X-rays must be secondary. It is possible that chromosome duplication occurs one cell generation before the two components can be differentiated by the induction of half-chromatid breaks and two cell generations before they separate at anaphase. Perhaps the actual duplication could be pushed back even further in chromosome synthesis, until DNA duplication is synchronized with chromosome duplication. If so, we would have to consider the chromosome as a multistranded unit at all stages in the nuclear cycle, emerging as a discrete unit only after many replications at the molecular level.

Summary.—In response to X-ray-induced aberrations, the *Tradescantia* microspore chromosomes react as single threads when irradiated during the resting stage, as double threads when rayed at prophase, and as four-partite strands when rayed at prometaphase and metaphase. The breaking of only one of the half-chromatids at metaphase, but of both at the resting stage, may be related to the nature of the half-chromatids at the time of duplication. The time of chromosome duplication as indicated by the types of X-ray-induced aberrations is compared with autoradiographs of DNA synthesis in microspore development and in the meiotic cycle.

* This work was supported in part by contract with the United States Atomic Energy Commission.

[1] C. P. Swanson, *J. Gen. Physiol.*, **26**, 485, 1943.
[2] Helen V. Crouse, *Science*, **119**, 485, 1954.
[3] Karl Sax and E. V. Enzmann, these PROCEEDINGS, **25**, 397, 1939.
[4] Karl Sax, *Cold Spring Harbor Symposia Quant. Biol.*, **9**, 93, 1941.
[5] D. E. Lea, *Actions of Radiations on Living Cells* (Cambridge: The University Press, 1947).
[6] B. P. Kaufmann, *Botan. Rev.*, **14**, 57, 1948.
[7] H. Swift, *Intern. Rev. Cytol.*, **2**, 1, 1953.
[8] J. H. Taylor, *Exptl. Cell. Research*, **4**, 164, 1953.
[9] Alma Howard and S. R. Pelc, *Heredity*, **6**, 261, 1952.
[10] W. S. Plaut, *Hereditas*, **39**, 438, 1953.
[11] A. H. Sparrow, M. J. Moses, and R. Steele, *Brit. J. Radiol.*, **25**, 182, 1952.
[12] B. R. Nebel, *Botan. Rev.*, **5**, 563, 1939.

*BREAKAGE OF CHROMOSOMES IN TRADESCANTIA WITH A CALCIUM DEFICIENCY**

BY DALE STEFFENSEN

BIOLOGY DEPARTMENT, BROOKHAVEN NATIONAL LABORATORY, UPTON, LONG ISLAND, NEW YORK

"Spontaneous" chromosome breakage in *Tradescantia paludosa* increases when plants are grown in decreasing amounts of magnesium.[1] Since calcium and magnesium show certain biochemical similarities and are components of chromosomes and nucleoprotein,[2] it seemed of interest to determine the effects of a calcium deficiency on chromosome stability. The present paper deals with attempts to demonstrate the importance of calcium in the structure and stability of chromosomes, since the motivating hypothesis of this work states that bivalent cations fulfil a structural requirement within nucleoprotein.

Materials and Methods.—The cultural methods were the same as those described previously.[1] Clonal divisions from a single plant of *Tradescantia paludosa* (clone

5 of Sax) weighing about 5 gm. each were placed in solution 1 of Hoagland and Arnon.[3] The concentration of calcium in the cultural solutions was adjusted to three different levels. The first solution contained 2.5 p.p.m. of calcium. Ten clones were grown in this solution for three months, after which the plants were transferred to solutions lacking calcium altogether. The second (suboptimal) solution, again with ten clones, contained 10.0 p.p.m. of calcium for the first three months and 2.5 p.p.m. thereafter. The third solution contained 250 p.p.m. of calcium, an optimal supply according to Hoagland and Arnon. Fourteen plants were started in this solution. For ten of these the pH of the initial cultural solution was 5.8. For the other four plants grown in optimal calcium, the pH of the solution was adjusted weekly to 5.5 and the solution replaced every two weeks. The other solutions were replaced every six or seven weeks. During the interim between replacements, none of the pH readings of these solutions exceeded 7.6.

Buds were collected during the sixth and seventh months of incubation. These were fixed in a 3:1 solution of ethanol and glacial acetic acid, and subsequently the anthers were stained with propiono-carmine.

Results.—Anthers from plants grown in the lowest calcium concentration contained only abortive cells, none of which were undergoing microspore division. Chromosomal alterations could not be analyzed in these anthers. Anthers from plants grown in optimal and suboptimal calcium solutions showed complete division cycles. From these, the frequency of chromosomal aberrations was determined. The frequency was much higher in the cells of the plants grown in the suboptimal level of calcium.

