EFFECTS OF VARIOUS CATION IMBALANCES ON THE FREQUENCY OF X-RAY-INDUCED CHROMOSOMAL ABERRATIONS IN TRADESCANTIA[1, 2]

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THE biological functions of metal ions, which are best understood, are involved in enzymatic reactions. Metal requirements for enzyme systems have been discussed recently by McELROY and NASON (1954). Metal ions have also been shown to influence permeability, coagulation, precipitation, electrical potential, and viscosity. The detailed functions and precise mechanisms of action of many metals have yet to be determined although their nutritional requirements and distribution have been established. This lack of specific evidence has indeed been the case for the metals occurring in the nucleus. One hypothesis followed here states that cations are directly involved with the stability of chromosomes.

Since it was found that "spontaneous" chromosomal aberrations were produced in Tradescantia when plants were subjected to magnesium or calcium deficiencies (STEFFENSEN 1953, 1955a), the possibility seemed likely that a particular metal might be effective in altering the frequency of X-ray-induced chromosomal aberrations. It is, therefore, proposed to examine (Steffensen 1954) the combined effects of X-irradiation with proportional dilutions of total nutrient solution and single cation deficiencies on chromosomal breakage frequency.

In the work with spontaneous breakage, the plants grown on suboptimal calcium showed the most striking increase in chromosome aberrations. The microspores and pollen of plants lacking calcium gave at least a 17-fold greater rate of chromosome aberrations than those of plants grown on optimal calcium. Of the individual metal ions examined here (calcium, magnesium, iron and manganese), only suboptimal calcium gave significant and reproducible results with X-rays. A hypothetical model will be discussed in which it is proposed that calcium plays an essential part in the chromosome's structure.

MATERIALS AND METHODS

The plants from clonal divisions of *Tradescantia paludosa* (clone 5 of Sax) were grown in nutrient solutions using slight modifications of solution 1 as given by HOAGLAND and ARNON (1950). Reagent grade salts were employed. Solution 1 consists of the following additions to distilled water, the final volume being one liter.

[1] A part of this work was taken from a thesis submitted in partial fulfillment for the degree of Doctor of Philosophy in the Department of Genetics, University of California, Berkeley.

[2] A part of research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

	ml
M KH_2PO_4	1
M KNO_3	5
M $Ca(NO_3)_2$	5
M $MgSO_4$	2
ferric iron solution (tartrate or EDTA)	1
trace solution (B, Mn, Zn, Cu and Mo)	1

In suboptimal calcium cultures the concentration of $Ca(NO_3)_2$ was reduced. Regular renewal of culture solution prevented a low nitrate concentration. In suboptimal magnesium cultures, the $MgSO_4$ concentration was lowered, and 0.5 M K_2SO_4 was used to make up the sulfate level to that of solution 1. Iron deficiency cultures were accomplished by reducing or omitting the iron solution. Iron was added as ferric tartrate in the 1950 series and as ferric ethylene diamine tetra acetic acid thereafter. Most of the experimental details are given in table 1.

TABLE 1

Experimental conditions of Tradescantia growing in water cultures and conditions of irradiation of inflorescences

Condition in water culture	Concentration	Vol. sol. in liters (b)	pH	X-ray conditions
Proportional dilutions of Hoagland's solution 1 (see text)	control	7	5.8–6.6	100 Kvp
	full conc.	7	5.4–7.6	3 ma
	1/10	7	4.25–7.6	inherent filtration
	1/20	7	4.6–7.6	
Low and deficient magnesium	control (49 ppm Mg)	7 (a)	5.5–7.2 (range) 5.5 (adjusted)	100 Kvp 3 ma
	1 ppm Mg	7 (a)	5.5–6.7 (range) 5.5 (adjusted)	1 mm Al plus inherent filtration
	no Mg added	40 (c)	5.5–6.8	
	control (49 ppm Mg)	40 (c)	5.5–7.4	
Low and deficient iron	control (1.4 ppm Fe)	7 (a)	5.5–7.2 (range) 5.5 (adjusted)	100 Kvp 3 ma
	.027 ppm Fe (as tartrate)	7 (a)	4.8–6.5 (range) 5.5 (adjusted)	inherent filtration
	no Fe added	40 (c)	5.5–7.6	
	control (5 ppm Fe as EDTA)	40 (c)	5.5–7.4	
High manganese (as $MnCl_2$)	control (0.5 ppm Mn)	7 (a)	5.5–7.2 (range) 5.5 (adjusted)	100 Kvp 3 ma
	14.2 ppm Mn	7 (a)	4.6–6.5 (range) 5.5 (adjusted)	inherent filtration
Suboptimal calcium	control (250 ppm Ca)	90 (c)	4.7–6.9	250 Kvp
	6.25 ppm Ca	90 (c)	5.1–6.8	15 ma
	3.1 ppm Ca	90 (c)	5.1–6.5	1 mm Al, plus inherent filtration

