Cytological Studies on the Effect of Herbicides on Plant Cells in vivo I. Hormonic herbicides

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Received August 15, 1963

Recent striking progress of medicamental industries has promoted greatly the production and researches in the branch of agricultural chemicals. Under present circumstances, not only for commercial purpose but also from a scientific standpoint, the biological significance of various farm drugs is strongly requested, and the answer of these problems are highly desirable to be elucidated fundamentally at the cellular level. The effect of chemicals on tissue cells hitherto has been studied cytologically on fixed materials treated with chemicals. However, such routine cytological techniques are wanting in accuracy to confirm the effectiveness of farm drugs on plant cells.

Wada has first begun to study the effect of chemicals on mitoses in vivo in the staminal hair cell of Tradescantia and reported the action of ammonium chloroform mixed vapour (1939), the effect of colchicine (1940) and a number of troponoid compounds (1952a,b, 1953) on mitotic cells. According to his techniques, the author studied the effect of sodium salts of 2, 4-dichlorophenoxyacetic acid (1953) and maleic hydrazide (1955) on mitosis of staminal hair cells of Tradescantia by means of in vivo observations. The author attempted further to search for materials available for in vivo observation of mitoses other than Tradescantia cells and found out the stipular cells of Vicia faba (1956), the petal cell of Allium fistulosum (1956), Allium cepa (1958) and the cell of pollen grains of Tradescantia (1957, 1962). However, these materials came to be utilizable to study the effect of chemicals on mitoses in vivo. Recently, the author has devised a micro-technique for in vivo observation of some isolated cells from the tissue of Triticum root tips. This isolated cell was also useful in this experiment.

Seventeen herbicides tested in this experiment were divided for convenience sake into two groups. The first group includes the chlorinated phenoxyacetic derivatives and related compounds and is known by the name of hormonic herbicides, and the second group comprises the other compounds and is called as non-hormonic herbicides. In this report, effects of various hormonic herbicides on mitoses and metabolic cells were studied and traced by means of the in vivo observation to clarify the biological significance of the farm drugs from the agricultural aspect and cell biology. Concerning
effects of non-hormonic herbicides, the results will be reported in the part 2 of this study.

Methods and materials

Among the herbicides tested, the liquid chemical was used immediately as the mother solution and diluted to various concentrations; in the case of a powdered chemical, 2% aqueous solution of the chemical was used as the standard solution. Microtechniques for in vivo observations and cultures of plant cells were described as follows.

1. The staminal hair cell of Tradescantia reflexa

According to Wada's microtechnique (1943, 1952), the staminal hairs arranged on a cover glass (24×24 mm) were covered with a small piece of agar plate (ca. 5 mm square) which contained 1% agar, 2% sucrose and a half concentration of the mother solution of chemicals. The concentration of chemicals contained in the agar plate, was changeable according to the requisition of the experiment. The covered material was kept upside down on a Van Tieghem's moist chamber (hereafter abbreviated moist chamber) for making observations and for culture. The other hairs obtained from the same bud were also kept in another moist chamber as a control.

2. The stipular cell of Vicia faba, the petal cell of Allium fistulosum and Allium cepa

Two percent agar solution containing 18% sucrose was first made and mixed with an equal volume of a mother solution of chemicals. In this case, the 1% agar plate contained generally 9% sucrose and a half concentration of the mother solution of chemicals. A growing apical tissue in Vicia faba was used (Fig. A). The techniques on in vivo observations of this material were already described in detail (Sawamura 1956) and omitted here. As a control, a half stipular piece of the same young sprout was sealed with the standard agar plate.