Variations from the normal chromosome constitutions of six haploid chromosomes of nearly equal length with medial or submedial centromeres were found in anthers from plants grown both in optimal and in suboptimal calcium. The types of variation and their respective frequencies are shown in Table 1. The constitutions of the spore types revealed much concerning their relationship in time

TABLE 1

FREQUENCY OF CHROMOSOME ABERRATIONS AT METAPHASE OF THE FIRST MICROSPORE DIVISION IN *Tradescantia*

	SUBOPTIMAL CALCIUM		OPTIMAL CALCIUM		SUBOPTIMAL ÷ OPTIMAL CALCIUM
	No. Obs.	Per Cent	No. Obs.	Per Cent	
Total cells*	405		1,390		
1. Six normal chromosomes + centric or acentric fragments	17	4.20	2	0.12	29.1
2. Chromosome fragments (5 chrs. with centric chr. + frag.)	10	2.47	2	0.14	17.1
3. Dicentrics, inversions, and acentric rings	2	0.49	1	0.07	6.9
4. Chromatid exchanges	1	0.25	0	..	..
5. Chromatid deletions	7	1.73	2	0.14	12.0
6. Six normal chromosomes + micronucleus	2	0.49	7	0.50	0
7. Seven normal chromosomes	1	0.25	0	..	..
Total cells with aberrations	40	9.88	14	1.01	9.7†

* Nine slides (buds) examined in each case.

† A comparison between the total number of aberrations of optimal and low calcium cells gave a chi-square value of 84.59, with D.F. = 1 .P <0.001.

of occurrence. Types 1, 6, and 7 originated prior to microspore formation; types 4 and 5 probably arose during development of the microspore; types 2 and 3 could not be determined.

The frequency of abnormal chromosomal alterations was higher in anthers from plants grown in suboptimal calcium. This was especially true for spore types 1, 2, and 5; also, types 4 and 7 occurred only in plants grown in suboptimal calcium. The chi-square test indicated a highly significant difference between the two groups of plants ($P < .001$).

The number of micronuclei was determined in microspores at a stage just prior to prophase. The results of these observations are shown in Table 2. Spores from plants grown in suboptimal calcium contained 4.42 times as many micronuclei as spores from plants grown in optimal calcium. The presence of micronuclei in the microspores reflected the occurrence of anomalous chromosome behavior at a previous spindle figure. Either chromosomal aberrations resulting in acentric chromatid formation or delayed chromosome separation could have accounted for the observed event. The variability in size of the micronuclei, together with the evidence presented in Table 1, suggested that structural alteration within chromosomes rather than faulty chromosomal separation was responsible for the origin of the majority of micronuclei.

TABLE 2

FREQUENCY OF MICRONUCLEI (MICN.) IN MICROSPORES PRIOR TO PROPHASE

	Optimal Calcium			Suboptimal Calcium		
	No.	Per 100 Cells	Total per 100 Cells	No.	Per 100 Cells	Total per 100 Cells
Total no. buds	7			4		
Microspores without micn.	9,680			2,954		
One micn. per cell	82	0.840	0.881	93	3.04	3.89
Two micn. per cell	2	0.020		13	0.42	
Aborted microspores	303	2.02		760	19.90	
Totals	10,067			3,820		

4.42 = Difference between optimal and suboptimal calcium

A third set of observations was made to determine the frequency of appearance of micronuclei in postdivision microspores or pollen. The number of pollen grains with 0, 1, 2, and 3 micronuclei from both groups of plants is recorded in Table 3. Here, again, differences between the two treatments were noted. Pollen grains containing micronuclei were 17.8 times more frequent in anthers from plants grown in suboptimal calcium than in anthers from plants grown in optimal calcium (Table 3). Abortive pollen grains were present in all anthers.

From Table 3 it can be seen that the number of pollen grains with micronuclei and the number of abortive grains varied from bud to bud. There is an obvious correlation between the frequency of these two types of cell within individual buds from plants grown in suboptimal calcium. Statistical tests show that no such correlation exists in buds from plants grown in optimal calcium. The relationship between the numbers of micronuclei and pollen abortion in buds from suboptimal calcium is presented graphically in Figure 1. Using the standard regression method, the equation for the line in Figure 1 is $y = 26.7 + 2.24(x - 12.4)$, and the