(a) Solution changed every 2 weeks.

(b) 7-liter cultures with four plants per tank (1950–1951), 40-liter cultures with ten plants (1951–1952) and 90-liter cultures with ten plants (1954).

(c) Solution changed every 2–3 months.

In the 1950 experiments, proportional dilutions (full, one tenth, one twentieth) of Hoagland's solution 1 were used. Four cuttings were put into each tank. Each tank contained a seven liter lot of solution. The plant material was irradiated in the tenth and eleventh months of cultural maintenance. In the first six months of growth of these dilution cultures, the solutions were changed monthly, while for three months previous to collection (the seventh through ninth months) no new salts were added. Preliminary irradiations to test chromosome sensitivity during the first six months showed no differences between cultures; hence, the more drastic treatment was used as explained above.

In the early experiments, a solution of three parts alcohol to one part acetic acid was used for fixing the inflorescences. Later specimens (1954) were fixed with NEWCOMER's fixative (NEWCOMER 1953) in parts by volume six isopropyl alcohol, three propionic acid, one petroleum ether, one acetone and one dioxane. It was found that after properly destaining propiono-carmine preparations, the new fixative gave less cytoplasmic staining and more chromosome-chromatid definition. Because buds and anthers became brittle in NEWCOMER's fixative, the inflorescences were stored and dissected in 70 percent alcohol.

Chi square tests were calculated in a *2* by *n* table by comparing the number of cells in columns with no aberrations, one aberration, two aberrations and three or more aberrations. If individual expected values in the last category were less than one the observed values were added to the column with two aberrations per cell. Degrees of freedom were calculated accordingly. The means of each column were used to derive individual expected values. Chi square values and degrees of freedom were omitted in order to simplify the presentation.

Catalase activity of the anthers was determined in the following manner. Twenty milligrams of fresh anther tissue were homogenized in the presence of levigated alumina and 0.5 ml of distilled water. The homogenate was added at zero time to 0.01 normal H_2O_2 in diluted McIllvane's buffer at pH 6.8. The reaction mixture was then agitated, and at given intervals 2 ml aliquots were removed and stopped with 1:4 dilutions of concentrated sulfuric acid. Unreacted H_2O_2 was determined by titration against 0.01 normal $CeSO_4$.

RESULTS

Dilutions of Hoagland's solution

During the ionic depletion period, a preliminary analysis indicated that the frequencies of chromatid aberrations and chromosomal aberrations in plants from the one tenth dilution were higher than in plants from the one twentieth dilution or full strength Hoagland's. Ionic depletion was attained since no new salts had been added for three months. Plants cultured on the one tenth Hoagland's solution also exhibited significantly higher frequencies of X-ray-induced aberrations than did plants from regularly maintained solutions or soil. A similar trend was evident in a second irradiation of material from the same plants. The respective frequencies of dicentrics and centric rings and interstitial deletions are shown in table 2. In another comparison where chromatid breakage was examined (table 3), chromatid interchanges were

TABLE 2

Frequency of chromosomal aberrations in microspores from two concentrations of Hoagland's solution given 360r at 40r/min and fixed in four days

Treatment	Dicentrics centric rings and tricentrics		Interstitial deletions		Total cells
	No.	No. per cell	No.	No. per cell	
Complete Hoagland's solution	331	0.455	275	0.378	728
One tenth dilution of Hoagland's solution	397	0.571	323	0.465	695
P values by χ^2 test	<.01		.02–.01		

TABLE 3

Frequency of chromatid aberrations in inflorescences grown on different nutritional conditions given 90r of X-rays at 49r/min and fixed in 24 hours

Treatment	Chromatid interchanges		Isochromatid		Chromatid		Total cells
	No.	Per cell	No.	Per cell	No.	Per cell	
Soil + Hoagland's solution	26	.054	99	.206	109	.227	480
One tenth dilution	25	.105	46	.193	54	.227	238
P by χ^2 test	.02–.01		.7–.5		.99		

significantly higher in the anthers of the one tenth dilution than in the control. Chromatid and isochromatid frequencies were similar for both treatments studied.