In the case of Allium petal cells, a small pedunculate flower bud was taken out in an involucre. Under a dissecting-microscope, young petals laid in a droplet of distilled water on the slide glass were taken out from a small flower bud by cutting off anthers and ovary. Further handling of the petal pieces was similar as stipular cells mentioned previously. Dif-
different from the hair cells of *Tradescantia*, the agar plate covered the piece of meristematic tissues can be replaced easily with other agar plate containing chemicals. In the standard agar plate, meristematic tissues of *Vicia* and *Allium* can survive more than few weeks and the resting nucleus can enter into *de novo* mitosis which finishes the mitosis in the normal way (Sawamura 1956, 1958).

3. The cell of pollen grains in *Tradescantia reflexa*

A new hanging-drop technique devised for *in vivo* observations of grasshopper germ cells and acites sarcoma of rats by Makino (1955), was found to be available also for plant male germ cells or pollen grains *in vivo*. In the fourth or fifth bud from the wanted apical flower, the mitoses of pollen grains are found. From an anther of such flower bud placed in a small drop of 9% sucrose solution on a cover glass (24×24 mm), pollen grains were flowed out in the droplet by dissecting carefully.

![Image](image.png)

Fig. B. Preparation for *in vivo* observation of pollen grain mitosis of *Tradescantia*. M, pollen grains. p, liquid paraffin. cg, cover glass. sg, hole slide glass.

This cover glass was put upside down on a hole-slide filled with liquid paraffin (Fig. B), and observed mainly under the phase microscope. According to the experimental purpose, a requisite concentration of chemicals was added to 9% sucrose solution used for the pollen grain culture. Under this culture condition, pollen grains of *Tradescantia* can survive from the beginning of their mitoses to the formation of the vegetative nuclei and the generative cells.

4. The isolation of *Triticum* root tip cells

A tip (about 5 mm in length) of *Triticum* root of 2 cm or 3 cm in length laid in a droplet of distilled water prepared on a cover glass (24×24 mm) was carefully cut into slices under dissecting-microscope. The slice was covered with an agar plate piece containing 1% agar and 7% sucrose. Then a few minutes later, the agar plate was stripped carefully from the cover glass and the tissue pieces sticking on the agar plate were taken off with a needle; this agar plate was put on a new clean cover glass. By this procedure, a number of isolated root tip cells still remained on the surface of the stripped agar. This cover glass was laid upside down on a moist chamber. The isolated cells sealed in the standard agar plate could survive for about two weeks (Sawamura unpublished). The effect of chemicals on these cells was investigated in the agar plate contained 1% agar, 7% sucrose and a requisite concentration of chemicals.

In this experiment, the staminal hair cell of *Tradescantia* and the
stipular cell of *Vicia* were used mainly and the other materials subsidiarily to compare the effectiveness of chemicals among these materials. The pH-value of the agar solutions used was found ranging from 6.0 to 5.6, in which the action of chemicals tested suffered scarcely from any suppression. The comparison of the difference of chemical effects on cells between *in vivo* and in fixed preparations was carried out on root tips of *Triticum*, *Vicia* and the *Tradescantia* pollen grains immersing for several hours in solutions of chemicals and the cells were observed with aceto-orcein staining.

**Observations and results**

The effect of five phenoxy hormonic herbicides on the staminal hair cell of *Tradescantia*, the stipular cell of *Vicia faba*, the petal cell of *Allium fistulosum*, the cell of pollen grains in *Tradescantia* and the isolated cell of *Triticum* root tips, were studied *in vivo*. These herbicides are 2,4-Dichlorophenoxyacetic acid, 2,4,5-Trichlorophenoxyacetic acid, 2,5-Dichlorophenoxyacetic acid, 2-Methyl-4-chlorophenoxyacetic acid and 2,4-Dichlorophenoxyethyl sulfate, and hereafter each chemical is described in abbreviated form (2,4-D), (2,4,5-T), (2,5-D), (MCP) and (SES) respectively. The experimental results arranged in Table 1, were summarized in three items: the effect on the metabolic cell, the effect on the occurrence of the mitosis and the abnormal behaviour of the mitotic cell, and the results were discussed from the standpoint of cell biology.

