AN ANALYSIS OF THE REJOINING OF X-RAY-INDUCED BROKEN ENDS OF CHROMOSOMES IN THE ROOT TIPS OF *ALLIUM CEPA*¹

NORMAN S. COHN²

Mergenthaler Laboratory for Biology, Johns Hopkins University, Baltimore, Maryland

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SINCE the early work of SAX (1938), the phenomena of chromosome breakage and the behavior of broken ends of chromosomes, especially as they are related to studies with ionizing radiations, have received considerable attention. Recently, special attention has been focused upon the possible mechanisms involved in the reunion of broken chromosome ends. In particular, attempts have been made to interpret this process in terms of certain current hypotheses on chromosome structure. The investigations of MAZIA (1954), STEFFENSEN (1955), and others have indicated that ionic bonds may play a role in chromosome organization. The work of WOLFF and LUIPPOLD (1955) on *Vicia faba* has led them to an hypothesis that radiation produces breaks in the chromosome that require energy for their rejoining, and that these breaks are breaks of covalent bonds.

The studies to be reported have been concerned with the establishment and investigation of an experimental system in which the behavior of broken ends of chromosomes could be readily analyzed. One of the initial aims of these studies with *Allium cepa* was to establish the time factor in the restitution of X-ray-induced chromosome breaks by means of fractionated dosage experiments. The modification of the behavior of induced breaks is most readily studied in a system which permits various experimental treatments during the interval between dose fractions.

The studies of SAX (1939) on *Tradescantia* microspores demonstrated that continuous radiation produces more aberrations than does intermittent radiation with respect to two-break types of aberrations. FANO and MARINELLI (1943) concluded that fractionation experiments, consisting of two high-intensity irradiations separated by a variable time interval are better suited to the investigation of the time-intensity factor than are experiments in which the intensity of continuous irradiation is varied. A number of independent investigations in *Tradescantia* (DE SERRES and GILES 1953; SAX, KING and LUIPPOLD 1955) have shown that the frequency of two-hit aberrations induced in *Tradescantia* micro-

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spore nuclei is reduced if the X-ray dose is fractionated, that this reduction is due to the restitution of breaks during the rest periods between fractionated exposures, and that with sufficiently long rest periods, the frequency of two-hit aberrations is approximately equal to that induced by a single exposure multiplied by the total number of exposures—the base line.

MATERIALS AND METHODS

Seeds of *Allium cepa*, variety Southport White Globe were used in these investigations. With the exception of certain portions of the radiosensitivity studies, seeds were immersed in distilled water for six hours, placed on moist filter paper in petri plates, and irradiated 24 hours from the initiation of soaking. The seeds were allowed to germinate for a total of three days from the time of initial immersion, and fixation of roots was done after a six hour colchicine treatment. For each X-ray exposure, Feulgen smears were prepared and at least 500 cells at metaphase of the first mitotic division were scored for two-hit aberrations. X-rays were administered with a G.E. Maxitron tube operated at 250 Kv peak and 30 ma with 1 mm Al filtration. The same intensity (1600r/min) was maintained for all experiments.

RESULTS AND DISCUSSION

Radiosensitivity of seeds

Preliminary experiments were performed with germinating seeds of *Allium cepa* to determine (1) the developmental stage of the roots at which irradiation would produce only chromosome aberrations, (2) the time at which a relatively high frequency of aberrations is produced, and (3) the length of time during which chromosome aberration frequencies remain constant during early root development. Seeds were soaked for varying periods of time—from 3 to 30 hours—prior to irradiation, and all roots were fixed three days from the time of initial soaking. Figure 1 shows that the frequency of aberrations, both exchanges and interstitial deletions, increases as the time of soaking is increased prior to irradiation, but reaches a plateau of radiosensitivity with about 14 hours of presoaking. There is a high degree of variation at the earlier soaking periods which may indicate some physiological variability in individual root development at early stages. As the duration of presoaking increases, the degree of variation decreases. This latter point is borne out in chi-square tests of homogeneity between slides of a single treatment which showed considerable heterogeneity for the shorter soaking times, but homogeneity of results at about 24 hours presoaking. The frequencies of aberrations induced by X-rays following soaking periods of from 14 to 30 hours are relatively constant and the aberrations are all of the chromosome type. Since several of the intervals in the fractionation studies are of long duration, it is imperative that the cells be in a comparable state of radiosensitivity, as measured by chromosome breakage, during this period.

