FRACTIONATED X-RAY DOSE AND CHROMOSOME ABERRATIONS IN BARLEY

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THE early experiments of Sax[1,2] which showed that the yield of chromosome aberrations was greater at higher intensities of irradiation and which were interpreted as indicating that restitution and reunion of broken ends take place within a very short time of their occurrence, pointed the need for studying the effect of dose fractionation on chromosome breakage. Experiments of this nature by a number of investigators with *Tradescantia* as the experimental material have given conflicting results leading to two opposite interpretations—one supporting the theory of immediate restitution and reunion, the other consistent with the conclusion that the breaks may remain open for 12 hours or more.

In the present study, barley root tips were irradiated with an X-ray dose of 2,400 *r* given continuously or divided into two equal fractions separated by 2, 4, 6 and 8 hours. The continuous dose or the first fraction of it was given to the root tips of seeds which had been kept for germination for 15 hours. Observations on the division cycle in the root tips had been made earlier and it had been determined that the cells, in the 15 hours sample, exist in resting stage and do not emerge from this condition, *i.e.*, enter prophase until about 9 hours later. In this way it was ensured that both the continuous dose and its variously separated fractions were given to cells in the same stage. In order to find out whether the sensitivity of the chromosomes and other factors determining breakage yield vary during the course of the resting stage, the continuous dose was also given to 17, 19, 21 and 23 hours old samples, thus covering the entire length of the resting stage over which the two fractions were spread. All the fixations were made 13 hours after the beginning of the first exposure, with the exception of the above four samples which were fixed after shorter periods as shown in Table I. The root-tips irradiated with the continuous or the variously fractionated dosages were treated with a 0·2% aqueous solution of colchicine for a period of 5 hours immediately before fixation in order to arrest the cells at metaphase for scoring the aberrations. The irradiation was done at an intensity of 2,400 *r* per minute at a distance of 15 cm.; the operating voltage being 50 kV. The germinating seeds before and after the treatment were kept at a constant temperature of 24 ± 1° C. in a Cenco incubator. The Feulgen squash method coupled with counterstaining in aceto-carmine was used for making the preparations.

The aberrations recorded as chromosome reunions were the dicentric chromosomes and the centric rings; those recorded as breaks,

TABLE I

Showing the frequencies of reunions and breaks due to (i) *a 2,400* r *continuous dose given at different periods of the resting stage.* (ii) *a single half-dose of 1,200* r, *and* (iii) *the 2,400* r *dose separated into two equal fractions*

Interval between the fractions (hrs.)	Interval between fixation and First Exposure (hrs.)	Interval between fixation and Final exposure (hrs.)	Chromosome reunions per 100 cells		Chromosome breaks per 100 cells	
0	13	13	21·43 ± 2·258	C.D. 6·42	98·57 ± 4·845	C.D. 16·11
0	11	11	20·58 ± 2·100		94·95 ± 6·357	
0	9	9	13·38 ± 2·295		98·03 ± 6·210	
0	7	7	17·62 ± 2·516		94·24 ± 5·787	
0	5	5	15·80 ± 2·361		93·66 ± 5·743	
0 (s.h.d.)	13	13	4·65 ± 0·830		32·88 ± 2·22	
2	13	11	6·15 ± 1·039		33·91 ± 2·441	
4	13	9	11·42 ± 1·420		50·53 ± 3·003	
6	13	7	14·67 ± 1·694		69·47 ± 3·686	
8	13	5	14·93 ± 1·590		98·09 ± 4·127	

the acentric fragments and the minutes. The chromotid aberrations were not taken into consideration because relatively few of them were produced with any of the treatments. Several hundred cells, without any selection, were analysed in each case for the scoring of the aberrations.

The observations in respect of each of the treatments are summarised in Table I. These show that when the radiation dose is given in two fractions with an interval of 2 hours between them, the frequencies of both types of aberrations are considerably reduced compared with those resulting from the continuous dose. When the interval between the fractions is extended to 4 hours, a reduction which however is less pronounced, can still be observed. As the gap between the fractions is still further increased, the aberrations frequency approaches more closely the breakage yield due to the continuous dose. The data thus show that the drop in the number of chromosome aberrations when the radiation dose is spread over two fractions with a relatively short interval between them is followed by a recovery which appears to reach a maximum for the reunion when the interval extends to 8 hours. Further, the aberration frequencies due to the single half dose of 1,200 *r*, also indicated in Table I, make it clear that the yield, on fractionation, drops below twice this value and rises above it, following the process of reduction and recovery.

A correct interpretation of results from investigations designed to study the dose fractionation effect, is rendered difficult because of the fact that radiation sensitivity of the chromosomes, as also the conditions determining restitution and reunion of breaks, are known to vary during the course of the nuclear cycle.[3] The possibility that the changed frequency of the aberrations following fractionation of the dose might be due to the altered sensitivity or the different conditions for restitution and reunion in the two samples, has therefore to be taken into consideration. The observations in Table I show that neither the frequency of the reunions nor that of the chromosome breaks differ significantly when the continuous dose is given to cells at different stages of the resting condition, except in the case of the sample fixed 9 hours after irradiation, in which the frequency of reunions is slightly reduced. It is obvious that neither the extent of this reduction, nor the stage at which it occurs, suggest a possible change in the reduction-recovery trend of the dose fractionation data. In view of these considerations, it may be supposed that the differences in the frequencies of the reunions and breaks due to the continuous and the fractionated dosages are primarily a function of dose fractionation.