Magnesium deficiency

Irradiated anthers collected from low magnesium (1 ppm) or magnesium deficient cultures exhibited frequencies of aberrations not consistently different from irradiated anthers of plants grown in control water cultures or soil. In these comparisons anthers which were given 90r and fixed 24 hours later were examined for chromatid aberrations at first microspore metaphase. Anthers given 360r were fixed three or four days later and were scored for chromosome aberrations at the first microspore division. A total of 814 cells was observed from the suboptimal or magnesium deficient plants, while 1571 cells were scored from plants with optimal nutrition from water culture or soil. In the plants lacking magnesium, the usual physiological symptoms were seen, the older leaves becoming pale green and necrotic.

Iron deficiency

Chromosomes from suboptimal or iron deficient plants were no more sensitive to X-rays than the chromosomes from plants grown in water culture with optimal iron or in soil. The experimental data for these conclusions are based on three separate irradiations of iron deficient material and material grown normally. Both chromatid and chromosomal aberrations were scored in a total number of 2816 cells, the combined number from all experiments and treatments.

Leaves, buds and pedicels from iron deficient plants exhibited typical iron chlorosis.

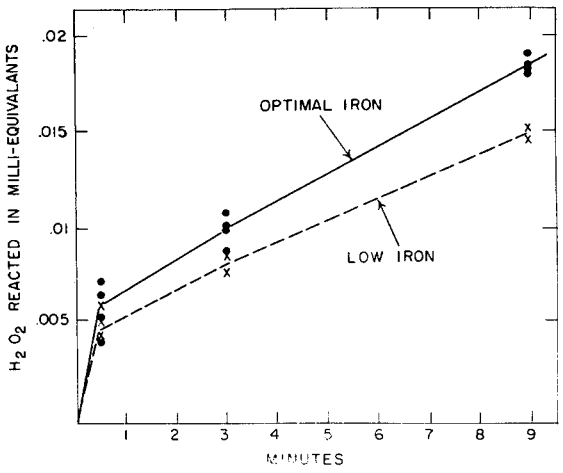

FIGURE 1.—The catalase activity (H_2O_2 reacted) of homogenates from anther tissue is plotted against time. The anther homogenates from the low iron cultures show a 20 percent reduction in activity as compared with anther homogenates from optimal iron cultures.

The color of buds ranged from yellow-green to near white, depending on the level of iron and the length of time in culture.

At the same time that a group of anthers was irradiated for examination of chromosomal aberrations, another group of anthers from optimal and low iron cultures was tested for catalase activity. The stages of the anthers were determined by microscopic examination of one anther out of each six from a single bud. Microspore to binucleate-pollen stages were used. The catalase activities of anther homogenates from the differently cultured plants are given in figure 1. A 20 percent reduction in catalase activity of anther tissue from low iron cultures (.027 ppm Fe^{+++}) was observed. These differences are similar to the values obtained in preliminary tests. The catalase activity should have been reduced still lower in anthers from cultures to which no iron was added. After irradiation the anthers from suboptimal iron and the anthers from iron deficient cultures gave no increase in the frequency of chromosomal aberrations (dicentrics, centric rings, and interstitial deletions) above normally cultured anthers similarly irradiated.

High manganese

An examination of small irradiated samples had suggested that less chromatid breakage was produced in plants grown on high manganese (14.2 ppm) than in plants cultured normally (0.5 ppm Mn). Examination of a larger irradiated sample taken from the two cultures showed that the frequencies of chromatid interchanges, chromatid breaks, and isochromatid breaks in the two treatments were not significantly different. A total of 1960 cells was scored in all treatments.

