COMMENT AND REVIEWS

THE EFFECT OF FRACTIONAL X-RAY DOSAGE ON THE FREQUENCY OF CHROMOSOME ABERRATIONS

KARL SAX

Bussey Institute, Harvard University

and

HENRY LUIPPOLD

University of Massachusetts

The effect of fractional dosage and radiation intensity on the frequency of induced chromosome aberrations has provided information of value in interpreting the nature of chromosome breaks and reunions. It has been found that fractional dosage of prophase chromosomes in *Tradescantia* microspores decreases the frequency of 2-hit aberrations (translocations, dicentrics and rings), but has no effect on the frequency of 1-hit aberrations (chromatid and iso-chromatid deletions) (Sax, 1941). Fractional dosage or low intensities also decrease the frequency of 2-hit chromosome aberrations induced in the resting chromosomes of *Tradescantia* microspores (Sax, 1940, 1941). These and other observations have been used by Lea (1946) and Catcheside (1948) to explain the nature of X-ray induced chromosome breaks and reunions. They show that the breaks necessary to permit the formation of a 2-hit chromatid or chromosome aberration must be limited in both time and space, and that many of the breaks undergo restitution.

Recently Lane (1951) has questioned the validity of the author's technique and results with fractional dosage. The technique of the earlier work has been questioned on the grounds that the temperature was not held constant between the time of irradiation and fixation of the microspores, and that cells fixed 24 hours after irradiation are excessively variable due to " relatively large effects of environmental variation." It is true that temperature does affect the frequency of X-ray induced chromosome aberrations (Sax and Enzmann, 1939), but the effect is limited to the period during and shortly after irradiation, and the normal laboratory temperature range would have relatively little effect. There is, of course, some variation in aberration frequency which might be attributed to " environmental variation," but we have found no evidence that this variation is any greater in prophase than in the resting stage. It is more difficult to score chromatid aberrations than chromosome aberrations, but in either case adequate numbers of observations have given consistent results.

Lane's experiments covered three growing seasons (1947, 1948 and 1949). Presumably he did not have a clonal line of *Tradescantia* which can be flowered throughout the year in a greenhouse. The irradiation was done at 18° C. and the inflorescences were held at 24° C. between irradiation and fixation. The X-ray dosage was 360 *r* given at the rate

of 25 *r* per minute in the more extensive experiments. The first year's work was too limited to be of any value. In 1948 it was found that two exposures of 180 *r* each separated by a rest period of 4 hours reduced the 2-hit aberration frequency, but only 480 chromosomes were analysed in each of the six experiments. In 1949 fractional dosage with rest periods of 1, 2, 4, 6 and 8 hours respectively showed a reduction in aberration frequency as the rest period increased up to and including 4 hours, but aberration frequency began to increase with the 6-hour rest period, and with an 8-hour interval between the two exposures the aberration frequency (9·0 per cent.) was nearly as high as that resulting from a single 360 exposure (9·9 per cent.). Only 960 chromosomes were analysed in each series of experiments with the exception of the single dose of 180 *r* where 1920 chromosomes were recorded.

These results led Lane to conclude that the decrease in aberration frequency following fractional dosage is due to physiological changes in the cell which makes the chromosomes less sensitive to the second exposure when the rest period is less than 8 hours. With an 8-hour rest period the cell has recovered from the first exposure and two doses of 180 *r* each then produce nearly as many aberrations as does a single dose of 360 *r*. Presumably it takes some time for the alleged physiological effect to become effective, since increasing rest periods up to 4 hours show an increasing decline in the frequency of aberrations. It is also claimed that two doses of 180 *r* each separated by a 4-hour rest period produces fewer aberrations than do two independent 180 *r* exposures.

Shortly after Lane's paper became available, a series of experiments were started for an analysis of the effects of fractional dosage, using the same dosage and rest periods used by Lane. The inflorescences of *Tradescantia paludosa* (Clone 3) were rayed at 180 *r* per minute, since at 25*r*/min. the aberration frequency is reduced by the intensity effect. After irradiation the inflorescences were placed in a greenhouse. Microspores were fixed on the fifth day after irradiation. An interval of only 2 or 3 days between irradiation and fixation often results in some chromatid aberrations as Lane has found. The control of temperature was not considered necessary because each series of experiments included an exposure of 360 *r* without fractionation which served as a control.

