


ABSENCE OF MUTAGENIC ACTION OF X-RAYED CYTOPLASM IN HABROBRACON

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The literature concerned with x-ray effects on cells includes few records of attempts to separate injury induced in chromosomes from that induced in cytoplasm. Vintemberger¹ working with frog eggs concluded that it is the nucleus of the cell which is sensitive to x-rays. "L'irradiation de la région nucléaire a donc les mêmes effets que l'irradiation de la cellule entière." Dose used was 115 r. Zirkle² found that injury to fern spores by α-particles can be induced by extra-nuclear irradiation alone if dose is sufficiently large but that it is much greater when the nucleus is treated. Astaurov³ obtained androgenetic males from x-rayed Bombyx eggs fertilized by untreated sperm. These males (from untreated chromosomes in treated cytoplasm) were normal and their production continued after doses completely lethal to the expected types of progeny, biparental males and females. Henshaw⁴ found a direct correlation between the presence of a nucleus at time of irradiation and the manifestation of an effect, delay in cleavage. He worked with nucleated and non-nucleated fragments of Arbacia eggs. Petrova⁵ compared results of exposure to α-particles of entire cells of the alga Zygnema with those obtained by the treatment of the cytoplasm alone. She found that the mean lethal dose of the former ("Kerntod") was to that of the latter ("Plasmatod") as 1 to 700. Types of response differed under the two conditions of treatment. Transmissible changes were induced only when the entire cell had been irradiated.

What appears to be a striking exception to the conclusions of these inves-
tigators is the behavior of Amphibian eggs x-rayed in the germinal vesicle stage. Duryee\textsuperscript{6–8} found that high doses of x-rays caused fragmentation of egg chromosomes, loss of lateral loops and separation of synaptic pairs as well as injury to the nucleolus when the nucleus was irradiated in the cell. Nuclei did not react markedly when irradiated after removal from the cell. Intermediate degrees of nuclear damage were produced by exposure of non-irradiated nuclei to x-rayed cytoplasm or to alkanilized Ringer's solution. These injuries appeared immediately following “as little as 30,000 r.” Some changes could be detected with “as little as 10,000 r.” Duryee concludes that “These data are consistent with a hypothesis that x-ray damage to nuclear components is not primarily a direct effect but an indirect one, probably caused by chemical changes in the cytoplasm.”\textsuperscript{8}

A consistent factor in the work of these authors is the high dose required for the induction of effect through cytoplasmic change. Duryee's statement that injuries can be induced by doses as low as 10,000 r stands in marked contrast to the reports of the cytogeneticists, most of whom work with relatively low doses. In fact, detailed analysis of chromosome changes induced by 50 r has been made several times.

Exceptionally favorable material for a further study of this problem is provided by Habrobracon. Normally, unfertilized eggs of this wasp develop into haploid males which are, of course, gynogenetic, while fertilized eggs develop into diploid biparental females. By suitable treatment with x-rays the chromosomes of an unfertilized egg can be made non-functional so that after fertilization the treated egg will develop as a haploid androgenetic male with paternal chromosomes only. If untreated sperm are used the possible mutagenic effects of the x-rayed cytoplasm on the untreated chromosomes can be tested. As indicated below, the experiments fail to show any evidence of mutagenic action on the part of the irradiated cytoplasm. Cytoplasm can be so seriously damaged by very high doses of x-rays as to prevent development, even of androgenetic males. The few androgenetic males surviving as this degree of injury is approached afford no evidence for mutagenic effect. This injury to Habrobracon cytoplasm is, in the opinion of the author, of the same nature as that induced by high doses administered to the cytoplasm and described in the works of the authors cited above.

The experiments on Habrobracon were conducted as follows: unmated homozygous wild type females were x-rayed and then mated to untreated males with one or more traits recessive to wild type. Dose ranged from 1350 r to 54,075 r. Three types of progeny were produced, the expected wild type biparental females and gynogenetic males and the exceptional androgenetic males, readily identifiable by their recessive paternal traits. The androgenetic males were carefully inspected for visible mutations.
Due to the fact that these males are haploid, both dominant and recessive mutations would be apparent in them. The biparental females were set unmated and their haploid sons studied for visible mutations. These females, half-sisters of androgenetic males, would be heterozygous for any visible mutations induced in the x-rayed egg chromosomes since they had developed from one set of x-rayed and one set of untreated chromosomes. The irradiated eggs were fertilized at times varying from one to several hours after exposure.

During the course of the experiments 6714 wild-type females were x-rayed, 2414 F1 females tested and 75,546 F2 males counted in the search for visible mutations (table 1). The number of androgenetic males at any one dose is too small to be significantly compared with their half-sisters in respect to visible mutation rate. A comparison of totals, 0/170° or 0% mutation rate for androgenetic males and 132/2414 or 5.470% for their half-sisters, indicates that the difference is highly significant. The exact method of treating contingency tables was used and 2 P was found to be 0.0002.