Suboptimal calcium

During the first three or four months of culture, plants grown on low concentrations of calcium exhibited few deficiency symptoms except for reduced root growth. The first and most definitive symptoms to appear after this time were failure to set

TABLE 4

Chromosomal aberrations produced in inflorescences grown on two different levels of calcium given 314r of X-rays (100r/min) and fixed four days later

	Di-centrics	Centric rings	Tri-centrics	Total interchanges per cell*	Percent increase	Interstitial deletions		Percent increase	Total cells
						No.	per cell		
Suboptimal calcium (3.1 ppm)	71	31	3	0.512	23.7	125	0.592	73.1	211
Optimal calcium (250 ppm)	123	55	9	0.414		162	0.342		474
P value by χ^2 test				.01–.001		<.001			

* Tricentrics counted as 2 interchanges.

seed and formation of incomplete flowers. Tradescantia plants in water culture with 3.12 ppm calcium usually died within six to seven months, while plants grown with 6.25 ppm calcium survived at least one year. Without addition of calcium to stock solutions, neither cuttings nor older plants would grow. The inflorescences used in these experiments were collected for irradiation when flower parts first began to show deficiency symptoms.

Two independent experiments showed that the frequency of X-ray-induced chromosomal aberrations of all types was higher in inflorescences grown on suboptimal calcium than in those grown on optimal calcium. In table 4 it is shown that the frequency of interchanges and interstitial deletions in material from 3.1 ppm calcium cultures is higher than in material grown in 250 ppm calcium cultures. In each case the higher frequency differs significantly. The proportional increase of interstitial deletions is 73.1 percent, while the increase of interchanges is 23.7 percent. This differential increase of interstitial deletions over dicentrics and centric rings suggests that interstitial deletions are produced at a higher rate. A chi square test suggests that this differential increase of one type above another is not quite significant ($P \sim .01$–$.05$).

The data presented in table 5 and figure 2 were taken on material from the same set of cultures three weeks later. A test of homogeneity on the frequency of interchanges between slides of the same treatment indicated that no significant deviations were present. The same was true for all aberrational frequencies in table 4. A comparison of interchange values obtained from X-irradiated inflorescences grown on the 250 ppm and the 3.1 ppm Ca^{++} cultures was made using a chi square test. A highly significant difference between treatments was indicated. ($P \sim .01$–$.001$). The 6.25 ppm Ca^{++} grown material had a higher frequency of interchanges but was not significantly different from that grown on 250 ppm Ca^{++} ($P \sim 0.1$–$.05$). As expected, the soil grown material did not differ significantly ($P \sim 0.5$–0.3) from the 250 ppm Ca^{++}.

No significant differences in interstitial deletion frequencies between slides within treatments were found for the 3.12 ppm Ca^{++} and soil-culture materials (P values were .70–.50, 0.9–0.8 and 0.5–0.3, respectively.) However, the same test for interstitial deletions in the 6.25 ppm Ca^{++} material showed a significant deviation ($P \sim .01$–$.001$), indicating a lack of homogeneity between slides. No particular trend was

evident in the slides which gave the largest chi square values. This would suggest experimental or random errors rather than a variable condition produced by Ca^{++} concentration differences. Additional support for the latter conclusion was found in the observed homogeneity (P $\sim$ .70–.50) between slides within the 3.15 ppm Ca^{++}

TABLE 5

Frequency of chromosomal aberrations in inflorescences grown on different levels of calcium and given 300r of X-rays (100r/minute)

	Di-centrics No.	Cen-tric rings No.	Tricen-trics* No.	Interchanges per cell	Percent increase	Interstitial deletions		Percent increase	Total cells
						No.	Per cell		
3.12 ppm calcium	96	39	3	.585	36.7	172	.712	52.9	241
P value†				.01–.001			<.001		
6.25 ppm calcium	169	94	10	.495	15.6	353	.618	32.3	571
P value				0.1–.05			<.001		
250 ppm calcium	170	76	3	.428	—	272	.467	—	582
Soil	150	82	8	.397	−7.8	190	.294	−58.8	645
P value				0.5–0.3			<.001		

* Tricentrics are counted as two interchanges.

† P values are obtained by χ^2 test from comparing individual treatments with the 250 ppm treatment. The percent difference is found by the same comparison.