In the analysis of chromosome aberrations only centric rings and dicentrics were included. Dot deletions were frequent, but were not included because as Rick (1940) has shown they are a mixture of 1-hit and 2-hit aberrations. The simple terminal deletions were not included because they occur with such a low frequency that significant results could be attained only by analysing enormous numbers of chromosomes. A series of three experiments were completed in August and September, including in each one a single 360 *r* exposure, a fractionate dose of 360 *r* with rest periods of 4, 8 or 12 hours, and a single exposure of 180 *r*. The results of the single exposures were similar in all cases and the results of the three series have been combined. The data are shown in table 1.

The single dose of 360 *r* produced a total of 2055 dicentric and ring chromosomes or 12·2 per cent. compared with Lane's results of 9·9 per cent. The difference can be attributed to the higher intensity of radiation (180 *r*/min.) than that used by Lane (25 *r*/min.). A 360 *r* dose at 18 *r*/min.

gave essentially the same results as were obtained by Lane. Two exposures of 180 r each separated by rest periods of 4, 8 and 12 hours respectively produced essentially the same aberration frequency. The fractionated dose

TABLE 1

Effect of fractional dosage on frequency of 2-hit Chromosome aberrations. 180 r/min.

Dose	Rest period	N-chromosomes	Dic and Ring	Per cent.
360	0	16,848	2055	12·2
180+180	4 hrs.	17,820	1534	8·6
180+180	8 hrs.	9,990	847	8·5
180+180	12 hrs.	6,000	487	8·1
180	0	16,900	726	4·3

with a 12-hour rest period produced a somewhat lower frequency of aberrations than did the fractionated doses with 4- or 8-hour rest periods, but only 6000 chromosomes were analysed in this case. There is no evidence of an increase of aberration frequency as the rest period is increased to 8 or 12 hours. A single exposure of 180 r produced 4·3 per cent. of dicentrics and rings, or essentially half of the values from the 360 r fractionated exposures.

These experimental results, based upon repeated experiments and adequate numbers of chromosomes, confirm earlier conclusions. Fractional dosage does decrease the frequency of 2-hit chromosomal aberrations even with rest periods of as much as 12 hours. There is no evidence of a physiological effect of radiation which reduces breakage of chromosomes. Nor is there any evidence that long rest periods restore radiation sensitivity. The earlier interpretation appears valid. Fractional dosage reduces the aberration frequency because of restitution of single breaks during the rest period. If the rest period is very short the simple breaks surviving the first exposure persist until breaks are induced at the second exposure when these breaks become involved in the production of dicentric and ring chromosomes and other 2-hit aberrations. With longer rest periods the breaks which have not undergone restitution or illegitimate reunion during the first exposure do so during the rest period so that no breaks survive to take part in the aberrations induced during the second exposure. Under such circumstances two fractionated doses should not be any more effective than two separate doses. With four or more hours of rest between the two 180 r exposures the frequency of 2-hit aberrations does not exceed twice the aberration frequency induced by a single exposure of 180 r. Nor is there any significant decline of aberration frequency below this base line. The base line (2×4·3 = 8·6) does not deviate significantly from the fractional dosage values with 4-, 8- or 12-hour rest periods. The value of 8·1 per cent. with the 12-hour rest period is somewhat below the base line, but this experiment involved only 6000 chromosomes. There was also considerable variation between 100 cell samples with the 180 r series, and even with an analysis of 16,900 chromosomes the aberration frequency of 4·3 per cent. might well have varied a few tenths of a per cent. in either direction.

