# THE EFFECT OF X-RAYS ON CHROMOSOMES IN DIFFERENT STAGES OF MEIOSIS

By A. Marshak

(*From the Biological Laboratories, Harvard University, Cambridge*)

Plate 1

(Accepted for publication, February 25, 1935)

The following investigation is concerned with the nature of the submicroscopic changes induced in the nucleus during meiosis and mitosis by the action of x-rays. The results indicate that cells irradiated in the early prophase of the first meiotic division (synaptene-pachytene) are markedly more susceptible to the effects of x-rays than other stages in the ontogeny of the cell. They also give some indication of the nature of one of the biological variables that has been so troublesome in attempts to determine the relationship of induced genetic and cytological effects to dosage, and the dimensions of the volume of the hypothetical portion of the cell sensitive to the lethal action of x-rays (Wyckoff, 1931–32; Glocker, 1932; Glocker, Langendorff, and Reuss, 1933).

One of the difficulties encountered in work on the biological action of x-rays is the lack of uniformity of material, since populations of cells in various stages of development have been used. In *Gasteria* it is possible to eliminate this variable. Each bud has six anthers containing pollen mother cells in approximately the same stage. The inflorescence is a spike with a sequence of buds in different stages arranged in an ascending spiral along the stem. The chromosomes are large, few in number, and show their chromonematic structure clearly. In all of the species used there are four long chromosomes of the same size and three very short ones (Marshak, 1934).

Mohr (1919) found that nuclei of very young spermatocytes (*Decticus verruccivorus*) were rendered pycnotic by treatments with radium and with low temperatures which did not affect other stages. Similar indications had been obtained by Regaud and his coworkers

(1906 and 1908) studying the effects of x-rays on the testes of mammals. Such pycnosis was observed in *Gasteria* pollen mother cells in the early prophase of meiosis when treated with doses of x-rays of 1,000 r. or more. Whether this is a cumulative effect of the linkage changes observed at low dosages or is due to the action of x-rays upon another constituent of the nuclear or cytoplasmic systems remains to be determined. Stone (1933) has observed that chromosome abnormalities are evident in the first meiotic anaphase of pollen mother cells 24 hours after treatment with x-rays although the same dose produced little or no effect on other stages. In the investigations of this author and the earlier work of Strangeways and his coworkers (1923, 1925) it is observed that the mitoses from nuclei irradiated in the "resting stage" have chromosome abnormalities. These results are in accord with the findings of the present investigation if the resting stage and what is here considered the early mitotic prophase be identical. There is little morphological difference between these early prophase stages and the resting stage. I refer to a condition of the nucleus which is still reticulate but in which the chromonemata are beginning to be resolved. There is a large subjective element in such a distinction.

A consideration that seems to have been overlooked by previous investigators is the possibility that the immediate effects of x-radiation on chromosomes may not be at once apparent. For example, the inactivation of the chromatin-producing mechanism at a given locus will not become evident until the new chromonemata are developed. Similarly induced chromonematic interchanges will not be microscopically visible until disjunction occurs.

### *Technique*

The distribution of division stages was determined by making smear preparations,[1] of the anthers of every bud on a young spike. The following procedure was used with the material irradiated: Beginning with the lower end of the spike, an anther from each bud was examined until one with the pollen mother cells in the first meiotic metaphase was found. This bud was left on the plant and all

---

[1] Fixed 15–30 minutes in Taylor's modification of Flemming's solution, rinsed in water, and placed in 50 per cent alcohol for 30 minutes to 1 hour. Stained in crystal violet.

the older ones removed. The remaining buds were numbered according to their position on the stem. At intervals along the stem a bud was removed and examined to determine the approximate location of buds with pollen mother cells in the various stages of the meiotic prophase and the premeiotic mitosis. The spike was then exposed to x-rays. The remaining anthers from the bud which had shown the metaphase figures were smeared ½, 1, and 2 hours after radiation. An anther from the bud immediately above was then examined. If anaphase or metaphase figures were found the bud was removed and smeared and the next bud examined until one with its pollen mother cells in the pachytene was found. This was usually two to four buds above the first one with anaphase I figures. The bud was again examined 24 hours later when most of the cells were in the first meiotic anaphase. In some cases this proved to be somewhat too early or too late (cells in metaphase or telophase). When the spike was approaching its maximum elongation, usually about 11–14 days after radiation, it required approximately 48 hours for cells in pachytene to reach the first anaphase.

In order to make enough determinations it was necessary to use more than one species of *Gasteria*. They were found to be similar in chromosome number and morphology, in the configurations at anaphase, and in the intervals between different stages on the spike. The species used were *G. lingua*, *G. glabra*, *G. disticha*, and *G. sp.* In this work only the long chromosomes were followed.

The source of x-rays was an air cooled Coolidge tube with a tungsten target. The primary current was kept at 110 volts, 10 milliamps. The secondary voltage was determined from the spectrum obtained with a spectrograph containing a rock salt crystal. The shortest wave length was found to be 0.15 Å.u., corresponding to a peak secondary voltage of 82,400 volts. The range of wave lengths was from 0.15 to 0.40 Å.u. with the maximum density near the 0.40 Å.u. region. In the calculations the mean wave length is taken as 0.30 Å.u.

The dosage was varied by altering the time of exposure and the distance from the target. Dosage was measured with a Victoreen dosimeter. I am indebted to Dr. J. C. Hudson for the determinations of the characteristics of the radiation used.

### *Observations*

The effects of x-rays as observed in the first meiotic anaphase may be put into three groups, namely, chromosome attachments, (Figs. 2, 4, 5, and 7), fragmentation (Figs. 2, 7, and 8), and achromatic spots (Fig. 6) (similar to "secondary constrictions") in the chromonema.