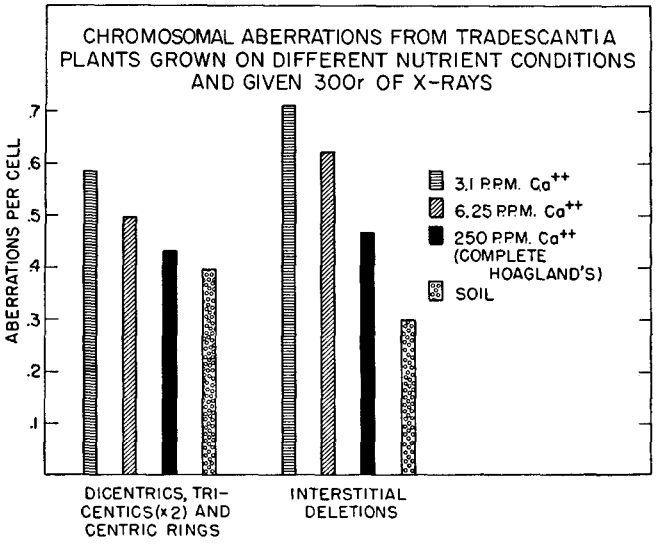

FIGURE 2.—The frequencies of chromosomal aberrations produced by X-rays in Tradescantia microspores when plants were grown on different concentrations of calcium and in soil.

material. In the 6.25 ppm Ca^{++} data, normal distribution was assumed for the χ^2 test between treatments. The frequencies of interstitial deletions in the 3.1 and 6.25 ppm Ca^{++} grown microspores were significantly higher than the 250 ppm Ca^{++} microspores. Inflorescences grown in soil exhibited significantly lower values than the 250 ppm Ca^{++}. This series of treatments was in contrast to most cases where soil and culture controls have shown similar values for all types of X-ray-induced aberrations.

When the values of suboptimal calcium treatments (tables 4 and 5) were pooled and compared with pooled 250 ppm Ca^{++} values, a chi square test showed that the proportional increase of interstitial deletions above interchanges differed significantly ($P < .05$). It was apparent that with X-rays interstitial deletions were produced at a higher rate in the inflorescences with reduced calcium than were dicentrics and centric rings. More extensive data (all unpublished) have confirmed this differential increase showing highly significant differences.

DISCUSSION

Data from the dilution experiments presented have indicated that the X-ray sensitivity of plant chromosomes can be increased by modifications of mineral nutrition. The mechanism of this nonspecific effect is by no means apparent. Two observations are, however, worth noting. First, a balanced depletion of available ions seems to have had little or no effect on the frequency of X-ray-induced chromosomal aberrations. In fact, the lowest dilution culture (one twentieth of Hoagland's solution) was the least variable. It is of interest that STUBBE and DÖRING (1938) found that starvation conditions did not increase the spontaneous mutation rate in Antirrhinum. When they studied specific anions, only a phosphorus deficiency gave an increased frequency of X-ray-induced mutations. Secondly, the pH of the one tenth dilution culture was the most acidic (pH at 4.3) of the treatments. A particular imbalance of one or more elements might best explain the pH change and the higher X-ray sensitivity in this dilution culture. It is known, for example, that the absorption of calcium is inhibited at pH 4.3 and is almost entirely suppressed at pH 4.0 according to HOAGLAND (1948).

This study of X-ray-induced chromosomal aberrations in Tradescantia plants from specific mineral deficiency cultures gave both positive and negative results. Magnesium deficient plants which were X-irradiated exhibited no consistent increase in the frequency of chromosome breaks over the control culture plants similarly irradiated. In contrast, the "spontaneous" rate of appearance of chromosomal aberrations was raised by a magnesium deficiency as previously shown by STEFFENSEN (1953, 1955b). Apparently chromosomes from plants grown in suboptimal magnesium cultures were not "sensitized" to X-rays.