TABLE 3

FREQUENCY OF MICRONUCLEI IN EARLY BINUCLEATE POLLEN

FLOWER BUD	CELLS WITHOUT MICN.	MICRONUCLEI PER CELL 1	2	3	CELLS ABORTED	TOTAL CELLS
Optimal Calcium:						
1	1,510	11	0	0	30	1,551
2	1,501	6	0	0	24	1,531
3	1,555	7	0	0	26	1,588
4	1,559	11	0	0	47	1,617
5	1,598	18	0	0	44	1,660
6	1,500	13	0	0	37	1,550
7	1,553	10	0	0	49	1,612
8	1,567	7	0	0	23	1,597
Totals	12,343	83	0	0	280	12,706
Per 100 cells		0.668	0	0	2.204	
Suboptimal Calcium:						
1	875	112	13	0	314	1,314
2	506	90	8	1	395	1,000
3	1,000	207	15	6	1,282	2,510
4	1,021	77	3	0	220	1,321
5	613	106	13	0	421	1,153
6	1,154	75	6	0	235	1,470
7	1,220	20	0	0	46	1,286
Totals	6,389	687	58	7	2,913	10,054
Per 100 cells		9.62	0.812	0.098		
Total per 100 cells		11.54			28.97	

17.28 = Difference between frequencies of micronuclei in optimal and suboptimal calcium cells

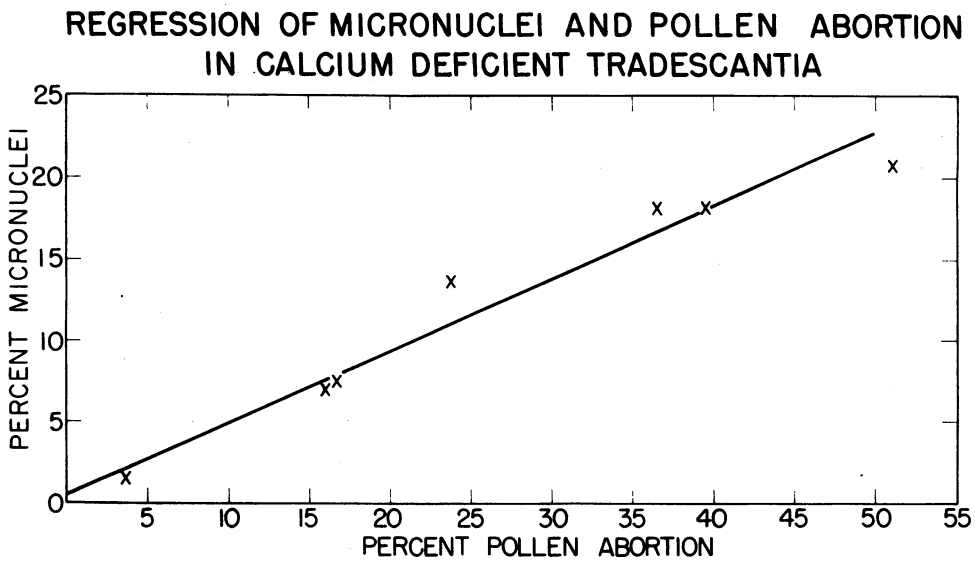

FIG. 1

slope of this line is 0.46, which differs significantly from one and zero. Chromatin duplication in one pollen grain of a tetrad would presumably produce a corresponding chromatin deficiency in another grain, causing it to abort. This could explain the relationship between the numbers of micronuclei in pollen and of pollen abortion.

Discussion.—The salient point of these experiments is that the frequency of chromosome fragmentation and the presence of micronuclei are functions of calcium concentration. This contention is further supported by unpublished experiments which involve the study of several different calcium levels.

Further evidence indicates that plants grown in decreasing concentrations of calcium are increasingly more sensitive to X-rays. Of particular interest to the case in point is a differential effect between types of chromosomal aberration observed following X-irradiation of buds grown in low calcium. The proportional increase of chromosome fragments and interstitial deletions is higher than the increase of dicentrics and centric rings. These observations were twice repeated, and each time differences were significant. These unpublished data support the conclusion that calcium is directly involved in the chromosome-breakage process in *Tradescantia.*

Bernstein and Mazia[4] report that chelating or sequestering agents, such as citrate and sodium phosphate, following treatment with distilled water produce a deoxyribonucleoprotein particle approximately 4300 Å by 250–300 Å in size. The ions removed by sequestering presumably hold the particles within the chromosome together by chemical bonds. In macromolecular states, it is indicated that calcium and magnesium form bonds with deoxyribonucleic acid (DNA)[5] and with protein.[6] Other studies show that cations provide stability for viruses[7] and for DNA.[8] The exact nature of bonding in DNA, nucleoprotein, or chromosomes is not known. But a priori bonding could occur both within and between the key groups of macromolecular constituents. Important is the fact that calcium and magnesium prefer to form complexes with oxygen, in particular with the oxygen of phosphate groups.[9]

The above biochemical evidence, together with the finding that a deficiency of calcium can significantly alter both spontaneous and X-ray-induced chromosomal aberrations, leads to the following hypothesis: Bivalent cations, e.g., calcium and magnesium, are involved with complexing and binding of chromosomal nucleoprotein and are thereby responsible for a part of the chromosome's structural constitution.