When the frequencies of chromosome abnormalities produced in buds of different ages are examined it is evident that there are pronounced maxima in buds which reached anaphase I at 1 and 5 days after radiation. These buds when irradiated were in pachytene

TABLE I*

*Frequency of Abnormalities Observed in Anaphase I shortly after Irradiation*

| Dose, in r | Time after radiation | Attached chromatids | Fragments | Achromatic spots | Total abnormalities | Total number chromatid pairs |
|---|---|---|---|---|---|---|
| | *hrs.* | *per cent* | *per cent* | *per cent* | *per cent* | |
| 264 | 0.5 | 0.6 | 0 | 0.02 | 0.8 | 3,368 |
| 264 | 1. | 0.9 | 0 | 0 | 0.9 | 1,200 |
| 79 | 1. | 0.94 | 0.07 | 0.02 | 1.03 | 2,891 |
| 15.8 | 1+ | 2.2 | 0 | 0 | 2.2 | 1,691 |
| 2.6 | 1+ | 1.7 | 0 | 0 | 1.7 | 2,068 |
| 528 | 1. | 0.3 | 0.06 | 0.06 | 0.4 | 2,520 |
| 528 | 1.5 | 25.0 | 24.1 | 0 | 49.1† | 112 |
| 528 | 2. | 26.4 | 4.2 | 7.7 | 38.3 | 72 |
| 147 | 2. | 6.1 | 0.09 | 0.3 | 6.4 | 1,184 |
| 30 | 2. | 0.9 | 0 | 0.1 | 1.0 | 3,952 |

* In Tables I, II, and III and Text-figs. 1 and 2 fragments have been counted as effects in one chromatid. In Text-figs. 3 and 4 and calculations from them each fragment is counted as the result of an effect in a pair of chromatids.

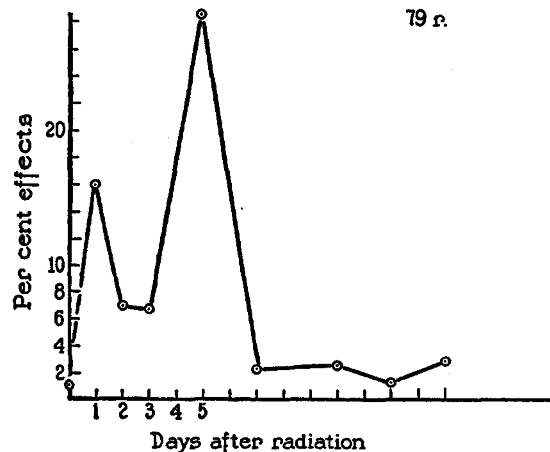
† At these dosages the frequency of fragmentation and spotting is so high that it is difficult to separate the two types of effects. For example an achromatic spot on a fragment may make it appear like two fragments. Obviously further investigation is necessary to determine whether there are definite maxima for achromatic spotting as for the other effects.

TABLE II

*Frequency of Induced Abnormalities Observed in Anaphase I at Various Times after Irradiation with 79 Roentgens*

| Time after radiation | Total chromatid pairs | $\frac{\text{Attached chromatids}}{\text{Total}} \times 100$ | $\frac{\text{Fragments}}{\text{Total}} \times 100$ | $\frac{\text{Achromatic spots}}{\text{Total}} \times 100$ | $\frac{\text{Total aberrant}}{\text{Total}} \times 100$ |
|---|---|---|---|---|---|
| *hrs.* | | | | | |
| 1 | 2891 | 0.94 | 0.07 | 0.02 | 1.03 |
| 24 | 1776 | 15.88 | 0 | 0 | 15.88 |
| 48 | 1969 | 3.81 | 2.0 | 1.1 | 6.91 |
| 72 | 624 | 3.21 | 1.8 | 1.7 | 6.71 |
| 120 | 573 | 16.23 | 10.9 | 1.3 | 28.43 |
| 168 | 2022 | 0.89 | 1.2 | 0.2 | 2.29 |
| 240 | 1392 | 1.37 | 1.0 | 0.2 | 2.57 |
| 288 | 2648 | 0.80 | 0.4 | 0.06 | 1.26 |
| 336 | 928 | 1.72 | 1.0 | 0.1 | 2.82 |

stage of meiosis and in the early premeiotic prophase, respectively. In Table II and Text-fig. 1 are shown the frequencies of abnormalities observed at the first meiotic anaphase in buds of the same spike removed at various times after treatment with 79 r. and similarly for 528 r. in Text-fig. 2. Maxima at approximately the same time were also observed in buds exposed to 16, 30, and 264 r. (Table III). In the 30 and 528 r. series there are indications of peaks probably



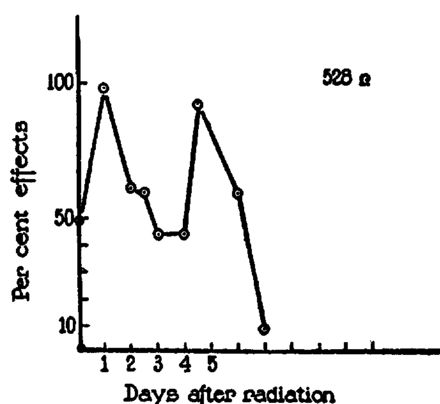

TEXT-FIG. 1. The frequency of chromosome abnormalities in x-ray-treated pollen mother cells (ordinate) plotted against time in days after irradiation (abscissa). The two peaks in the curves are taken to indicate periods of maximum sensitivity to x-rays. The first peak coincides with irradiation during the synaptene-pachytene stage of meiosis, the second with the early premeiotic prophase. Total dosage—79 roentgens.

corresponding to mitoses prior to the one shown by the buds which took 5 days to reach the first meiotic anaphase.

Of 6,723 pairs of chromatids from untreated plants of *G. lingua*, *G. conspurcata*, and *G. sp.* examined at the first meiotic anaphase, 0.5 per cent were attached so that the chromonematic coils were drawn out, 0.007 per cent (one case) had an achromatic spot in the chromonema, and there were 0.02 per cent of chromosome fragments. In Table I are given the frequencies of these chromosome aberrations observed shortly after irradiation. There is no marked increase

until somewhat more than an hour has elapsed between irradiation and examination of the cells.