A striking increase in the frequency of X-ray-induced chromosomal aberrations was observed in plants cultured on suboptimal Ca^{++}. The differential increase of particular types of aberrations should be considered. Interstitial deletions have been described as small paired dots of chromatin, presumably rings, averaging about one micron in size, according to RICK (1940). In suboptimal calcium, these interstitial deletions were increased at a higher rate than were dicentrics and centric rings. The

increase was found to be significant by a chi square test. Recent findings from microspores of low calcium cultures (STEFFENSEN 1956) indicated that interstitial deletions could be produced at nearly double the rate, while the increased frequency of interchanges was similar to the value reported here. In studies concerning the role of oxygen in X-irradiated Tradescantia, GILES and RILEY (1949) found that dicentrics and centric rings and interstitial deletions increased with dose at essentially the same rate. The higher rate of production of interstitial deletions with X-rays in low calcium inflorescences may have special significance.

With respect to the behavior of the chromosome breaks produced by X-rays, three points should be made: (1) more breaks were observed in calcium deficient material than in control material; (2) interstitial deletions were produced at a higher rate than was expected; (3) independent breaks were able to form interchanges at a higher rate in plants grown on suboptimal Ca^{++}, indicating that movement of broken-chromosome ends was not hindered.

If we consider the effect of a calcium deficiency in physiological and physical-chemical terms, at least two explanations seem tenable. The first concerns the evidence amassed by HEILBRUNN (1943) on the role of calcium in the viscosity of protoplasm. When calcium concentration was low, cytoplasm was less viscous. In connection with the viscosity idea (as discussed by HEILBRUNN) it could be supposed that less restitution of breakage-ends to the original state might occur in nucleoplasm with reduced viscosity because the chromosomes could move more freely. The breakage-ends might thereby form aberrations at a higher rate. The higher production of interstitial deletions by X-rays could not be explained by the viscosity hypothesis nor could the high "spontaneous" rate of breakage (STEFFENSEN 1955a). The viscosity hypothesis has merit and should not be excluded, but it is less satisfactory in accounting for the many variables than the hypothesis discussed below.

The chromosome has been proposed to be, in part, a giant chelate polymer, an aggregation of macromolecules bound by divalent cations (STEFFENSEN 1955b). This hypothesis, given further treatment here, was based primarily on the following considerations: (1) Calcium and magnesium have been demonstrated to be associated with nucleoprotein and chromosomes (see STEFFENSEN 1953, 1955a for early references). A sensitive chemical test of HOLOWAY and BERGOLD (1955) determined that magnesium was present in an insect virus. FRICK (1956) demonstrated a requirement of magnesium or calcium for stabilization of desoxyribonucleoprotein. (2) Calcium deficient Tradescantia exhibited a high frequency of "spontaneous" chromosomal aberrations (STEFFENSEN 1955a), and X-ray-induced breaks were increased by a calcium deficiency as presented here. (3) BERNSTEIN and MAZIA (1952 a, b) have suggested that the chromosome may be particulate, and MAZIA (1954) has postulated that metal ions, like calcium or magnesium, hold the particles of chromosomes together. (4) It was established before the beginning of this century that calcium and phosphate could form fairly stable, long chain polymers or aggregation polymers (VAN WAZER 1950; VAN WAZER and CALLIS, in press). (5) The sugar to phosphate bonds along the DNA chains were quite resistant to irradiation according to BACQ and ALEXANDER (1955), and CAVALIERI *et al.* (1955). Breakage within bundles of DNA molecules with their attached protein should be extremely difficult; hence,

chromosome breaks should be produced primarily *between* macromolecule species. (6) Some recent experiments on crossing over may be applicable to the argument. LEVINE (1955) and STEFFENSEN, ANDERSON and KASE (1956) have found that crossing over in the X chromosome of Drosophila can be increased by the chelating compound ethylene diamine tetraacetic acid (EDTA or Versene). This compound will bind calcium and other metals to a degree more or less proportional to their electronegativity. EVERSOLE and TATUM (1956) also used EDTA to produce striking increases in crossing over in Chlamydomonas. With Drosophila again, LEVINE (1955) found that calcium added to food would reduce the frequency of crossing over.

The average molecular weight of DNA has been estimated to be around six million (FLUKE, DREW and POLLARD 1952; BROWN, M'EWEN and PRATT 1955; THOMAS and DOTY 1956). Moreover, within a cell DNA was demonstrated to be heterogeneous both as to base ratios and to molecular weights as discussed by CHARGAFF (1955) and MARMUR and FLUKE (1955), respectively. This heterogeneity concept leads to the idea that DNA molecules might have individuality and belong to different molecular "species" as denoted by ALLFREY, MIRSKY and STERN (1955) and to the considerations which follow.