Summary.—*Tradescantia* plants from the same clone were grown in different calcium concentrations. Observations from microspores and pollen showed that plants grown in suboptimal calcium concentrations exhibited at least seventeen times more chromosomal aberrations and micronuclei than did plants grown in optimal calcium. Coupling these data with the biochemical evidence presented, it was concluded that bivalent cations may bind together the macromolecular constituents of the chromosome and thereby provide structural stability.

* Research carried out at Brookhaven National Laboratory under the auspices of the United States Atomic Energy Commission.

[1] D. Steffensen, these PROCEEDINGS, 39, 613–620, 1953.

[2] E. S. Horning, in G. Bourne (ed.), *Cytology and Cell Physiology* (London: Oxford University Press, 1942), pp. 160–88; L. G. Allgén, *Acta Physiol. Scand.*, Vol. 22 (Suppl. 76), 1950.

[3] D. R. Hoagland and D. I. Arnon, *Calif. Agr. Exptl. Sta. Circ.*, No. 347, 1950.
[4] M. H. Bernstein and D. Mazia, *Biochim. et biophys. acta*, **10**, 600–606, 1953; *ibid.*, **11**, 59–68, 1953.
[5] G. Jungner, *Acta Chem. Scand.*, **5**, 168–174, 1951.
[6] W. G. Crewther, *Australian J. Biol. Sci.*, **6**, 597–616, 1954.
[7] Karl G. Lark and Mark H. Adams, *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 171–183, 1953.
[8] S. Zamenhof, G. Griboff, and N. Marullo, *Biochim. et Biophys. Acta*, **13**, 459–470, 1954.
[9] R. J. P. Williams, *Biol. Rev.*, **28**, 381–415, 1953.

---

*ON THE NICKEL, COBALT, AND COPPER CONTENTS OF DEEP-SEA SEDIMENTS**

BY G. E. HUTCHINSON, R. J. BENOIT,† W. B. COTTER, AND P. J. WANGERSKY

OSBORN ZOOLOGICAL LABORATORY, YALE UNIVERSITY, NEW HAVEN, CONNECTICUT

Communicated January 9, 1955

In two recent papers Pettersson and Rotschi,[1] using a magnificent series of analyses of deep-sea cores collected by the Albatross expedition and analyzed by Rotschi,[2] have suggested that the nickel content of Pacific red clay is mainly of extraterrestrial origin. They observed considerable differences between typical Pacific red clays and the series of Atlantic samples from a single core that they analyzed, the Pacific material ordinarily containing at least five times as much nickel as the Atlantic. They attribute this to the greater lithogenous sedimentation in the Atlantic, which masks the effect of the extraterrestrial contribution.

Goldberg,[3] in a stimulating study of the role of electrochemical precipitation in the sea, concludes that the nickel content of the sediments analyzed by Rotschi is in a broad way dependent on their manganese content. He supposes that, during the electrochemical precipitation of manganese oxides, positively charged nickel is adsorbed on the precipitate. He further points out that in manganese nodules cobalt and copper as well as nickel are enriched; the ratios of these elements to nickel in such nodules are much greater than in meteoritic material in which the Ni:Co ratio is at least 10:1 and the copper content much less than that of nickel.[4] If an extraterrestrial origin for the nickel is postulated, special hypotheses for the enrichment of the other two elements must also be entertained.

Since we have been engaged in the chemical study of certain deep-sea cores, during which a number of analyses of nickel, cobalt, and copper have been made, it has seemed proper to complete Goldberg's argument by publication of our data for these elements. This is particularly desirable since Goldberg's original analyses relate solely to manganese nodules, while Rotschi's great body of data does not include determinations of cobalt and copper.

Three cores have been available, two from the Atlantic and one from the Pacific ocean. A 160-8 is for the most part a typical red clay, from 5,030 meters at latitude 16°54′ N., longitude 59°31′ W. It is 870 cm. long; twenty samples were analyzed, but, in presenting the data, two are omitted. One of these is from a sharply defined band of volcanic ash and is obviously irrelevant; the other is from a man-