If the frequency of effects produced in buds rayed in pachytene be plotted against dosage, a straight line passing through the origin is obtained as the best fit to the points (Text-figs. 3 and 4). A similar relationship is obtained from the values of the second peak (109 to 144 hours after radiation), given by buds irradiated during the premeiotic stage. Buds taken at 48 and at 72 hours after radiation also show a rectilinear relationship to dosage. Such a linear




TEXT-FIG. 2. Data plotted as in Text-fig. 1. Total dosage—528 roentgens.

function is taken to indicate that photoelectrons produced in the absorption of x-rays within the tissue act directly[2] upon the biological agents involved in the binding together of the constituent portions of the chromonema. Although the linear relationship between dosage and the frequency of induced effects does not in itself prove a direct effect of x-rays on the biological units in question, any other explanation would involve assumptions which at present seem improbable.

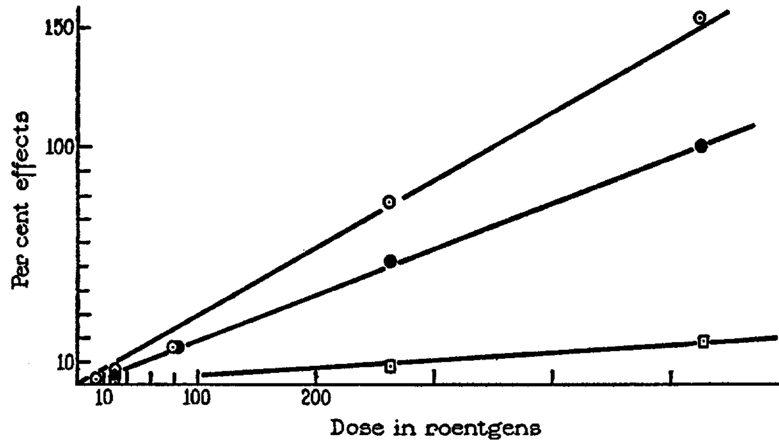
[2] We are not concerned here with the nature of the physical action of photoelectrons in the cells, *i.e.* whether effects are produced by ionization or other means, but with the problem of whether any particular category of effects observed is a consequence of the alteration of a single biological unit or system, or whether one system shows changes only after another has been modified by the radiation.

TABLE III*

*Per Cent Total Chromosome Abnormalities with Different Dosages*

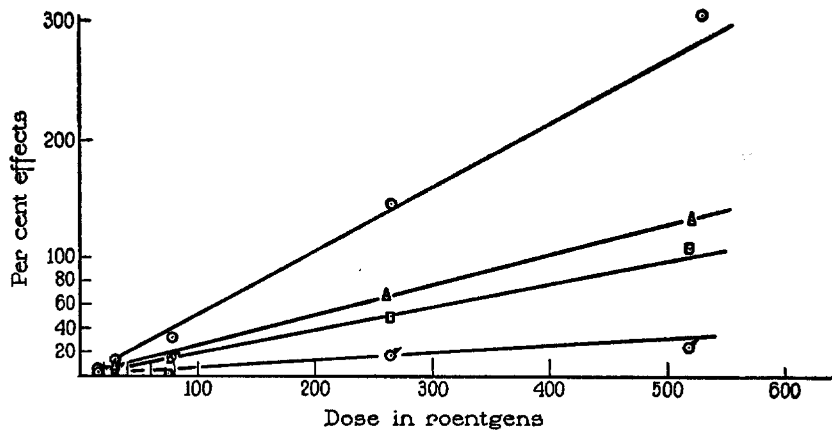
| Time after radiation | Dose in r. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2.6 | 5.3 | 15.8 | 30 | 79 | 264 | 528 |
| *hrs.* | | | | | | | |
| 0.5 | | | | | | 0.64 | |
| 1 | 1.71 | | | | (1.1)<br>1.0 | 0.91 | (0.46)<br>0.40 |
| 1.5 | | | | | | | (73.2)<br>49.1 |
| 2 | | | | 1.01 | | | |
| 24 | | | 2.20 | | 15.9 | (76.9)<br>51.4 | (152.6)<br>98.1 |
| 36 | | | | (6.5)<br>5.3 | | | |
| 48 | 0.97 | 1.35 | 0.98 | (3.4)<br>3.0 | (8.8)<br>6.9 | | (97.0)<br>61.2 |
| 60 | | | | | | (28.2)<br>18.5 | (97.0)<br>59.2 |
| 72 | 0.74 | 0.26 | | (3.05)<br>2.75 | (8.4)<br>6.7 | | (57.6)<br>57.0 |
| 96 | | | (0.68)<br>0.60 | | | | (67.3)<br>44.1 |
| 109 | | | | | | | (150.9)<br>92.6 |
| 120 | | (1.58)<br>1.24 | | | (39.3)<br>28.4 | | |
| 144 | | | (1.65)<br>1.42 | (4.1)<br>3.54 | | | (97.8)<br>59.2 |
| 168 | | | | (3.93)<br>2.96 | (3.4)<br>2.3 | | (13.6)<br>8.96 |
| 216 | | | | (1.36)<br>1.05 | | | |
| 240 | | | | (0.71)<br>0.52 | (3.5)<br>2.6 | | |
| 266 | | | | | | | |
| 288 | | | | (1.24)<br>0.80 | (1.8)<br>1.3 | | |
| 312 | | | | (2.45)<br>2.00 | | | |
| 336 | | | | | (3.7)<br>2.8 | | |
| 424 | | | | (0.81)<br>0.61 | | | |

* The figures in parentheses are the percentages of chromosome abnormalities when fragments are counted as resulting from effects induced in pairs of chromatids. Attached chromatids have in all cases been counted as effects in pairs of chromatids and achromatic spots as effects in single chromatids.




