THE EFFECT OF FAST NEUTRONS ON CHROMOSOMES IN MEIOSIS AND ITS BEARING UPON PACHYТЕNE PAIRING

A. MARSHAK AND W. S. MALLOCH

Radiation Laboratory and the Department of Botany,
University of California

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PREVIOUS experiments have shown that quantitatively the response of chromosomes of *Vicia faba* in pachytene to X-rays is approximately the same as that of the same species at the onset of the somatic prophase (MARSHAK 1939a). CREIGHTON (1941) using methods similar to those employed in the Vicia studies found that meiotic chromosomes are only a sixth as sensitive as the mitotic chromosomes in Chorthippus. SAX and SWANSON (1941) have asserted that meiotic chromosomes of *Tradescantia* are more sensitive to X-rays than mitotic, although the meiotic stages used in their comparison are not identified.

The experiments reported here show that the somatic chromosomes of *Vicia faba* at the onset of the somatic prophase have the same sensitivity to neutrons as chromosomes in pachytene.

MATERIALS AND METHODS

In a previous report (MARSHAK 1939a) the method of identifying the meiotic stages of *V. faba* was briefly outlined, but will be described here in greater detail. In the axil of each leaf of the upper portion of the mature plant is an inflorescence bearing about six blossoms. Subsequently it was found that the stamens in each flower bud are in two sets, one longer than the other, and that all the stamens of any one set are approximately in the same stage. Since the microsporocytes of the longer set are usually more advanced in the early stages, it is necessary that comparable stamens be used in following meiotic development (table 1). In these experiments only the longer set of stamens was used. The buds were slit with a needle which had been ground to a cutting edge, and a single anther was removed. The slit was covered with a small patch of Scotch tape. The bud was then left on the plant and the inflorescence covered with moist absorbent paper and waxed paper to prevent desiccation. Buds found to have anthers with microsporocytes in pachytene were left on the plant for various time intervals, after which the entire bud was removed and fixed in a mixture of equal parts of 50 percent acetic acid and 50 percent ethyl alcohol. After fixation each of the anthers was separately smeared and stained in acetocarmine and counts were made of the frequency of different stages.

The data on the frequency of different meiotic stages in anthers at vari-

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1 This work was supported by the Columbia Fund for Medical Physics of The Columbia Foundation.
ous intervals after the examination of the first anther are shown in table 2. It is evident that cells found to be in anaphase I were in pachytene 22 to

### Table 1

Distribution of meiotic stages in the different anthers of a bud. Pk = pachytene, Dk = diakinesis, Dp = diplotene, M1 = first metaphase, AI = first anaphase, TI = first telophase, MII = second metaphase, AII = second anaphase, TII = second telophase, Td = tetrad. Anthers 1-5 are the long set and 6-10 are the short set.

<table>
<thead>
<tr>
<th>FIRST COUNT</th>
<th>TIME INTERVAL IN HOURS</th>
<th>SECOND COUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANther</td>
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</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
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<td>5</td>
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</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pk 163</td>
<td>16</td>
<td>Dk 702</td>
</tr>
<tr>
<td>Pk 101</td>
<td>16</td>
<td>Dk 616</td>
</tr>
<tr>
<td>Pk 103</td>
<td>24</td>
<td>Dk 509</td>
</tr>
<tr>
<td>Pk 127</td>
<td>24</td>
<td>TI 33</td>
</tr>
<tr>
<td>Pk 270</td>
<td>24</td>
<td>MI 81</td>
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</table>

### Table 2

Distribution of stages at various time intervals after pachytene.

<table>
<thead>
<tr>
<th>FIRST COUNT</th>
<th>TIME INTERVAL IN HOURS</th>
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</tr>
</thead>
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<tr>
<td>TOTAL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PACHYTENE</td>
<td></td>
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</tr>
<tr>
<td>1361</td>
<td>100.0</td>
<td>6,7,8</td>
</tr>
<tr>
<td>487</td>
<td>100.0</td>
<td>12½ 1070</td>
</tr>
<tr>
<td>990</td>
<td>100.0</td>
<td>16 699</td>
</tr>
<tr>
<td>177</td>
<td>100.0</td>
<td>16 614</td>
</tr>
<tr>
<td>221</td>
<td>100.0</td>
<td>17 1006</td>
</tr>
<tr>
<td>416</td>
<td>100.0</td>
<td>17 352</td>
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<tr>
<td>182</td>
<td>100.0</td>
<td>18 517</td>
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<tr>
<td>239</td>
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<td>18 510</td>
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<td>235</td>
<td>100.0</td>
<td>19 1284</td>
</tr>
<tr>
<td>137</td>
<td>100.0</td>
<td>20 287</td>
</tr>
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<td>100.0</td>
<td>21 386</td>
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<td>454</td>
<td>100.0</td>
<td>21 3010</td>
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<td>222</td>
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<td>22 1123</td>
</tr>
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<td>56</td>
<td>100.0</td>
<td>22 462</td>
</tr>
<tr>
<td>324</td>
<td>100.0</td>
<td>22½ 389</td>
</tr>
<tr>
<td>336</td>
<td>100.0</td>
<td>23 330</td>
</tr>
<tr>
<td>128</td>
<td>100.0</td>
<td>24 405</td>
</tr>
<tr>
<td>429</td>
<td>100.0</td>
<td>24 625</td>
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<tr>
<td>343</td>
<td>100.0</td>
<td>24 1669</td>
</tr>
<tr>
<td>163</td>
<td>100.0</td>
<td>24½ 214</td>
</tr>
<tr>
<td>166</td>
<td>100.0</td>
<td>24½ 226</td>
</tr>
<tr>
<td>61</td>
<td>100.0</td>
<td>25 168</td>
</tr>
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</table>
24 hours earlier. From these data one may also determine the approximate duration for pachytene, diakinesis, metaphase I, and anaphase I. In figure 1 the percentage cells in pachytene is plotted as a function of time after examination of the first anther. It appears from the graph that pachytene lasts from eight to 12 hours with a mean at ten hours. Similar graphs are shown for diplotene in figure 1, for diakinesis in figure 1 and for MI and AI plus TI in figure 2. Cells in diplotene appear with a high frequency 12–16 hours after pachytene, with a smaller percentage as late as 23 hours.