<table>
<thead>
<tr>
<th>Chemical Structure</th>
<th>Description</th>
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<tbody>
<tr>
<td>OCH&lt;sub&gt;2&lt;/sub&gt;COOH</td>
<td>2, 4-Dichlorophenoxyacetic acid (2, 4-D)</td>
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<td></td>
<td>2, 4-D sodium salt...the power containing the effective ingredient of 95% by the formula of sodium salt</td>
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<tr>
<td></td>
<td>2, 4-D amine salt...the aqueous solution dissolving the effective ingredient of 20% by the formula of amine salt</td>
</tr>
<tr>
<td>OCH&lt;sub&gt;2&lt;/sub&gt;COOH</td>
<td>2, 4, 5-Trichlorophenoxyacetic acid (2, 4, 5-T)</td>
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<td></td>
<td>2, 4, 5-T amine salt...the aqueous solution dissolving the effective ingredient of ca. 20% by the formula of amine salt</td>
</tr>
<tr>
<td>OCH&lt;sub&gt;2&lt;/sub&gt;COOH</td>
<td>2, 5-Dichlorophenoxyacetic acid (2, 5-D)</td>
</tr>
<tr>
<td></td>
<td>2, 5-D sodium salt...the powder containing the effective ingredient of ca. 90% by the formula of sodium salt</td>
</tr>
<tr>
<td>OCH&lt;sub&gt;2&lt;/sub&gt;COOH</td>
<td>2-Methyl-4-chlorophenoxyacetic acid (MCP)</td>
</tr>
<tr>
<td></td>
<td>MCP sodium salt...the aqueous solution dissolving the effective ingredient of 22.2% by the formula of sodium salt</td>
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<tr>
<td></td>
<td>MCP potassium salt...the aqueous solution dissolving the effective ingredient of ca. 20% by the formula of potassium salt</td>
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OCH₂CH₂OSO₄Na 2,4-Dichlorophenoxyethyl sulfate (SES)

SES sodium salt...the powder containing the effective ingredient of 90% by the formula of sodium salt

1. The effect on the metabolic cell

   a) Staminal hair cells of *Tradescantia*

Figs. 1-6. 1-2. Postmortem modification of staminal hair cell of *Tradescantia* treated with 1% MCP potassium salt at 10:13. ca. ×880. 1, photo. 10:28. The mitotic cell in early telophase shows gelation gradually after treatment. 2, on the third day after treatment. Coagulated nuclear regions change to a homogeneous state. 3-6. Various states of cultivated cells. 3, staminal hair cell of *Tradescantia* cultured one month after treatment with 0.02% of 2, 4-D sodium salt at Oct. 18, contains fully grown plastids. ca. ×1100. 4, stipular tissue cells of *Vicia faba* embedded in 0.01% of 2, 4-D sodium salt, show active growth of chloroplasts on the 8th day of treatment. ca. ×330. 5, isolated cells of *Triticum* root tip treated with 0.01% of 2, 4-D sodium salt. They show growth on the 4th day of treatment. ca. ×270. 6, pollen grain cells of *Tradescantia* treated with 0.1% of 2, 4-D amine salt at 10:43. ca. ×590. Nuclei become homogeneous after coagulation. Photo. 15:45.
In the concentrations ranging from 1% to 0.1% of 2, 4-D amine salt, 2, 4, 5-T amine salt, from 1% to 0.3% of 2, 4-D sodium salt, MCP potassium salt and from 1% to 0.5% of 2, 5-D sodium salt, the hair cells coagulated gradually and died about in 10 minutes or within 2 days after the treatment. Likewise, the cells died in the concentration of 1% MCP sodium salt. The gelated state of the protoplasm induced by the treatment with the high concentrations (1%–0.5%) of hormonic herbicides except SES, became liquefactive and homogeneous within 5 days (Figs. 1–2). This phenomenon is considered to be due to the hydration of the protoplasm caused by the salt action of the chemicals, and this postmortem modification of cells occurred more markedly in the case of treatment with the potassium salt of MCP than with other salts. In the concentration of 1% SES sodium salt, the majority of hair cells survive about 10 days and in that of 0.5% more than 35 days.