FIGURE 1.—The effect of presoaking on X-ray-induced chromosome aberrations. All roots fixed three days from initiation of soaking.

Effect of fractionated doses

The frequency of chromosome exchanges (only exchanges are reported because of the greater reliability in their scoring as compared with interstitial deletions) was determined for each of the following irradiations: a full, continuous dose of 1600r given in one minute; a single, half-dose of 800r given in one-half minute; two dose fractions of 800r each, with intervals between fractions of 2.5, 5, 10, 15, and 30 minutes, 1, 2, 4, 5, 6, 10, and 15 hours. In most cases (Table 1) each experimental point represents the results of two or more identical exposures. The theoretical base line (Figure 2) was calculated by multiplying by two the frequency induced by a single, half-dose of 800r.

When the dose is divided into two equal fractions of 800r separated by intervals of 2.5 or five minutes, the frequency of exchanges is statistically the same as that induced by the full, continuous dose (Table 1). This indicates that the breaks induced by the first dose fraction do not undergo restitution before the second

TABLE 1
Effect of fractionated doses on aberration frequency

Dose in r	Interval between dose fractions	Total cells	Number of exchanges	Exchanges per cell
1600		2435	2141	.91±.03
800		1516	333	.22±.01
800 + 800	2.5 min	1262	1137	.90±.03
800 + 800	5.0 min	1569	1273	.90±.06
800 + 800	10.0 min	1128	881	.75±.04
800 + 800	15.0 min	1663	1080	.64±.03
800 + 800	30.0 min	524	354	.65±.04
800 + 800	1 hr	500	317	.63±.04
800 + 800	2 hrs	1179	764	.64±.03
800 + 800	4 hrs	1082	683	.60±.06
800 + 800	5 hrs	497	262	.53±.04
800 + 800	6 hrs	907	421	.45±.04
800 + 800	10 hrs	500	236	.47±.02
800 + 800	15 hrs	503	221	.44±.01

(Exchanges 800r) x 2 = base line = .44±.014

FIGURE 2.—Comparison of the effects of fractionated doses in air and with carbon monoxide treatment.

dose is delivered, and consequently, these breaks are available for complete interaction with the breaks produced by the second dose. As the interval between fractions increases from five to 15 minutes, the frequency of exchanges decreases and then remains relatively constant for the intervals up through four hours. With a six-hour interval there is no interaction of the breaks produced by the two dose fractions and the frequency of exchanges is equal to twice the frequency induced by a single, half-dose—the base line (Figure 2). These data indicate that in *Allium cepa* root tips two classes of X-ray-induced broken ends of chromosomes can be distinguished on the basis of their average restitution times. One class of breaks includes those that undergo restitution within 15 minutes, and the other those that remain available for reunions for as long as four hours. The fact that the frequency of chromosome exchanges is of a magnitude intermediate between the frequency induced by 1600r alone and the base line, indicating partial restitution and partial interaction, is the basis for this conclusion.

In considering the effect of fractionated dose treatments *per se*, these results are in substantial agreement with those found by SAX *et al.* (1955) and others (*op. cit.*) in *Tradescantia*, but are in contrast to the results of LANE (1951), and strongly suggest that in *Allium* the original hypothesis of SAX is applicable. An indication that a certain portion of X-ray-induced broken ends of chromosomes in *Tradescantia* do not undergo immediate restitution was also found by SAX (1939). However, at the end of about an hour restitution and reunion are complete (SAX 1940). LEA and CATCHESIDE (1942) calculated that for chromatid exchanges in *Tradescantia* the average time between breakage and reunion is about 3.5 minutes, but a few breaks may remain open for a longer period of time. Later experiments of CATCHESIDE *et al.* (1946) suggested that those breaks which do not undergo rapid restitution remain open for a time measurable in hours. The findings of these authors, then, indicate the occurrence of more than one type of induced chromosome break—those that undergo rapid restitution and those that remain open for a prolonged period. The effect of fractionated doses reported here provides substantial evidence for the production in *Allium* of two classes of chromosome breaks as judged by their average time of restitution.