Figure 1.—Percentage cells in pachytene, diplotene and diakinesis as a function of time after first examination.

Figure 2.—Percentage cells in metaphase I and anaphase I as a function of time after first examination.
The average duration of the diplotene stage thus appears to be about four hours. Diakinesis reaches a maximum frequency at 17–18 hours after the first examination, while metaphase I is most frequent at 21 hours and anaphase I at 23 hours. From these curves it also appears that cells may be in diakinesis for two to four hours, in metaphase for a similar period, and in anaphase for one to two hours. The duration of diakinesis was obtained by allowing buds whose anthers were found to be in this stage to remain on the plant and fixing such buds at hourly intervals afterwards. Data obtained in this way are given in table 3 and show, as did the graphs

![NORMAL ANAPHASES AS A FUNCTION OF DOSE](image)

**Figure 3.**—Percentage normal anaphase plotted on a log scale against dose in neutrons.

**Table 3**

<table>
<thead>
<tr>
<th>TIME INTERVAL</th>
<th>TOTAL</th>
<th>% DIPLO-</th>
<th>% DIAKINESIS</th>
<th>% MI</th>
<th>% AI-TI</th>
<th>% DIAKINESIS</th>
<th>% MI</th>
<th>% AI-TI</th>
<th>% II</th>
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<td></td>
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<td>48.5</td>
<td>51.5</td>
<td></td>
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<td>99.2</td>
<td>0.8</td>
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<td></td>
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<tr>
<td></td>
<td>545</td>
<td>34.1</td>
<td>37.5</td>
<td>24.5</td>
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<tr>
<td></td>
<td>665</td>
<td>27.4</td>
<td>72.6</td>
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<td></td>
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<tr>
<td></td>
<td>565</td>
<td>20.7</td>
<td>52.4</td>
<td>6.8</td>
<td>20.1</td>
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<td>0.2</td>
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<td>43.8</td>
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</table>

Distribution of stages following diakinesis and metaphase.
described above, that diakinesis and metaphase I each last two to four hours. It may be noticed in tables 2 and 3 that some buds have a considerable percentage of the microsporocytes in the second meiotic division by 24 hours after the removal of the first anther. These plants were all older, with shoots three to four feet tall. Younger plants (one to two feet) have a more uniform distribution of stages (table 1). Similar behavior was found in Gasteria. In these and previous experiments all plants were grown in 8 inch pots in the greenhouse during the months April, May, and June and were irradiated when one to two feet tall. All plants were grown from a single lot of seed (variety Bell Windsor) obtained from a commercial seed firm.

From the data presented above it seems evident that microsporocytes found in anaphase I were in pachytene 23 to 24 hours earlier.

Conditions for irradiation of the buds with X-rays were the same as those previously described (MARSHAK 1939a). The source of neutrons used was a beryllium target bombarded by 16 million volt neutrons produced by the cyclotron. The neutron beam was collimated by a lead-lined channel embedded in a large block of paraffin (AEBERSOLD 1939). The plants were placed so that the buds to be treated were in the center of the 10×10 cm field at the end of the collimator. The neutron dose was measured in arbitrary "n" units, an "n" unit being that amount of ionization produced by neutrons which gives the same reading on a 100 r Victoreen thimble ionization chamber as does one roentgen of X-rays. We are indebted to DR. P. C. AEBERSOLD for measurements of the neutron doses.

Twenty-three to 24 hours after treatment the buds were fixed in the acetic acid-alcohol mixture and stained in acetocarmine. Only cells in mid- or late anaphase I were examined. These were classified as either normal or abnormal, an abnormal cell being one containing one or more chromosome or chromatid attachments or fragments.