TEXT-FIG. 3. The frequency of x-ray-induced chromosome abnormalities observed in anaphase I 24 hours after irradiation. These data represent effects induced at the synaptene-pachytene stage of meiosis.

Abscissae, dose in roentgens; ordinates, per cent effects. Open circles represent total chromosome abnormalities when fragments are considered as being produced by an x-ray effect on a pair of chromatids, closed circles the same totals when a fragment is considered as being produced from a single chromatid. Squares represent frequencies of achromatic spots. The first meiotic division is considered reductional.




TEXT-FIG. 4. Circles represent total chromosome abnormalities as in Text-fig. 3, but with the first division considered both equational and reductional with equal frequency. Circles with arrows represent chromosome attachments, rectangles fragments, and triangles attachments plus fragments, all with the first division always reductional.

There are no very pronounced maxima in the frequencies of induced achromatic spots at low dosages, although there is considerable variability probably due to the difficulty of detection at the higher dosages. There is a noticeably lower frequency in buds which are quite young when irradiated. The data for attachments and fragments are similar to those for achromatic spots in this respect and suggest that cells affected in any of these ways have a greater tendency to die off than non-affected cells. In Text-fig. 3 the frequency of achromatic spots in buds examined 24 hours after irradiation is plotted against dosage. Here too the curve is rectilinear.

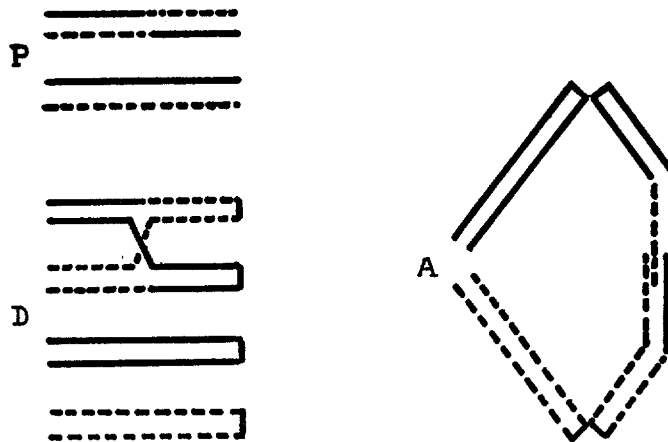
*Interpretation*

Accepting the hypothesis of direct action, it becomes necessary to explain by means of it the higher frequency of chromosome aberrations induced in cells irradiated at particular stages of division. Such an explanation must apply to some premeiotic condition as well as to the synaptene-pachytene stage of the meiotic prophase. It must also account for the fact that there are no pronounced maxima in the frequency of induced achromatic spots.[3] Of several schemes devised to indicate the manner in which the condition leading to attached chromonemata might be produced, only one seems to satisfy the conditions adequately. This requires that each synapsed homologue be at least two-parted at the time of irradiation, that a change of linkage take place between a pair of strands when a photoelectron intersects both, and that a chromonematic division occur between this stage and anaphase, as illustrated in Text-fig. 5.[4] Careful examination of anaphase chromosomes from unrayed plants showed that the chromonema of each chromatid was visibly two-parted at least along some portions of its axis (Fig. 3). From the same untreated material there are visible indications that the attachment of chromonemata that leads to attenuation of the chromonematic helix is

[3] There are indications of maxima for induced achromatic spots in the data from the 264 and 528 r. series but not at the lower dosages. (See footnote to Table VI.)

[4] It has been suggested that the chromatid attachments may be produced by reverse cross-overs; *i.e.*, lateral attachments between chromonemata. This would require that every attachment be accompanied by a fragment. The data do not show such a correlation between fragments and attachments.

due to an interlocking of half-chromonemata (Fig. 3). The condition in the x-rayed material (Fig. 4) is very much the same, and it seems reasonable to suppose that the mechanisms immediately responsible are similar in the two cases. In the irradiated cells there is another type of attenuation in which a portion of the chromonema becomes drawn out to a slender thread which finally breaks, leaving the distal portion of the chromatid pair on the median plate (Figs. 5, 7, and 8). Since chromonemata may remain attached through the second meiotic anaphase (as far as they were followed), it seems possible that




TEXT-FIG. 5. Diagram to illustrate formation of attached chromatids. Dotted and solid lines represent homologous chromatids. $P$ = pachytene; $D$ = diplotene; $A$ = anaphase.

we are dealing with two really different conditions. The type of attenuation leading to fragmentation may be produced by assuming an inactivation of the chromonematic division mechanism in the region of a pair of chromonemata intersected by a photoelectron, which may or may not be followed by another division in the rest of the chromonema. Another explanation alternative to the inactivation of division might be the alteration of forces holding the chromonema together; *i.e.*, weakening of linkage. In either case pairs of chromonemata must be affected, since the condition observed is usually an attenuation of a portion of both members of a pair of chromatids. The schemes for both types of attenuation require the presence of

pairs of very intimately associated strands, a condition which may be common to the early prophase of the last premeiotic mitosis and the pachytene stage of meiosis. In the one case (mitosis) the close association may be considered to be due to the division of the units of the chromonematic strand forming the new units of the still microscopically undetectable chromonema. In meiosis the condition is produced by synapsis or by another chromonematic division.

Since attenuations are in pairs of chromatids,[5] not threes or fours, there follows as a result of this interpretation an interesting implication concerning the nature of synapsis, namely, that although there is a pairing of four strands, at any given locus there is a very intimate association between only two of them. It appears as though the association between the members of a pair of strands is of a different order of magnitude from the association of the two pairs of the tetrad, although this difference cannot be detected optically in the pachytene chromosome. If the members of the intimately associated pair are different at different loci, it follows that there will be an alternate close association between homologous and sister chromatids. If under particular conditions the members of a pair are the same for a considerable length of the chromosome the more or less secondary association of the pairs might not, along this region of the chromosome, be an exact locus for locus combination. The "non-homologous" pairing described in *Zea* (McClintock, 1932) may be interpreted as such a condition.