The initial drop following fractionation of the dose as described here is in full agreement with the corresponding observations of Sax and Luippold,[4] Lane[5] and others[6,7] on *Tradescantia*. Sax interpreted this drop as due to a possible property of the broken ends to reunite, restitute or heal within an hour, usually within a few minutes of the breakage and therefore failing to take part in reunions with those produced by the second fraction given after an interval of more than one hour.

Experimental observations opposed to this interpretation of the decline in the breakage yield were first described by Lane (*loc. cit*). These showed that the initial drop was followed by a recovery as the interval between the fractions was extended beyond 4 hours. This recovery which is obviously not consistent with the theory of rapid reunion and restitution was not apparent in the experiments of Sax and a number of other authors. It has been suggested that the different conditions of experiments may be responsible for the contradictory results.

The present study in barley indicates a recovery in the frequency of aberrations similar to that reported for *Tradescantia*, although the two differ in their timings. The extent of this recovery suggests that the breaks due to first fraction remain open for reunion with those produced by the second, several hours later. Similarly the extent of the drop preceding the recovery is not fully explained by the hypothesis of rapid reunion and restitution. An alternative explanation of the dose fractionation results, however, is not very evident. Lane has suggested that the drop and recovery are due to a physiological effect of the radiation treatment, which makes the chromosomes temporarily less sensitive to subsequent irradiation.

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THE PROBLEM OF THE BLAST DISEASE OF RICE*

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DESPITE much work done on the blast disease of rice caused by *Piricularia oryzæ* Cav. there is still a lacuna in our knowledge on the physiology of the fungus and its host-parasite relationships.

Our investigations and those of others indicate that the vitamins thiamine and biotin and the heavy metals Fe, Zn, Mn and, to a certain extent, Cu are indispensable for growth and sporulation of the fungus *in vitro*[1] and considerably greater amounts of the nutrilites are needed for sporulation than for growth.[2] Taking thiamine alone, the fungus does not need the intact molecule but only the pyrimidine fraction on a sucrose-nitrate medium. Curiously, however, the biosynthetic abilities of the organism towards thiamine seems to depend upon the nature of the substrate sugar. With maltose, pyrimidine is equivalent to the whole thiamine molecule, whereas with glucose as the carbon source, pyrimidine is not fully effective thus indicating that pyrimidine is probably active only when present with the labile γ form of glucose. The utilization of pyrimidine under these restricted conditions appears to be a temperature phase when disaccharides are hydrolysed.[3,4] Inorganic nitrogen metabolism of the pathogen *in vitro*[5] shows that while the fungus uses NO_3 nitrogen with ease, inorganic NH_4 nitrogen is not assimilated due to the development of a high physiological acidity in the case of ammonium salts of strong acids like ammonium sulphate. Should this acidity be neutralized with $CaCO_3$ or if certain organic acids of the Kreb's cycle like succinic, fumaric and citric acids are added in small amounts, normal growth of the fungus is evident with ammoniacal nitrogen. Thus, the action of the organic acids appears to be two-fold: either they act as buffers or enter the metabolic cycle to combine with the ammonium ions to form the primary amino acids.

Among the metabolic products of interest synthesized by this fungus *in vitro* is the identification of the toxin piricularin and α-piconilic acid.[6] What role thiamine and the specialized nitrogen sources this fungus seems to prefer play in the synthesis of these toxins is a point of interest and offers great scope for future investigations.

Little is known of the biologics of parasitism of *Piricularia oryzæ* and normal suscepts fail to take infection under temperatures of 24-26° C. and above 95% humidity which have been found optimum for infection. Quite recently we have succeeded in demonstrating that a low night temperature (about 20° C.), is intimately connected with host susceptibility in altering the nitrogen metabolism of the host and favouring amide synthesis especially glutamine, by facilitating greater nitrate reduction.[7] At high nycto-temperatures, nitrate reduction is possibly low and the photosynthates are mainly utilized in the building up of complex cell-wall materials which might combine with high concentrations of silicon observed in rice plants and form organo-silicon complexes which are relatively resistant to attack by extra-cellular enzymes of *P. oryzæ*.[8] Earlier results on the nitrogen metabolism of the rice plants, resistant and susceptible to the blast disease,[2] viewed in light of our recent findings indicate that the susceptible types possess a more keyed up enzyme system(s) for the efficient utilization of the absorbed N than the resistant ones. This appears to be true of glutamine synthesis in the two types.

Earlier investigations on the cuticular excretions of the rice plant in relation to disease incidence showed that a variety of amino and organic acids are found on the leaf blades of rice.[9] Recent studies have, however, revealed that among the metabolites, glutamine crystallizes on the leaf surfaces in sizeable quantities under conditions of heavy nitrogenous manuring and markedly stimulates the germination of *Piricularia* spores.[10]

Current investigations on the resistance of rice to *Piricularia* indicate that resistance can be broken down with maleic hydrazide, but only if the plants have been subjected to a low nycto-temperature (20° C.).[11] This only strengthens our view that the resistance-susceptibility mechanisms in relation to the blast disease though primarily gene controlled, is intimately interrelated with the physiology of the host as influenced by the environment, particularly low nycto-temperatures.

All these experimental findings indicate exacting growth requirements of the fungus

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