The frequency and percentage of normal anaphases after doses of 5–40 n is given in table 4A. The controls showed 5 percent abnormal anaphases. Previous experiments with plants from the same lot of seed showed only 1–2 percent abnormalities in the controls for both mitosis and meiosis. The increase in abnormalities may be due to changes in growing conditions and hence may be real or may be only apparent due to changes in fixing and smearing technique introduced by the second author. In figure 3 the logarithm of the percentage normal anaphases from the corrected values is plotted as a function of the dose. The curve is of the form $Y = e^{-kx}$ as in the case of X-rays (MARSHAK 1939a). Table 5 gives the value of the slope (k) obtained by fitting the percentage normal anaphases by least squares. In fitting the curve the weight assigned to each point was the number of cells observed. Tables 4C, 4B, and 4D give the data for mitosis following neutron treatment and meiosis and mitosis following X-ray
treatment. The slopes for each of these is given in column k of table 5. In column s of the same table are given the root mean square deviations and in column R, a measure of the goodness of fit where

\[ R^2 = \frac{1 - \sum (z - Z)^2}{n \left( \frac{\sum z^2}{n} - \frac{(\sum z)^2}{n} \right)} \]

\[ z = \text{observed log percentage normal anaphase} \]

\[ Z = \text{calculated log percentage normal anaphase} \]

\[ n = \text{number of points} \] (Yule and Kendall 1937)

### Table 4

<table>
<thead>
<tr>
<th>Meiosis—24 Hours After Irradiation</th>
<th>Mitosis—3 Hours After Irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose</strong></td>
<td><strong>Normal</strong></td>
</tr>
<tr>
<td>A</td>
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<td>0</td>
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</tr>
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<td>4</td>
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<td>68</td>
</tr>
<tr>
<td>500</td>
<td>30</td>
</tr>
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</table>
The constants given have all been corrected to the base e of natural logarithms.

With X-rays, the slopes and thus the sensitivities to X-rays of chromosomes in meiosis and mitosis agree to within 2 or 3 percent. The difference in slopes is $0.0003 \pm 0.002$ and is clearly not significant. With neutrons using the uncorrected slope the difference between meiosis and mitosis is $0.010 \pm 0.017$, and with the corrected values $0.010 \pm 0.008$, in both cases it is not significant. Thus if there is a difference in the response of meiotic and mitotic chromosomes to neutrons it must be less than 15 percent.

### DISCUSSION AND SUMMARY

Somatic chromosomes of several different species irradiated at the onset of prophase have all given $n/x$ ratios of approximately 6 (Marshak 1938a). However, with chromosomes of a single species, $V. faba$, $n/x$ varies from 6.6 to 15, depending on the stage of the mitotic cycle irradiated (Marshak 1938b). The close similarity of the ratios for somatic chromosomes in the onset of prophase and meiotic chromosomes in pachytene thus indicates the existence of similar functional conditions with respect to ionizing radiation or particles in both these stages which was postulated previously on other grounds (Marshak 1935, 1939a). It was further suggested that the condition common to the onset of the somatic prophase and to pachytene was the presence of closely approximated chromonematic surfaces during these stages of the nuclear cycle (Marshak 1939a, 1939b, 1942).

The lower limit of the diameter of the sensitive portion of the chromonema was obtained from calculations based on the theory of sensitive volumes. The value of the upper limit is obtained from the comparison of the relative efficiency of X-rays and neutrons and is based only on the average distance between ion pairs along a proton track and is thus a direct physical measurement in the sense that the dimension sought is compared with a known dimension—that is, the ion spacing in the proton track.

<table>
<thead>
<tr>
<th>Type</th>
<th>Meiosis a*</th>
<th>Meiosis</th>
<th>Mitosis</th>
<th>Mitosis</th>
</tr>
</thead>
<tbody>
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<td>0.0150</td>
<td>0.0102</td>
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</tr>
<tr>
<td>$S_e$</td>
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<td>0.0033</td>
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<tr>
<td>$R$</td>
<td>0.982</td>
<td>0.998</td>
<td>0.999</td>
<td></td>
</tr>
</tbody>
</table>

* In no allowance is made for the 5 percent abnormal anaphases observed in the controls. The values in $b$ are obtained from the corrected percentage normal anaphases.
From these data and those previously published, the upper as well as lower limit of the diameter of the sensitive portion of the meiotic and mitotic chromonema is of the order of $10^{-7}$ cm. This may represent material within the chromonema having the dimensions and properties of small proteins—for example, histones (Marshak 1938a, 1938b)—or may represent the average distance between the approximated chromonematic surfaces. It has commonly been assumed that chromonemata are made up of large protein molecules. If the dimensions obtained in these experiments are referred to the chromonema, they indicate that the fundamental constituents are not necessarily chains of unusually large proteins but of some of the smallest known—that is, histones. If the dimensions are referred to inter-chromonematic spacing during pairing, then chromonemata approach to within molecular distances of each other. In either case it would seem that in seeking an explanation of pairing and crossing over, consideration should be given to molecular forces appropriate for structures of these dimensions.

LITERATURE CITED