Inhibition of restitution by carbon monoxide

On the basis of the behavior of the broken ends induced in the chromosomes of *Allium cepa*, and taking into account the mechanisms proposed by STEFFENSEN (1955) and by WOLFF and LUIPPOLD (1955), the hypothesis was suggested that in *Allium* the more rapidly restituting breaks are of ionic bonds in the chromosome and the more slowly restituting breaks are of covalent linkages (COHN 1956). A series of experiments was then designed to test whether the two classes of breaks detected in the fractionation experiments could be distinguished from each other by their responses to carbon monoxide. The results of studies in *Vicia faba* (WOLFF and LUIPPOLD 1955) have indicated that carbon monoxide prevents the restitution of broken ends of chromosomes, and the effect has been interpreted as due to an interference with oxidative mechanisms required for the synthesis

of covalent bonds. If the more rapidly rejoining breaks in *Allium* chromosomes are ionic in nature, carbon monoxide would be expected to produce no change in their time of rejoining, while if the more slowly rejoining breaks are of a covalent nature, carbon monoxide would be expected to inhibit their rejoining. On this hypothesis, the introduction of carbon monoxide during an interval up to 15 minutes between two dose fractions would be expected to result in an aberration frequency the same as that induced by X-rays in air. By contrast, the presence of CO during an interval longer than four hours between fractions should produce an exchange frequency higher than that found in the fractionation studies in air. That is, the presence of CO should delay the rejoining of the slowly rejoining breaks produced by the first dose, and complete interaction of breaks of this type produced by the two dose fractions would occur. An equal response of the two types of breaks to CO would indicate that the two classes of breaks are not separable on the basis of ionic vs. covalent bonds, but might suggest that oxidative metabolism is necessary for the rejoining of both types of X-ray-induced chromosome breaks.

Prior to the experiments with CO, two experiments of a preliminary nature were carried out. The first was a test for a possible effect of carbon monoxide in producing chromosome breakage. The roots of germinating seeds which had been kept in carbon monoxide in the dark for six hours were scored for metaphase aberrations. In 300 cells, the frequency of exchanges was less than 0.01/cell, a figure within the range of spontaneous breakage. There was no apparent effect of the CO in causing chromosome breakage in the absence of irradiation. The second experiment involved the determination of an X-ray dose given *in vacuo* which would induce an aberration frequency equal to that produced by 800r in air. If the first dose fraction in a treatment combining fractionated doses and carbon monoxide exposure is performed *in vacuo*, carbon monoxide can be introduced rapidly into the evacuated chamber and so into the roots immediately following X-ray exposure. The establishment of the dose *in vacuo* which produces the same effect as 800r in air permits better comparisons between the effect of fractionated doses in conjunction with carbon monoxide treatment and the effect of fractionated doses in air. With a dose of 1100r, a frequency of 0.22 exchanges/cell was observed from an analysis of 350 cells. This frequency is approximately the same as that induced by 800r in air.

The general procedure for the experiments with fractionated doses involving carbon monoxide treatments is indicated in Table 2. In all treatments, the roots were maintained *in vacuo* for five minutes prior to dose I. Dose I was administered *in vacuo* to ensure the rapid and relatively uniform entrance of CO into the roots following irradiation. These experiments included pre- and postirradiation treatments with carbon monoxide, and treatments comparable to a combination of pre- and postirradiation carbon monoxide exposure in which carbon monoxide was present between the two dose fractions. Each of the four experimental series—5 minutes, 10 minutes, 1 hour, and 6 hours—incorporated the six treatments as listed in Table 2, differing only in the time of CO exposure.

TABLE 2
The effect of carbon monoxide on rejoining

Treatment No.	Dose I (r) (all <i>in vacuo</i> 6 min)	CO treatment time	Dose II (r) (all in air, in light)	Exchanges per cell		
				Observed	Calculated	
					No interaction	Complete interaction
1	1100	dark, 5 min	800	1.28±.10	.70	1.41
		10 min		1.33±.05	.61	1.23
		1 hr		1.30±.08	.68	1.37
		6 hrs		1.20±.03	.68	1.34
2	1100	light, 5 min	800	1.02±.08	.48	.96
		10 min		.81±.05	.43	.87
		1 hr.		.61±.03	.48	.96
		6 hrs		.43±.02	.45	.90
3	0	dark, 5 min	800	.33±.04		
		10 min		.30±.02		
		1 hr		.31±.03		
		6 hrs		.34±.01		
4	0	light, 5 min	800	.25±.03		
		10 min		.22±.01		
		1 hr		.24±.01		
		6 hrs		.22±.01		
5	1100	dark, 5 min	0	.37±.01		
		10 min		.31±.01		
		1 hr		.37±.03		
		6 hrs		.34±.01		
6	1100	light, 5 min	0	.23±.01		
		10 min		.21±.01		
		1 hr		.24±.02		
		6 hrs		.23±.01		

In all treatments, light and air were introduced immediately after CO exposure.