In the concentrations ranging from 0.3% to 0.1% of 2, 5-D sodium salt, from 0.5% to 0.3% of MCP sodium salt and in those of 0.1% of MCP potassium salt and 0.05% of 2, 4-D amine salt, the young hair cells died gradually, but the adult cells could survive more than 15 days. In the concentration of 0.1% of MCP sodium salt, the majority of hair cells could survive more than 33 days. After the treatment with the low concentrations (0.03%–0.01%) of hormonic herbicides except SES, some of the hair cells became a gel state and died within a week. However, during this lapse of days, some of them acquired resistance to the toxicity of these chemicals and recovered vital forces. Such cells grew and their plastids also grew within 10 days. Thus, some cells grew unusually and survived containing fully grown plastids for a long time (Fig. 3). Afterwards, with the senile degeneration of the survived cells, the grown plastids changed into fine granular form and became gradually invisible in cytoplasm. This cell, however, could survive more than 130 days. The hypertrophied cell was found also in the hair cells treated with 0.1% of MCP sodium salt. In the concentration of 0.0001% of 2, 4, 5-T amine salt, the dead cells were crushed by the growth of the survived cells. From these results, it was considered that the chemicals displayed the effect as a growth promoter.

In the concentrations of less than 0.01% solution of the hormonic herbicides, the hair cells survived vigorously for a long time. After their death, the cell contents disappeared by the liquefaction and in many cases, there remained only their cell membranes. In some cases, the lipoproteid-like granules appeared in the cell continuing Brownian movement.

b) Stipular cells of Vicia

The stipular cell became a gel state and died after 30 minutes or within 3 days in the concentrations of the hormonic herbicides except SES ranging from 1% to 0.05%. Although the cell treated with 1% of SES sodium salt coagulated on the second day, in the treatment of 0.5% solution, the
cell could survive more than 14 days and the development of chloroplasts started on the second day. In the stipular tissue treated with the concentration of 0.03 % of 2, 4-D amine salt, the younger cells died one by one from the second day and the adult ones within 6 days, and they showed no development of chloroplasts. In the treatment with the concentration of 0.03 % of 2, 4-D sodium salt and 2, 5-D, the stipular cells could survive for about 10 days without developing of chloroplasts.

In the stipular cells treated with the concentration of 0.03 % of 2, 4, 5-T amine salt and MCP sodium salt, the cells could survive for about 20 days and the growth of the chloroplasts appeared in a certain degree on the third day of culture. Similar development of the chloroplasts was found only in the adult cells in the stipular tissue treated with 0.03 % solution of MCP potassium salt. These cells could survive more than two weeks. In the concentration of 0.01 % of the chemicals mentioned above and 0.1 % of SES sodium salt, the stipular cells could survive for a long time and active growth of chloroplasts was found in each cell on the second day after the treatment (Fig. 4). Similar development of chloroplasts takes place also in the stipular cells embedded in the standard agar plate containing 9 % sucrose. From these phenomena, the growth of the chloroplasts is considered to be an important mark suggesting the metabolic state of the stipular cells treated with chemicals, whether or not the cells are healthy or senile.

c) Petal cells of *Allium fistulosum*

The petal cell treated with 0.3 % solution of 2, 4-D amine salt, coagulated gradually and died within 15 minutes, while the cells treated with 0.1 % solution, only the adult ones could survive more than 10 days and the cells treated with 0.05 %, more than 16 days. The plastids in the survive cells showed development.

d) Isolated cells of *Triticum* root tips

The cells treated with 0.1 % of 2, 4-D amine salt coagulated and died within 2 days and in those treated with 0.05 % solution, plastids became visible on the third day. The cell treated with 0.01 % of 2, 4-D sodium salt, could grow to a certain degree (Fig. 5).

e) Cells of *Tradescantia* pollen grains

In the pollen grains treated with 0.1 % of 2, 4-D amine salt, the nucleus coagulated at first, but became homogeneous later (Fig. 6). Although the mitotic cell in early prophase treated with 0.05 % of this chemical, showed no proceeding of mitotic stages, the nucleus remained without coagulation in the first day of the experiment.