The achromatic spots observed in the x-rayed chromonemata may be considered as originating from an inactivation of the chromatin-producing mechanism in localized regions of these chromonemata. If each chromonema is single at or prior to pachytene, one would expect the achromatic spots to occur in pairs in the 24 hour buds. This does not agree with the observations. On the other hand, if only one of the four pachytene strands is affected at a given locus one might expect by the previous schemes that the newly formed chro-

[5] At the lower dosages attachments occur only between pairs of chromatids in the 24 hour buds, but in the 48 hour buds attachments in threes do occasionally occur. For example, there were two complexes of three chromatids to twenty-seven of pairs of attached chromatids at 48 hours, 15.8 r., and thirty-eight pairs of attached chromatids and no complexes of three at 24 hours.

monema would replace the necessary constituent of the system altered by the photoelectron. However, it is possible that the chromatin-producing system may be functionally related to the reproductive one of the chromonema in such a way that when one is altered the other is also affected, or, that the production of chromatin or its precursors requires the presence of both chromonematic units at a given locus.

*Sensitive Volume of the Chromonema*

An estimate of the sensitive volume of the chromonema was obtained by using the equation given by Glocker (1932) for the number of photoelectron paths traversing a given volume.

$$(1)\ N = N_0 \cdot \frac{R + a}{a} \cdot v,$$

where

$v$ = the volume in question.
$N$ = number of electron paths in $v$.
$N_0$ = photoelectrons per cm.³ tissue per roentgen.
$R$ = range of photoelectron in tissue.
$a$ = mean path length of photoelectron inside volume $v = \frac{4}{3} r$.

The energy per roentgen per cm.³ air at 0°C., 760°mm. Hg is taken as 0.11 ergs (Kuhlenkampf, 1929), and the density of the nucleus as approximately 1.035 (Harvey, 1932), of air as 0.001293. To get the relative electron density of tissue as compared with air the atomic composition of the cells used is taken as approximately that of a young embryo (Armsby and Moulton, 1925) and an average value for $b$ of the equation $V_o^2 - V_x^2 = bx$, where $b = \frac{\text{Density} \times \text{atomic number}}{\text{Atomic weight}}$ is obtained as follows:

| Atom | Density | Atomic No. | Atomic weight | b |
|---|---|---|---|---|
| | *per cent* | | | |
| O | 70.3 | 8 | 16 | 39.6 |
| H | 10.3 | 1 | 1 | 10.3 |
| C | 5.1 | 6 | 12 | 2.55 |
| N | 1.3 | 7 | 14 | 0.65 |
| Ash | 2.2* | 17* | 16* | 2.34 |
| | | | | 55.44 |

* Values are the weighted means of the constituents of the ash.

$b$ for *Arbacia* eggs is somewhat less (though it can only be estimated from the data given by Harvey), while $b$ for air is 60.41. Then taking $\lambda = 0.3$ Å.u., $E = 6.54 \times 10^{-8}$ ergs, and $N_o = \frac{0.11}{6.54 \times 10^{-8}} \times \frac{1.035}{0.00129} \times \frac{55.4}{60.4} = 12.4 \times 10^{8}$.

Using Glocker's method in which a correction is made for the argon content of air but the cell is considered as water at a density of 1.0,

$$N_o = 3.82 \times 10^{9}\, \lambda \text{ in Å.u.}$$
$$= 11.5 \times 10^{8}.$$

$R$ for air is calculated from the equation

$$R = \frac{V_o^4}{a'} \text{ where } V_o = \text{velocity of photoelectron}$$

and $a'$ = number of electrons per unit volume of material traversed $= 5.5 \times 10^{39}$ (Compton, 1926; Wilson, 1923).

$$\text{In air } R = \frac{2.1 \times 10^{40}}{5.5 \times 10^{39}} = 3.78 \text{ cm.}$$

$$\text{in tissue } R = 3.78 \times \frac{0.00129}{1.035} = 47 \times 10^{-4} \text{ cm.}$$

Glocker's relationship, obtained from Kuhlenkampf's determinations $V = 21.4\sqrt{R(\text{air})}$, gives $R = 46 \times 10^{-4}$ cm.

Using $N_o = 12.4 \times 10^{8}$, $R = 46 \times 10^{-4}$ cm., and $N = 2.8 \times 10^{-3}$ obtained from the slope of the uppermost line in Text-fig. 3, equation (1) may be solved by considering the volume spherical and solving for $r$ with the result that $r = 1.25 \times 10^{-5}$ cm. The total sensitive volume of the pachytene chromosome (one of the pair of homologues) is $v = 8.2 \times 10^{-15}$ cm.$^3$ if the first anaphase is reductional. If chromatids disjoin at random, $N = 5.2 \times 10^{-3}$ (derived from Text-fig. 4, uppermost line) and $r = 1.7 \times 10^{-5}$ cm., and $v = 20.6 \times 10^{-15}$ cm.$^3$

As measured under the microscope the diameter of the pachytene chromosome is $13 \times 10^{-6}$ cm. The chromosomes at this stage are too long to be measured directly but an estimate of their approximate length can be obtained if analogies are made with the behavior of other meiotic chromosomes. The small chromosomes in *Gasteria* are essentially small sections of the long ones. They are also very similar in appearance to those of *Zea mays*. The length at pachytene could be measured from photographs of the B chromosome of *Zea*

which doubled back upon itself (McClintock, 1933, Fig. 2). This was compared with the length of the metaphase chromonema, assuming that the relative length of the chromonema to the external dimension of the chromosome was the same as in *Gasteria*. Making these assumptions one may determine the reduction in length of the chromonema from pachytene to metaphase, which proves to be a reduction of 11 times. Knowing the length of the *Gasteria* metaphase chromonema by measurement, the length at pachytene may then be estimated as $21.2 \times 10^{-3}$ cm.

