AGEING AND CHROMOSOME ABERRATIONS IN MALE MAMMALIAN GERM CELLS

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Abstract—The present observations have been performed on male mice from 30 to 750 days of age in order to elucidate whether the ageing process in male mammals is associated with an increased number of germ cells showing numerical or structural chromosome aberrations which could eventually be transmitted to the progeny. Cytological observations performed on dividing spermatocytes I showed that 76-83 Yo of the cells appear normal, 2.8-5.0% are polyploid, 1.2-5.9% have autosomal univalents and 9.75-14.6% show X-Y univalents. No reciprocal translocation was observed in the dividing spermatocyte I. The absence of evident relation between age and the incidence of anomalies suggests that the differences observed result of technical features.

INTRODUCTION

AGEING is often thought to be associated with an increase in chromosome abnormalities. Thus, Jacobs et al. reported, in 1961, that the proportion of aneuploid cells in cultures of human leucocytes increases with the age of the subject, beginning during the sixth decade of life in women and during the seventh decade in life in man (Jacobs et al., 1963). This increased aneuploidy was shown to be a result of the loss of the Y chromosome in the male somatic cells and of an X chromosome in the female ones. In the same year Stevenson and Curtis (1961) reported that the incidence of structural aberrations such as bridges and fragmentations in dividing cells from mouse liver increases approximately linearly with age up to 12 months. In man, an advanced maternal age is well known to be associated with an increased incidence of chromosome anomalies such as the Down's syndrome resulting from an error of disjunction (Turpin and Lejeune, 1965 for review).

The present observations were performed in order to detect in the male a possible relation between the ageing process and an increased number of numerical or structural chromosome aberrations in germ cells which then could eventually be transmitted to the progeny.

METHODS AND PROCEDURE

Male C57Bl mice were killed at an age of 30, 350 or 750 days. The testes were removed immediately and meiotic preparations were made by the air-drying method of Evans et al. (1964). In each testis one hundred spermatocytes I at the diakinesis-first metaphase stage of meiosis were examined for the presence of such chromosome anomalies as X-Y or autosomal univalents, reciprocal translocations or polyploidy. Ten animals (2000 dividing spermatocytes) from each group were analysed.

RESULTS

The results of cytological analysis are summarized in Table 1. From 76 to 83 Yo of the cells appear normal, 2.8-5.0Yo are polyploid, 1.2-5.9Yo have autosomal univalents and 9.75-14.6Yo show X-Y univalents. No reciprocal translocation was observed in the dividing spermatocyte I. The \( \chi^2 \) test revealed that the different groups are significantly heterogenous (\( \chi^2 = 112.79; d.f. = 6; p < 0.001 \)) but no evident relation between the age of the animals...
and the incidence of the different types of anomalies can be discerned. Thus, the middle aged animals appear to have more abnormalities than either the young or the old ones.

### Table I. Results of cytological analysis

<table>
<thead>
<tr>
<th>Age of the animals (days)</th>
<th>Total number of cells analyzed</th>
<th>Normal cells</th>
<th>Polyploid cells</th>
<th>Cells with autosomal univalents</th>
<th>Cells with X-Y univalents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Mean per 1000 cells</td>
<td>Total</td>
<td>Mean per 1000 cells</td>
<td>Total</td>
</tr>
<tr>
<td>50</td>
<td>2,000</td>
<td>40.52</td>
<td>45</td>
<td>4.25</td>
<td>9.5</td>
</tr>
<tr>
<td>300</td>
<td>2,000</td>
<td>50.03</td>
<td>100</td>
<td>2.40</td>
<td>17.5</td>
</tr>
<tr>
<td>750</td>
<td>2,000</td>
<td>60.06</td>
<td>110</td>
<td>5.00</td>
<td>24</td>
</tr>
</tbody>
</table>

$\chi^2 = -10.73$ df = 6, $p < 0.05$. In parentheses are given contributions of the respective groups to the total $\chi^2$.

### DISCUSSION

At diakinesis, the $X$ and $Y$ chromosomes of the male mouse display a terminal association between the distal ends of their long arms (Kofman-Alfaro and Chandley, 1971). This association is generally sufficiently strong to prevent separation of the $X$ and $Y$ chromosomes during the preparation of meiotic cells, the $X$ and $Y$ chromosomes appear separated (univalents) during diakinesis and metaphase in a certain percentage of cells. On the other hand, autosomal bivalents remain paired until this end of metaphase 1 and autosomal univalents are rarely seen under normal condition. Schleiermacher (1970) reported an incidence of 1.3–3.9 of $XY$ univalents and 0 to 1.9% of autosomal univalents in adult C3H mice whereas Adler (1966) and Adler and Röhrborn (1969) using the same technique found less than 1% univalents in ten weeks old males from the same strain and in $101 \times C3H$ F1 hybrids. Cohen and Mukherjee (1968) and Ohno et al. (1959) observed less than 1% $XY$ univalents whereas Chyi-Chyang Lin et al. (1971) found more than 6% in 3-4 month-old inbred Swiss mice and Beechey (1973) 8–10% in (C3H/He × 101/H)F1 hybrids. The observations of Ohno et al. (1959) and of Chyi-Chyang Lin et al. (1971) on second metaphase spermatocytes suggest that precociously separated sex chromosomes migrate normally to opposite poles. On the other hand, a failure of $X-Y$ to associate in meiosis has been found to be related with several cases of sterility in male mice (Cattanach et al., 1968; Beechey, 1973) or human azoospermie (McIlree et al., 1966; Chandley and Edmond, 1971). Studies on spontaneous human abortuses (Carr, 1971 for review) demonstrate that most chromosome aberrations observed result from errors of disjunction (autosomal trisomies of XO embryos). Our results demonstrate clearly that the incidence of autosomal and $XY$ univalents is not increased in dividing spermatocytes from old mice. It can be inferred that numerical chromosome anomalies resulting from errors of disjunction are not more frequent in the offspring of old male mammals than in that of young ones. This differs from the behaviour of female mice which display a significant increase in univalents with age (Henderson and Edwards, 1968).

Ford and Evans (1971) claim that the apparent polyploid spermatocytes observed in the mouse probably represent artefacts of preparation arising from the spreading together of the contents of adjoining cells. The low incidence of apparent polyploids observed
in the different groups, which is about the same as the 3% reported by Cyi-Chyang Lin et al. (1971) in 3- to 4-month-old inbred Swiss mice, supports this conclusion.

Our negative findings, with respect to the presence of reciprocal translocations, show that the risk of transmissible structural chromosome rearrangements does not increase in old male mice, a danger which was suggested by the preliminary results of Leonard and Deknudt (1970) and Muramatsu (1974).

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REFERENCES