If the chromosome can be assumed to be an aggregate macromolecule of DNA and protein, the problem arises as to how such macromolecules could be bound together. Numerous configurations might be taken, but recent X-ray diffraction and electron microscope studies narrow down the number of possibilities. The chromosome model in figure 3 is a simple and reasonable fit to the current physical evidence. This hypothetical model considers only a small section. A discussion on the binding of protein to DNA follows later.

The WATSON-CRICK model of DNA, a double-chained complementary structure, (CRICK and WATSON 1954) and the corrected DNA model of FEUGHELMAN *et al.* (1955) places phosphate groups and sugar groups at each end of the molecule. These phosphate end groups, are the proposed points of attachment of calcium between DNA "species". The two acid groups of the phosphate should be ionized at the pH of the nucleus (pH 6.8–6.9 according to HEILBRUNN 1943) because the pK values of phosphate esters are 1 and 6 to 6.5 (JORDAN 1955).

The diester phosphate groups within each DNA chain would have only single negative charges. A bundle of two or more adjacent DNA "species", which should be exactly the same length as a result of template reproduction, could then be bound laterally at their point of contact by negative charges through a metal link. The macromolecules would thereby be aligned in exact juxtaposition. An analogous example of this kind of binding has been proposed by STERN and STEINBERG (1953) wherein rare earths form complexes between DNA molecules. Precise alignment has been shown to occur in the sodium salt of DNA as determined by the X-ray patterns of FEUGHELMAN *et al.* (1955). Another very important observation of FEUGHELMAN *et al.* was the orientation of the macromolecules in a different plane. Both DNA and protamine molecules were seen to aggregate in a hexagonal pattern in nucleoprotein. Such a hexagonal state might well be the condition of DNA and its attached protein *in vivo*.

At the microscopic level, the half chromatid evidence of NEBEL (1939), CROUSE

(1953), LaCour and Rutishauser (1954), and Sax and King (1955) would indicate that the chromosome has at least four strands. Actually there are probably multiple numbers (8, 16 or 32) of strands at the subchromatid level. Ris (1955) has done electron microscopy of chromosomes from a number of different organisms including Tradescantia. In Tradescantia eight subchromatid strands were seen, the widths of the smallest filaments being 500–600 Å at prophase and 250 Å at interphase. This 250 Å width was the same found by Bernstein and Mazia (1953b) for their chemically isolated desoxyribonucleoprotein particles. The number of calcium atoms bound to DNA end groups at any one cross section of the chromosome should be the number of filaments or subchromatid strands multiplied by the number of DNA molecules in a strand.

The hypothetical model proposed here should not interfere with binding of DNA to basic protein as considered by Butler (1955). In his picture of the chromosome, histone held DNA molecules together by "salt-like" bonds. He has stated that these bonds might be the weak points of breakage which would be true for the metal bound sites proposed here. Feughelman *et al.* (1955) have suggested that protein might be bound to DNA diester phosphate groups through the amino groups of arginine side chains. The protein as a single strand would follow the spiral of DNA. Protein could form a "splint" between DNA molecular species. Ambrose and Gopal-Ayengar (1953) have proposed that hydrogen bonds are the longitudinal cohesive forces within the chromosome. According to their scheme it would be necessary to connect phosphate to phosphate or phosphate to deoxyribose sugar. In such end-group linkages,