The meiotic nucleus in *Gasteria* is spherical and can be measured accurately. It has a diameter of $35 \times 10^{-4}$ cm. and a surface area therefore of $a'' = 3.85 \times 10^{-5}$ cm.$^2$ The number $N'$ of photoelectrons traversing the nuclear volume may be calculated from equation (1). This may be taken as the number of photoelectrons intersecting the surface of the nucleus. Assuming a cubical cross-section for the pachytene chromosome, it will have an area on one face of $a''' = 27.6 \times 10^{-8}$ cm.$^2$ Since $N' = 82.5$, the number of photoelectrons entering the pachytene chromosome will be approximately $5.9 \times 10^{-1}$ per roentgen.

If $w$ be the width of the sensitive volume $v$ of a pair of chromonemata whose volume is $v'$, then

$$v = v' \times \frac{N}{N'}$$

$$l \times w \times \frac{w}{2} = l \times 1.3 \times \frac{1.3}{2} \times 10^{-10} \times \frac{2.8 \times 10^{-3}}{5.9 \times 10^{-1}}$$

and $$w = \sqrt{\frac{1.3 \times 1.3 \times 10^{-10} \times 2.8 \times 10^{-3}}{5.9 \times 10^{-1}}} = 9.0 \times 10^{-7} \text{ cm.}$$

With random disjunction of chromatids

$$w = \sqrt{\frac{1.3 \times 1.3 \times 10^{-10} \times 5.2 \times 10^{-3}}{5.9 \times 10^{-1}}}$$
$$= 12.2 \times 10^{-7} \text{ cm.}$$

If the sensitive volume obtained by the previous method be dis-

tributed along the length of the chromonema the width of the chromonematic pair $w'$ will be

$w' = \sqrt{\frac{2v}{l}}$, where $v$ = the sensitive volume of the pair of chromonemata and $l$ their length.

$$w' = \sqrt{\frac{2 \times 7.22 \times 10^{-15}}{21.2 \times 10^{-3}}} = 8.8 \times 10^{-7} \text{ cm.}$$

and with random disjunction

$$w' = \sqrt{\frac{2 \times 20.6 \times 10^{-15}}{21.2 \times 10^{-3}}} = 13.9 \times 10^{-7} \text{ cm.}$$

Although these values are obviously rough approximations it is interesting to compare them with the diameters of protein molecules and enzymes. Albumin = $4.34 \times 10^{-7}$ cm. (Svedberg and Sjogren, 1929; Nichols, 1930), trypsin = $5.2 \times 10^{-7}$ cm. (Northrop and Kunitz, 1932–33), insulin = $4.36 \times 10^{-7}$ cm. (Sjogren and Svedberg, 1931), hemocyanin = $2.4 \times 10^{-6}$ cm. (Svedberg and Chirnoaga, 1928).

The width of the sensitive portion of the single chromonema $\left(\frac{w}{2}\right)$ determined by either of the above methods falls well within the limits of the sizes of protein molecules and approximates the size of the smaller protein molecules and of insulin and trypsin. If we assume that the sensitive portion of the chromonema is composed of more or less spherical units we obtain for the number of such units $n = \frac{l}{\frac{w}{2}}$, which is $4.7 \times 10^4$, $3.5 \times 10^4$, $5.1 \times 10^4$, and $3.0 \times 10^4$ for the different values of $w$. If the metaphase chromonema be considered to represent a condition in which there is a maximum condensation of sensitive material along the axis of the chromonema without any corresponding increase in width, then since the length of the chromonema is $1.93 \times 10^{-3}$ cm., $n = 4.8 \times 10^3$, $3.2 \times 10^3$, $4.7 \times 10^3$, $2.8 \times 10^3$. These two sets of values of $n$ might be taken as representing the maximum and minimum limits of the number of such units in the chromonema, but since the shape and changes in size (if any) along different axes of such units are unknown the values have little meaning.

TABLE IV

*Sensitive Volumes—Method II*

| Category | $N$ ($\times 10^{-3}$) | $w$ ($\times 10^{-7}$ cm.) | $v$ ($\times 10^{-16}$ cm.$^3$) |
|---|---|---|---|
| Achromatic spots | 0.3 | 2.9 | 0.9 |
| Attachments | 0.7 | 4.5 | 2.2 |
| Fragments | 1.9 | 7.4 | 5.8 |
| Attachments and fragments | 2.5 | 8.5 | 7.7 |
| Total | 2.8 | 9.0* | 8.6 |

* Gowen and Gay (1933) have calculated the maximum size of a gene as $1 \times 10^{-18}$ cm.$^3$; *i.e.*, $w \underset{ca.}{=} 1 \times 10^{-6}$. They considered the absorbed quantum as the agent effective in producing gene changes. If their calculations were made in terms of effects produced per ion pair the size would be smaller by a factor of about 500.

TABLE V

*Per Cent Chromosome Fragments—Each Fragment Counted as Effect in a Pair of Chromatids*

| Time after radiation | Dose in r. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2.6 | 5.3 | 15.8 | 30 | 79 | 264 | 528 |
| *hrs.* | | | | | | | |
| 0.5 | | | | | | 0 | |
| 1.0 | 0 | | | | 0.1 | 0 | 0.1 |
| 1.5 | | | | | | | 48.2 |
| 2.0 | | | | 0.03 | | | |
| 24 | | | 0 | | 0 | 51.1 | 109.0 |
| 36 | | | | 2.4 | | | |
| 48 | 0 | 0 | 0 | 0.8 | 3.9 | | 71.7 |
| 60 | | | | | | 19.5 | 75.3 |
| 72 | 0 | | | 0.7 | 3.5 | | 29.2 |
| 96 | | 0.03 | 0.09 | | | | 46.2 |
| 109 | | | | | | | 117.0 |
| 120 | | 0.3 | | | 21.8 | | |
| 144 | | | 0.2 | 1.1 | | | 77.0 |
| 168 | | | | 1.9 | 2.3 | | 9.3 |
| 216 | | | | 0.6 | | | |
| 240 | | | | 0.4 | 1.9 | | |
| 266 | | | | | | | |
| 288 | | | | 0.9 | 0.9 | | |
| 312 | | | | 0.9 | | | |
| 336 | | | | | 1.9 | | |
| 424 | | | | 0.3 | | | |