Androgenetic males of Habrobracon owe their origin to injury of irradiated egg chromosomes, their survival to absence of injury in the x-rayed egg cytoplasm.16,11 If these were the only factors involved in the incidence of androgenetic males, however, they would increase with increase in dose until the limit for their production was reached, the number of fertilized eggs capable of developing into them. Maximum number of such eggs per female is about thirteen. Maximum number of androgenetic males per female is about 0.12. This is attained at the relatively low dose of about 15,000 r. At higher doses a factor which reduces androgenetic males begins
to take effect and they decrease in number until none appears at about 54,000 r.\textsuperscript{12} They have the same dose limit for survival as do gynogenetic males and biparental females. The single factor which all these classes of progeny have in common is x-rayed cytoplasm. Cytoplasm can, therefore, be injured by x-rays. Nevertheless it has exhibited no mutagenic effect. Of androgenetic males studied in the present work, 123 or 72.3% developed in cytoplasm which had been treated with doses high enough to induce detectable cytoplasmic injury. In spite of this they were normal in appearance and all tested were found to be fully fertile.

A question might be raised concerning the relative sensitivity to x-rays of the mitotic stages of the nuclei under discussion. The irradiated nuclei taking part in the formation of biparental females were treated in the diffuse stage of the first meiotic prophase. Sperm chromosomes become diffuse very soon after entering the egg. The chromosomes do not differ strikingly, either in degree of condensation or in tension, therefore, at times of exposure to possible mutagenic influences.

All results obtained from the study of the responses of Habrobracon eggs to x-rays are consistent in their indication that two kinds of change may be induced in the cell: (1) chromosome alterations connected with the production of dominant and recessive lethal and visible mutations and (2) a lethal effect directly cytoplasmic. The former are induced directly in the chromosomes or by transitory effect on the cytoplasm and have no lower threshold. The dose limits above which there is no survival may vary greatly in different mitotic stages of the same kind of cell. The latter has a high threshold and could not be detected in any cell with sensitive chromosomes except by such a method as the one used in this study. In the Habrobracon egg this cytoplasmic injury is constant in respect to dose for its incidence and dose for complete lethal action, irrespective of stage of the chromosomes at time of treatment.\textsuperscript{10-12}

It is possible that mutagenic changes in chromosomes are induced by temporary alteration of irradiated cytoplasm since the shortest period between exposure of the egg and fertilization in the present study was one hour. This allowed time for recovery of cytoplasm before fertilization. If, however, there is such temporary cytoplasmic injury, there remains the conclusion, unavoidable to the author, that this is quite distinct from that induced by high doses which has been described by Duryee and Petrova.

The kinds of change with which the cytogeneticist works, "point" mutations, inversions, translocations, deletions, could not be identified in Duryee's material. These changes may have been induced in nuclei x-rayed after removal from the cytoplasm. The fact that such nuclei do not appear to have reacted markedly does not rule out mutational effects. The significance of the changes observed by Duryee in the three sets of eggs, nucleus x-rayed, cytoplasm x-rayed and both x-rayed, can be deter-
mined with certainty only if progeny can be obtained from them or if
detailed analysis of chromosome behavior in subsequent meiotic divisions
can be made. No author has reported induction of visible mutations by
means of irradiation of the cytoplasm alone.

Conclusion.—X-rays can induce permanent change in the cytoplasm of
Habrobracon eggs. This change may have a lethal effect on the egg but
has not induced visible mutations in untreated chromosomes.

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3 Astaurov, B. L., Biol. Zhur., 6, 3 (1936).
7 Duryee, W. R., Anat. Record, 75, 144 (1939).
9 Since this paper was written the students in the Genetics Seminar at Swarthmore
College have obtained twenty-nine additional androgenetic males. All were normal in
appearance. Dose used was 28,000 r.
12 Whiting, Anna R., Ibid., 97, 210 (1949).

A NOTE ON THE EXCEPTIONAL JORDAN ALGEBRA

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An associative algebra $\mathfrak{A}$ over a field $\mathfrak{F}$ is a vector space over $\mathfrak{F}$ together
with an associative bilinear operation $xy$. When the characteristic of
$\mathfrak{F}$ is not two we can use the same vector space and define a new algebra
$\mathfrak{A}^{(\dagger)}$ relative to the operation $\frac{1}{2}(xy + yx)$. This algebra is a Jordan
algebra. Any Jordan algebra $\mathfrak{J}$ is called a special Jordan algebra if $\mathfrak{J}$ is
isomorphic to a Jordan subalgebra of some $\mathfrak{A}^{(\dagger)}$.

In 1934 it was shown that the Jordan algebra $\mathfrak{G}$ of all three-rowed
Hermitian matrices with elements in the simple eight-dimensional Cayley
algebra $\mathfrak{C}$ is exceptional in the limited sense that $\mathfrak{G}$ is not isomorphic to a
subalgebra of a finite-dimensional $\mathfrak{A}^{(\dagger)}$. In the present note we shall give
a simpler proof of the fact that $\mathfrak{G}$ is not a special Jordan algebra and shall