The experimental scheme was based on the assumption that the frequencies of exchanges induced by CO pretreatment in the dark (treatment 3) and by CO posttreatment in the dark (treatment 5) could be used to calculate the frequency of exchanges to be expected from the combined treatment (treatment 1). The summation of the frequencies induced by treatment 3 and 5 would give a value to be expected if there is no interaction between the breaks induced by dose I and dose II. Such a value would suggest that CO is ineffective in preventing restitution. However, if CO does prevent restitution and breaks induced by dose I remain available for complete interaction with those produced by dose II, the frequency of exchanges will be equal to the square of the sum of the square roots of the frequencies induced by treatments 3 and 5 (WOLFF and ATWOOD 1954). The cytological and irradiation procedures were the same as those previously described.

Table 2 and Figure 2 present those portions of the data dealing with combined CO and fractional X-ray dose treatments in comparison with the previously

studied effects of fractionation treatments in air. The most striking feature of Figure 2 is the consistently high level of effect of carbon monoxide treatment in the dark. The data show that carbon monoxide prevents the restitution of X-ray-induced chromosome breaks for all four of the tested intervals. This conclusion is based on the fact that, for all four intervals, calculations utilizing the frequencies induced by treatments 3 and 5 indicate that in treatment 1, complete interaction has occurred between the breaks produced by dose I and dose II (Table 2). That is, the observed values are comparable to the expected values calculated on the basis of complete interaction. Furthermore, it appears evident that both types of breaks—the rapidly and the slowly restituting types—are prevented from undergoing restitution by carbon monoxide. That this effect is light reversible is also clear, since the frequencies of exchanges induced by fractionated dose treatments with CO in the light are essentially the same as those induced in air. This light reversibility of the CO effect was also found in the comparisons of the pre- and postirradiation CO treatments in the dark and in the light (Table 2).

An additional aspect of these results is indicated by the observed frequencies of exchanges induced by the pre- and postirradiation CO treatments in the dark and by the combined CO-fractionation treatments in the dark. Since, in all treatments, the seeds were exposed to air and light immediately before dose II was administered, it was anticipated that the frequency of exchanges induced by treatment 3 would be equal to the frequency induced by 800r in air. Actually, the frequency induced by the preirradiation CO treatment is significantly higher than expected. Furthermore, on the average there appears to be an even more marked effect of CO post-treatment in increasing the aberration yield, as indicated by treatment 5. These results apparently account for the exceedingly high frequencies of aberrations found after the CO-fractionation treatments in the dark. The increased frequency of exchanges following CO exposure prior to irradiation (treatment 3) might be due to a residual CO-cytochrome complex in some of the cells as a result of delayed or incomplete light reversal, which could give rise to an increase of initial breakage or to a modification of restitution. An interpretation of these data will be presented in more detail subsequently.

In conclusion, the results indicate that all the induced breaks are influenced by carbon monoxide, and that the effect is primarily an inhibition of restitution. There is no differential effect of CO on the rapidly or slowly restituting classes of breaks. On the basis of the original hypothesis that CO should affect the formation of "covalent" but not of "ionic" bonds, no distinction can be made between "ionic" and "covalent" bonds in this system. An explanation of the biphasic curve elaborated from the fractionated dosage studies is still necessary. On the basis of the CO investigations, the two classes of breaks are not qualitatively different.

CONCLUSIONS

Results similar to those found in the fractionated dose experiments with *Allium cepa* have subsequently been presented by WOLFF and LUIPPOLD (1956).