In Table IV are given the results of calculations of sensitive volumes for the different types of observed effects whose frequencies are given in Tables V and VI. Fragmentation and attachments make up a large proportion of the abnormalities. If both these categories be

TABLE VI

*Per Cent Achromatic Spots*

| Time after radiation | Dose in r. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2.6 | 5.3 | 15.8 | 30 | 79 | 264 | 528 |
| *hrs.* | | | | | | | |
| 0.5 | | | | | | 0.02 | |
| 1.0 | 0 | | | | 0.02 | 0 | 0.06 |
| 1.5 | | | | | | | 0 |
| 2.0 | | | | 0.09 | | | 7.7 |
| 24 | | | 0 | | 0 | 8.5* | 18.3* |
| 36 | | | | 1.2 | | | |
| 48 | 0 | 0 | 0 | 0.8 | 1.1 | | 10.1 |
| 60 | | | | | | 2.8 | 9.4 |
| 72 | 0 | | | 1.0 | 1.7 | | 13.3 |
| 96 | | 0 | 0 | | | | 10.6 |
| 109 | | | | | | | 22.4* |
| 120 | | 0 | | | 1.3 | | |
| 144 | | | 0 | 1.0 | | | 4.2 |
| 168 | | | | 0.6 | 0.2 | | 1.3 |
| 216 | | | | 0.2 | | | |
| 240 | | | | 0.07 | 0.2 | | |
| 266 | | | | | | | |
| 288 | | | | 0.08 | 0.06 | | |
| 312 | | | | 0 | | | |
| 336 | | | | | 0.1 | | |
| 424 | | | | 0.05 | | | |

* Whether these apparent peaks are real or the result of confusion with fragmentation which is pronounced at these periods has not yet been proven. Since at the lower doses (30 r., 79 r.) where such inaccuracy is reduced there are no pronounced maxima, it would seem that the apparent peaks are produced by the inclusion of fragments in this category.

considered consequences of effects in the linkage mechanism, then this system makes up about 90 to 95 per cent of the total sensitive volume of the chromonema. If the linkage mechanism is distributed primarily along the long axis of the chromonema, then its reduction

to a minimum will reduce the length of the chromonema to about one-tenth of its extended size. This is in good agreement with the reduction in length from pachytene to metaphase of 11 times as previously calculated. It would seem therefore that the contraction of the chromonema from prophase to metaphase is due largely to a reduction in the linear extension of the linkage material.

Of particular interest is the probable relation of these sensitive volumes to the genetic unit, whether this be a discrete body or a portion of one of the systems mentioned. If it can be shown that the frequency of x-ray-induced mutations varies with the distance between chromonemata then it would seem likely that the gene was a part of the linkage mechanism and that mutations were not changes in a more or less independent unit. For example one would expect a greater frequency of mutations in cells irradiated at pachytene or early mitotic prophase. There already exist indications of such a variation in mutation frequency from studies of effects on sperm and eggs in different developmental stages of *Drosophila melanogaster* (Moore, 1934; Hanson and Heys, 1929; Harris, 1929). If the genetic unit be considered a portion of the linkage mechanism one would expect unequal crossing-over and translocations to be accompanied by position effects of varying intensities, but if the gene is a discrete unit a position effect is not a necessary concomitant of unequal crossing-over or translocation.

## SUMMARY

1. Pollen mother cells exposed to low dosages of x-rays at various stages show different frequencies of chromosome abnormalities in the first meiotic anaphase.

2. Maximum frequencies of abnormalities were obtained in buds irradiated in the pachytene stage of the meiotic prophase and in the preceding mitosis.

3. These results are taken to indicate that the x-ray-sensitive portions of the chromonemata are closely approximated in pairs in pachytene and in the early mitotic prophase. The significance of this in relation to non-homologous pairing is indicated.

4. From the nature of the chromosome configurations observed it is concluded that chromonemata are two-parted when they synapse

and that a chromonematic division occurs between pachytene and anaphase and during the mitotic prophase.

5. The frequencies of abnormalities show a linear relationship to dosage.

6. The diameter of the sensitive volume of the chromonema is calculated and found to approximate the diameter of some known protein molecules.

7. The linkage mechanism is found to make up about 90 per cent of the total sensitive volume which corresponds with the approximate reduction in length of the chromonema from pachytene to anaphase.

8. The relation of these sensitive volumes to the gene is discussed.

## REFERENCES


Armsby, H. P., and Moulton, C. R., The animal as a converter of matter and energy, New York, The Chemical Catalog Co., 1925.

Compton, A. H., X-rays and electrons, New York, D. Van Nostrand Co., 1926.

Glocker, R., 1932, *Z. Phys.*, **77**, 653.

Glocker, R., Langendorff, H., and Reuss, A., 1933, *Strahlentherapie*, **46**, 517.

Gowen, J. W., and Gay, E. H., 1933, *Genetics*, **18**, 1.

Hanson, F. B., and Heys, F., 1929, *Am. Naturalist*, **63**, 511.

Harris, B. B., 1929, *J. Hered.*, **20**, 299.

Harvey, E. N., 1932, *Biol. Bull.*, **62**, 143.

Kuhlenkampf, H., 1929, *Z. Phys.*, **30**, 777.

Marshak, A., 1934, *Am. J. Bot.*, **21**, 592.

McClintock, B., 1932, *Proc. 6th Int. Cong. Genetics*, **2**, 126.

McClintock, B., 1933, *Z. Zellforschung. u. mikr. Anat.*, **19**, 191.

Mohr, O., 1919, *Arch. mikr. Anat.*, **92**, 300.

Moore, W. B., 1934, *Genetics*, **19**, 209.