These results are in accord with earlier experiments (Sax, 1941) with fractional dosage involving chromatid breaks where it was possible to

compare the effects on both 1-hit and 2-hit aberrations. It was found that fractional dosage reduced the frequency of 2-hit aberrations, but produced as many simple deletions as did a single dose. Similar differential effects on the frequency of 1-hit and 2-hit aberrations were also found by reducing radiation intensity. High intensities of X-rays produce a much higher frequency of 2-hit aberrations than do low intensities, but the production of 1-hit aberrations is independent of intensity. These results can best be explained on the limitations of breaks in time and space. If two adjacent breaks occur within certain limits of time or space it is possible to obtain a 2-hit aberration. Lea (1946) has calculated the space factor as about 1 micron and the time factor as quite variable but with an average value of 3 or 4 minutes.

Lane dismisses the absence of any decrease in frequencies of 1-hit aberrations following fractional doses or low intensities as due to excessive variability of the cells when rayed at prophase and given only a 24-hour interval between irradiation and fixation. He has, however, made no tests to confirm his assumption. Nor has he presented any valid explanation of the differences in relative frequencies of 2-hit aberrations following irradiation with X-rays and neutrons. Although the variation in sensitivity of the chromosomes to X-rays during the cell cycle has little bearing on the effects of fractional dosage, Lane attempts to explain the decreased sensitivity of the chromosomes following the first exposure as due to an increased nucleic acid charge induced by radiation. He accepts the assumption of Darlington and La Cour (1945) that increased accumulation of nucleic acid decreases sensitivity to X-rays, although there is ample evidence to the contrary (Sparrow, 1949 ; Bishop, 1950). Lane's results can best be attributed to his inadequate data and inadequate analysis.

SUMMARY

Repeated experiments have not confirmed Lane's (1951) conclusions that the reduction of chromosome aberration frequency following fractional dosage can be attributed to a " depressing effect on sensitivity to breakage." Fractional dosage of *Tradescantia* microspores results in essentially the same reduction of chromosome aberrations with 4-, 8- or 12-hour rest periods. With these rest periods between two exposures of 180 r each the aberration frequency is much less than that obtained with a single dose of 360 r, and is approximately twice that obtained from a single exposure of 180 r. The effect of fractional dosage can be attributed to the failure of breaks induced at the first exposure to survive the longer rest periods, so that broken ends induced at the first exposure cannot unite with broken ends induced at the second exposure to produce the 2-hit chromosome aberrations.

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REMARKS ON SAX AND LUIPPOLD

Sax's new data confirm the general conclusion drawn from previous experiments, that fractionation of X-ray dose reduces the yield of chromosome reunions. But, for the following reasons, I cannot accept the conclusions which Sax draws from the discrepancy between his results and my own. Nor can I take the view that the new experiments throw further light on the cause of the reduction. In the first place, in the experiments which Sax compares with my own, the temperature at which the material develops is not controlled nor is it even recorded so far as we are told. In the second place, Sax employs different material, a different intensity and a different time period between irradiation and fixation.

Since temperature affects the rate of physiological processes, time alone without controlled temperatures cannot serve as a measure in the comparison of experiments in which physiological processes are involved. The usefulness of comparing time-scaled experiments in which the temperature is uncontrolled, particularly if the other conditions and the materials differ in the different experiments, is therefore open to question.

A comparison between some earlier experiments carried out by Sax (Sax, K., 1939, *P.N.A.S.*, 25, 225-233) and the present experiments serves to illustrate this point. In these earlier experiments he observes that the rate of decrease in yields of aberrations with increasing intervals between fractions varies with the intensity. It is much greater at lower intensities of 25 r/min. or 50 r/min. than it is at 160 r/min. As Sax puts it " at high intensity, 160 r/m, there is little or no effect of the single rest period." These results are clearly pertinent to any comparison made between Sax's present experiments (intensity 180 r/min.) and mine (25 r/min.).

In discussing the temporary physiological effects of radiation (*e.g.* the effect on the rate of development and the effects on nucleic acid metabolism), I remarked that the timing and magnitude of these effects depend upon the dose and possibly the intensity, as well as upon the material used and the external conditions. If, as I have suggested, the reduction in breakage frequency with fractionation of dose is, in the main, the result of a physiological effect, the timing and magnitude of this effect would be expected to vary with all these factors. It is therefore not surprising that Sax's results differ from mine, but without a knowledge of the experimental conditions we are not in a position to attempt to interpret his results.