Studies on the root tips of *Vicia faba* have indicated that two classes of breaks are induced by X-rays in this system as well as in the root tips of *Allium*. In *Vicia faba*, the experimental procedure involved intensity studies rather than fractionation techniques and the results differ from those with *Allium* in that the rapidly rejoining breaks undergo restitution within about three minutes, whereas the slowly rejoining breaks undergo restitution within about two hours. The hypothesis proposed by these workers to explain the nature of the two types of breaks is the same as that previously proposed by COHN (1956) for *Allium*. From the results of their studies with such substances as Versene (EDTA) and metabolic inhibitors, WOLFF and LUIPPOLD have concluded that the hypothesis involving ionic and covalent linkages may be applicable. However, the evidence is indirect, and the two classes of breaks may not be clearly separable in these terms. The recent studies of KAUFMANN *et al.* (1957a) have, in fact, indicated that the effect of EDTA in increasing crossing over in *Drosophila* may not be reliable evidence for the direct chelation of divalent cations such as Mg and Ca within the chromosomes. The results of these investigators have suggested that the effects of such an agent on crossing over might best be interpreted in terms of changes in the general ionic environment of the organism. Additionally, cytochemical studies of the salivary gland cells and chemical studies of onion root tip cells have indicated that structural alterations of the chromosomes induced by a modification of the ionic environment of the cell may involve RNA (KAUFMANN and McDONALD 1957b). In essence, these results do not support the proposition that EDTA-induced chromosomal changes depend on the removal of specific divalent cations from the chromosomes.

The possibility that one of the two types of X-ray-induced chromosome breaks in *Allium cepa* is ionic in nature may not be entirely eliminated by the present experimental results. POULSON and BOWEN (1952) have presented evidence for the presence of iron in the nuclei of *Drosophila* with the indication that the bound iron is associated with protein. The recent investigations of POSSINGHAM and BROWN (1957) with Fe⁵⁹ provide additional evidence that iron is an important component of the nuclear material. On the basis of their studies and those of other workers, POSSINGHAM and BROWN have suggested that iron and/or other heavy metals may promote the linkage of DNA with protein. KIHLMAN and others (1957a,b) have investigated the effect of heavy metal complexing agents on the induction of chromosomal aberrations in *Vicia faba*. They have proposed that Fe and/or other heavy metals are localized in the chromosomes and that cyanide as well as other heavy metal complexing agents produce chromosomal aberrations by reacting with these metals. There are two aspects of the results of the carbon monoxide treatments in *Allium cepa* that lead to the development of an hypothesis involving iron linkages in the chromosome. The combined CO-fractionated X-ray treatments resulted in an aberration frequency considerably higher than expected, and the results of the postirradiation CO exposure were similar in this respect (Table 2). The effect of CO might be the formation of a complex with the Fe of a broken linkage, thereby prohibiting the restitution of the

broken ends. The effect of preirradiation CO exposure in increasing the aberration frequency could be the result of complex formation which enhances X-ray breakage. In this case, it would appear that either the complex of chromosomal iron and carbon monoxide (unlike that of CO-cytochrome oxidase) that is formed during the pretreatment is not light dissociated, or that the effect of such a complex on chromosome stability is maintained after the complex has been light dissociated. Assuming this interpretation, the rapidly restituting breaks would be breaks of ionic linkages in the chromosome in which Fe is the cation involved, whereas the slowly restituting breaks could still be explained as requiring energy for their synthesis during restitution.

An alternative explanation of the results presented here is still feasible. It is possible that the difference between the two types of breaks is somehow related to the spatial relations of broken chromosome ends. If chromosome movements are the cause of two classes of breaks, one might expect in fractionation studies a curve with less pronounced plateaus because of the randomness of such movements. However, if the distance separating broken chromosome ends is critical in the rejoining process, as calculated by LEA (1947) for *Tradescantia* microspore chromosomes, and if the time factor is equally crucial with regard to chromosome movements, then a sharp biphasic curve of the type reported in *Allium* might occur. Under these circumstances the formation of a CO-cytochrome complex would still be effective in preventing respiratory oxidation, but the effect on aberration yield would not necessarily require an explanation in terms of "covalent" bonds or "ionic" linkages. The results of these investigations in *Allium* however, tend to support the hypothesis that heavy metals such as Fe play a role in chromosome organization.

SUMMARY

Experiments were performed with the roots of *Allium cepa* seedlings in order to analyze the rejoining behavior of X-ray-induced broken ends of chromosomes, and to test several hypotheses relating the results of such studies to the nature of chromosome organization. Studies with fractionated doses have shown that two classes of breaks, as judged by their average time of restitution, are induced by X-rays—those that undergo relatively rapid restitution within 15 minutes, and those that remain open for a relatively long period of time e.g., four hours. In an effort to distinguish the two classes of breaks on a qualitative basis, experiments utilizing carbon monoxide treatments were performed. Carbon monoxide was found to produce a light-reversible inhibition of the restitution of both types of breaks. The results of these studies indicate that the two classes of breaks cannot be distinguished on this basis. The possibility that iron provides an ionic linkage in the chromosome is discussed.