Nichols, J. B., 1930, *J. Am. Chem. Soc.*, **52**, 5176.

Northrop, J. H., and Kunitz, M., 1932–33, *J. Gen. Physiol.*, **16**, 295.

Regaud, C., and Blanc, J., 1906, *Compt. rend. Soc. biol.*, **61**, 163.

Regaud, C., and Dubreuil, G., 1908, *Compt. rend. Soc. biol.*, **65**, 393.

Sjogren, B., and Svedberg, T., 1931, *J. Am. Chem. Soc.*, **53**, 2657.

Stone, L. H. A., 1933, *Ann. Bot.*, **47**, 815.

Strangeways, T. S. P., and Oakley, H. E. H., 1923, *Proc. Roy. Soc. London, Series B*, **95**, 373.

Strangeways, T. S. P., and Hopwood, F. W., 1925, *Proc. Roy. Soc. London, Series B*, **100**, 283.

Svedberg, T., and Chirnoaga, E., 1928, *J. Am. Chem. Soc.* **50**, 1399.

Svedberg, T., and Sjogren, B., 1929, *J. Am. Chem. Soc.*, **51**, 3594.

Wilson, C. T. R., 1923, *Proc. Roy. Soc. London, Series A*, **104**, 1.

Wyckoff, R. W. G., 1931–32, *J. Gen. Physiol.*, **15**, 351.

## EXPLANATION OF PLATE 1

Photomicrographs of meiotic chromosomes in *Gasteria*.

FIG. 1. Meiotic anaphase and early telophase—not irradiated.

FIG. 2. Meiotic anaphase, telophase, and interphase—264 r.—24 hours after irradiation.

FIG. 3. A chromosome with both pairs of chromatids attached to each other—not irradiated. Notice interlocking of half-chromatids.

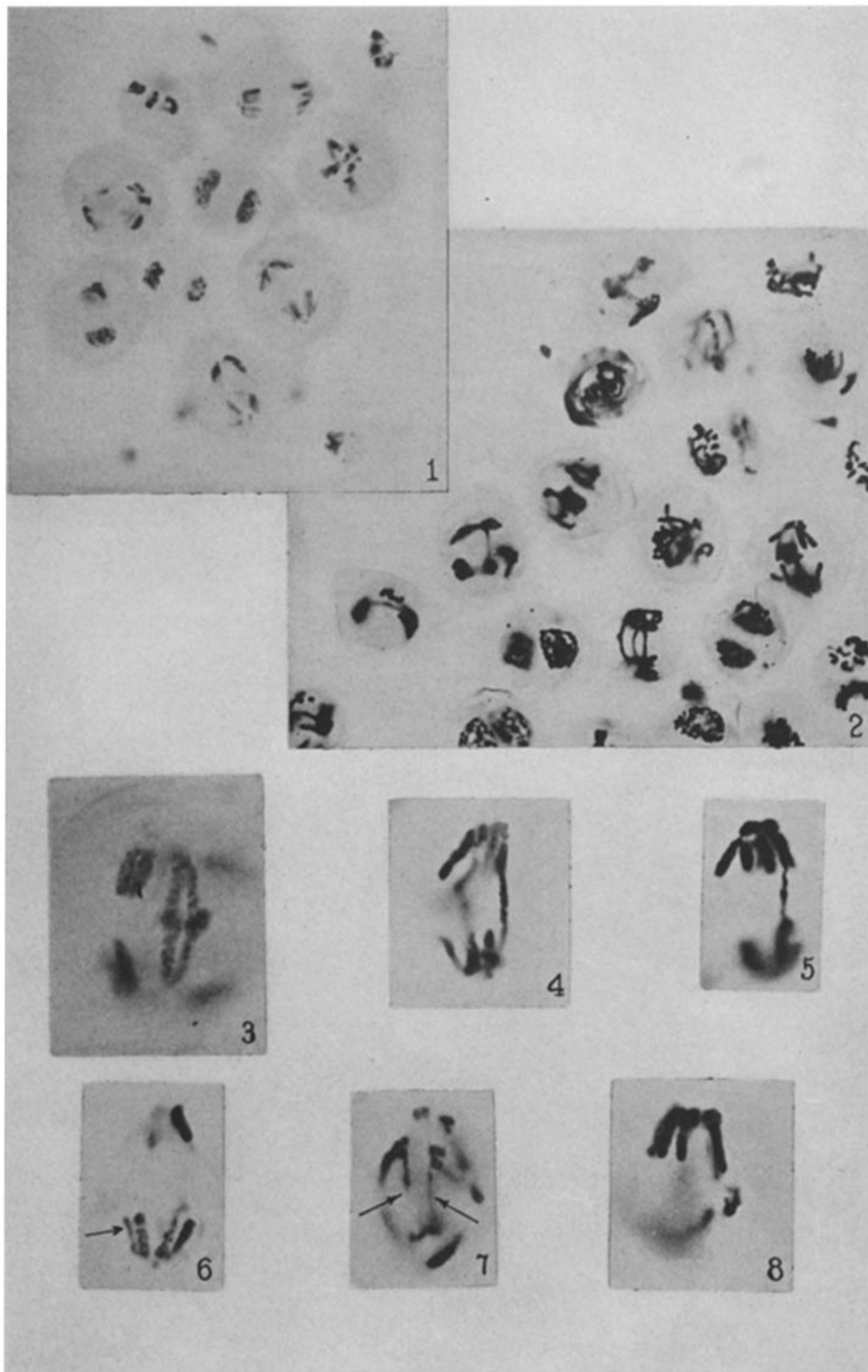
FIG. 4. A pair of attached chromatids—79 r.

FIG. 5. A pair of attenuating chromatids—79 r.

FIG. 6. Same as (5) at another focus. Arrow indicates an achromatic spot.

FIG. 7. Attenuating chromatids just broken (left) and about to break (right)—528 r.

FIG. 8. A fragment left on the equatorial plate—30 r.




(Marshak: X-rays and chromosomes in meiosis)