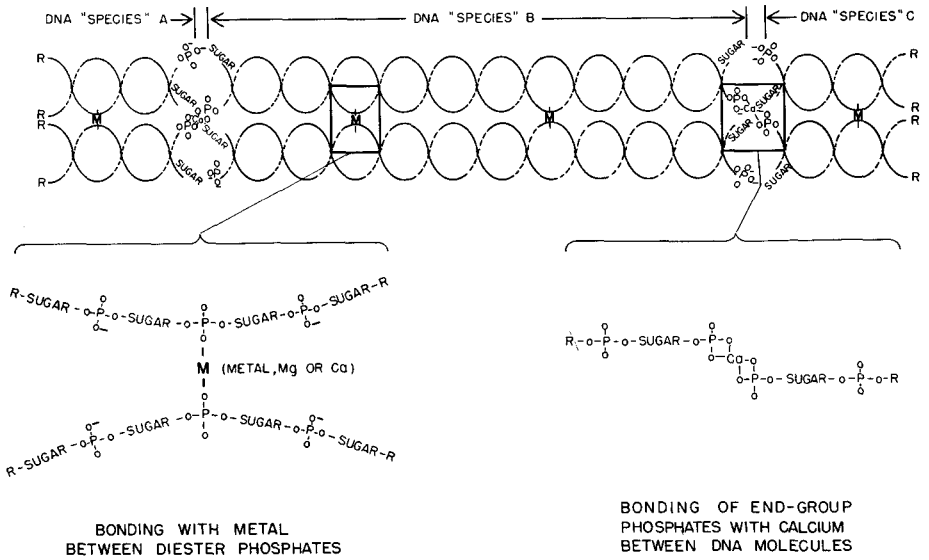

Figure 3.—This hypothetical model of a chromosome section considers possible bonding by metals between negatively charged phosphates of DNA. Three different DNA "species" are shown linked to phosphate end-groups by calcium. Bundles of the same DNA "species" might form cross links with cations between molecules. The attachment of protein to diester phosphates along the DNA chains could be accomplished with little hinderance to metal bonds.

metal bonds would seem more feasible because of the negative charges of the phosphates. In their treatment of the lateral dimension of the chromosome, AMBROSE and GOPAL-AYENGAR suggested that the forces of cohesion were electrostatic in nature which would be compatible with the hypothesis offered here.

Compounds which react with or esterify phosphate groups would be expected to greatly effect chromosome stability. ELMORE, GULLAND, JORDAN and TAYLOR (1948), and ALEXANDER (1954) found that nitrogen mustard esterified phosphate groups of DNA. Esterification might be predicted as the principal reaction responsible for the chromosome breaking properties of mustards. Terminal or diester phosphates could not form proper secondary bonds if tied up by mustard derivatives. Such alterations of DNA might give rise to a chromosome break immediately or in later cell divisions.

Assuming that the model of the chromosome (fig. 3) is a close approximation, it might be predicted that chromosome breaks, whether spontaneous or X-ray-induced, would occur at the links between DNA molecular species. In addition crossover exchanges in chromosomes of higher organisms should occur at these contact points between macromolecules. Chelate bonds formed by metals between DNA "species" and "salt-like" bonds occurring between protein and DNA have relatively low energy when compared to covalent bonds. The former bonds would be stable under typical conditions in the nucleus but would become unstable in the presence of high energy or certain metabolic regulators. For example specific chemical control of chromosome behavior by the cytoplasm might occur through temporary changes in hydrogen ion, phospha te, or citrate concentrations.

At this time it can be said with some degree of certainty that electrostatic or secondary bonds occur between the macromolecules in the chromosome. Future experiments must decide whether they are chelate or metal complex-type bonds, hydrogen bonds, or "salt-like" bonds and their relative importance. Some of the predictions indicated by the hypothetical model have been fulfilled although much of the evidence is circumstantial and proof by direct experiment is lacking. The model, whether correct or not, is useful because as a precise hypothesis it can be tested either by genetic or physical-chemical studies.

SUMMARY

Microspores of Tradescantia plants grown on proportional dilutions of Hoagland's solution were irradiated with X-rays. Results indicated that an ionic depletion had increased the chromosome sensitivity of the plants and that some particular ionic imbalance might be responsible for the effect. Specific cation imbalances were then tested for their effects on microspore sensitivity. Chromosome aberrations induced by irradiation were not increased over control values in cultures under conditions of excess manganese, deficient iron, or deficient magnesium concentrations. Only those plants grown on suboptimal calcium showed a significant increase in the occurrence of interchanges and interstitial deletions. It was observed that the rate of increase of interstitial deletions in this material was greater than the rate of increase of interchanges.

The above observations on the effect of a calcium deficiency are compatible with a general hypothesis that calcium is required for stability of the chromosome. A

hypothetical model is discussed, which proposes that calcium forms chelate bonds with phosphate end-groups between different DNA "species" along the chromosome.

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