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LITERATURE CITED

- CATCHESIDE, D. G., D. E. LEA, and J. M. THODAY, 1946 The production of chromosome structural changes in *Tradescantia* microspores in relation to dosage, intensity, and temperature. *J. Genet.* **47**: 137-149.
- COHN, N. S., 1956 An analysis of the rejoining of X-ray-induced broken ends of chromosomes. *Genetics* **41**: 639 (Abstr.)
- DE SERRES, F. J., JR., and N. H. GILES, 1953 The effect of radiation dose fractionation on chromosome aberration frequencies in *Tradescantia* microspores. I. Studies with X-rays. *Genetics* **38**: 405-417.
- FANO, U., and L. D. MARINELLI, 1943 Note on the time intensity factor in radiobiology. *Proc. Natl. Acad. Sci. U.S.* **29**: 59-66.
- KAUFMANN, B. P., H. GAY, and M. J. McELDERRY, 1957a Effect of ribonuclease on crossing over in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.* **43**: 255-261.
- KAUFMANN, B. P., and M. R. McDONALD, 1957b The nature of the changes effected in chromosomal materials by the chelating agent EDTA. *Proc. Natl. Acad. Sci. U.S.* **43**: 262-270.
- KIHLMAN, B. A., 1957a Experimentally induced chromosome aberrations in plants. I. The production of chromosome aberrations by cyanide and other heavy metal complexing agents. *J. Biophys. Biochem. Cytol.* **3**: 363-380.
- KIHLMAN, B. A., T. MERZ, and C. P. SWANSON, 1957b Experimentally induced chromosome aberrations in plants. II. The effect of cyanide and other heavy metal complexing agents on the production of chromosome aberrations by X-rays. *J. Biophys. Biochem. Cytol.* **3**: 381-390.
- LANE, G. R., 1951 X-ray fractionation and chromosome breakage. *Heredity* **5**: 1-35.
- LEA, D. E., and D. G. CATCHESIDE, 1942 The mechanism of the induction of chromosome aberrations in *Tradescantia*. *J. Genet.* **44**: 216-245.
- LEA, D. E., 1947 *Actions of Radiations on Living Cells*. Cambridge University Press.
- MAZIA, D., 1954 The particulate organization of the chromosome. *Proc. Natl. Acad. Sci. U.S.* **40**: 521-527.
- POULSON, D. F., and V. T. BOWEN, 1952 Organization and function of the inorganic constituents of nuclei. *Exp. Cell Research* **2**: 161-180.
- POSSINGHAM, J. V., and R. BROWN, 1957 The intracellular incorporation of Fe^{59} into the root cells of *Pisum*. *Nature* **180**: 653-654.
- SAX, K., 1938 Chromosome aberrations induced by X-rays. *Genetics* **23**: 494-516.
- 1939 The time factor in X-ray production of chromosome aberrations. *Proc. Natl. Acad. Sci. U.S.* **25**: 225-233.
- 1940 Analysis of X-ray-induced chromosomal aberrations in *Tradescantia*. *Genetics* **25**: 41-69.
- SAX, K., E. D. KING, and H. LUIPPOLD, 1955 Effect of fractionated X-ray dosage on the frequency of chromatid and chromosome aberrations. *Radiation Res.* **2**: 171-180.
- STEFFENSEN, D., 1955 Breakage of chromosomes in *Tradescantia* with a calcium deficiency. *Proc. Natl. Acad. Sci. U.S.* **41**: 155-160.

- WOLFF, S., and K. C. ATWOOD, 1954 Independent X-ray effects on chromosome breakage and reunion. *Proc. Natl. Acad. Sci. U.S.* **40**: 187-192.
- WOLFF, S., and H. LUIPPOLD, 1955 Metabolism and chromosome break rejoining. *Science* **122**: 231-232.
- 1956 The production of two chemically different types of chromosomal breaks by ionizing radiation. *Proc. Natl. Acad. Sci. U.S.* **42**: 510-514.