the results at present are on the borderline of statistical significance, more work will be necessary before definite conclusions can be drawn.

Plant No.	Slide No.	Treatment	No. of cells	Average No. of strands	Grouped average	Diff.	df t	p
Daj 619-18	200	50R-24 hr.	25	1.88				
Daj 620-16	250	100R-24 hr.	50	1.78	1.81		141 1.88	0.05- 0.10
Daj 619-18	212	control	50	1.26		0.55		
Daj -----	---	control	18	1.28	1.26			

At present, therefore diakinesis stranding does not seem to be an accurate index to small differences in radiation received by dividing cells. Because of theoretical interest, however, in the nature of the process responsible for the production of strand material, further work is being done on this effect.

Development of the Technique for Determination of Synergism and Antagonism of Specific Substances to the Effects of Radiation on Chromosomes

A method is being developed for bringing chemical substances into contact with meiotic tissues. Inflorescences are removed from the plant and submerged in the solution. The system is evacuated, and air drawn out of the plant tissue, is replaced by the solution. After evacuation, the inflorescence is placed in a small vial of the same solution so that it will continue entering the vascular system. Sterile precautions are not attempted since it is impossible to disinfect the living surface tissues of the inflorescence. However, by the choice of initially healthy plant tissue, it is possible to conduct experiments for brief time periods during which little or no bacterial or fungal action

would produce effects. Inflorescences show normal meiosis four hours after treatment with distilled water. Some specimens kept twenty-eight hours show, however, definite damage from bacteria or fungi. This method, therefore is strictly limited to experiments of brief duration.

The goal in working out this new technique has been to enable the testing of specific substances for their synergistic or antagonistic action to radiation. Plants make invaluable experimental material in this regard since the vascular water system is not subject to such strict control as are animal vascular systems. Another drawback, at present, is the qualitative nature of the technique. No estimates are available of the amount of substance drawn into the inflorescence while it is being evacuated or later while it is standing in the vial of solution. An attempt will be made to get a partial answer to this question by the use of vital dyes, although even with this technique it may be difficult to find out how much of the test solution actually comes in contact with or enters the cells undergoing meiosis.

~~RESTRICTED~~

II. Studies of the Biological Effects due to Nuclear Fission

The studies reported last month were continued with the main new result being definite data on the presence of uranium in the bone marrow of experimental mice given UO_2 colloid type C. It was measured that the concentration of uranium in bone marrow scooped out from the femur was as high as .2 mg. of uranium per gm. bone marrow.

There is one difficulty with the technique of measuring the radioactivity of bone marrow in small animals. When removing the bone marrow, one often gets bone spicules mixed with it. Thus, it is difficult to determine whether the bone cells or the marrow cells are the ones containing radioactive material.

Technique of radioautography on bone marrow and blood samples.

A dilute sample of bone marrow was used to make thin smears on a microscope cover glass. The smear is pressed against an Eastman alpha plate for a suitable time. After development the photographic plate and the cover glass were superimposed and examined under the microscope simultaneously. By aligning the two plates carefully it was possible to establish the identity of the cells from which the alpha particles originated. So far it appears that most of the alpha activity was in the cells of the bone marrow. It is believed that the autoradiographic technique here described was not used previously for the study of blood and marrow smears and it appears that the technique when sufficiently well developed will have important application in the study of specific uptake of radioactive materials in the various types of cells found in blood and marrow. When the smear is made thin enough it will be possible to identify the individual cells containing the radioactive material. In some cases it may even be possible to point out some parts of the cell as origin of the alpha particle tracks. The technique is sensitive to very low intensity alpha radiation.

Transplantation of bone marrow

In collaboration with another project one of us has developed a technique to transplant red bone marrow from one rat into the liver of another rat. The transplanted marrow grew well in the liver for a period of a month, when the animal was sacrificed. A layer of newly formed bone surrounded and protected the marrow. This work is being continued.