From the results of these experiments tested in different cell types and genera, it has been revealed that the petal cell of *Allium* shows the strongest resistance and the stipular cell of *Vicia* the weakest one to the chemicals among other materials. This may be due to some differences in physiological conditions as well as biocolloidal states of the cell organization. Further it
is assumed that the specific toxicity of the hormonic herbicides to broad-leaved plants may be attributed to another aspect of cell organizations at the cellular level.

2. The effect on the occurrence of the mitosis

a) Staminal hair cells of *Tradescantia*

As shown in Table 1, the mitotic cells treated with 2, 4-D sodium ranging from 1 % to 0.5 % solutions, coagulated in 30 minutes. Although the mitotic cells could proceed from anaphase to telephase during this half an hour, they showed gradually various fatal alterations. However, the mitosis could proceed in 0.3 % solution from metaphase further to succeeding stages, in 0.1 % from mid-prophase and in 0.05 % from early prophase respectively. Furthermore, in 0.03 % or in more dilute solutions, the resting nucleus could enter into mitosis on the second day. In 0.1 % solution of 2, 4-D amine salt, the postmitotic cell and the mitotic cell coagulated in 15 minutes after the treatment. However, in 0.05 % solution the mitotic cells could proceed from metaphase to succeeding stages and in 0.03 % solution from mid-prophase respectively. In 0.01 % solution of this chemical and in
more dilute ones, the mitotic cells in early prophase could proceed and *de novo* mitoses were found on the second day.

The hair cells treated with 0.05% of 2, 4, 5-T amine salt, 2, 5-D sodium salt and MCP sodium salt, could proceed from early prophase to succeeding stages and the mitosis occurred *de novo* on the second day. In 0.05% solution of MCP potassium salt, no *de novo* mitosis was observed on the second day, although the mitotic cell in early prophase could proceed. However, in 0.01% solution, *de novo* mitoses were found on the second day. In the treatment with SES sodium salt, *de novo* mitoses were observable even in high concentration of 0.3% on the second day of the treatment.

Concerning the suppression of the mitosis in *Tradescantia* hair cells, the action of 2, 4-D amine salt was found to be the strongest and that of MCP potassium salt stronger than the others. Thus, with regard to the suppression of the mitosis, 2, 4-D sodium salt, 2, 4, 5-T amine salt, 2, 5-D sodium salt and MCP sodium salt were found to be in the decreasing order and the action of SES sodium salt was the weakest among these chemicals. Moreover, the effect of 2, 4-D on *Tradescantia* hair cells appeared differently between sodium salt and the amine salt, and also the effect of MCP on the hair cells showed a striking difference between the sodium salt and the potassium salt as shown in Table 1. It is assumed, that such different effectiveness of the drugs with regard to different salts seems to be due to the different permeability caused by the salt solutions. From the results of above experiments and standing on a cytological basis, it becomes clear that the amine salt of 2, 4-D is generally superior to its sodium salt in the application to agricultural purpose.

b) Stipular cells of *Vicia*

Although the mitosis of stipular cells treated with 0.05% solution of SES sodium salt, could proceed from early prophase to succeeding stages, the mitotic cells in mid-prophase treated with 0.05% solutions of 2, 4-D, 2, 4, 5-T and MCP sodium salt and those in metaphase treated with the same concentration of MCP potassium salt, coagulated gradually during succeeding mitotic stages and died. In the concentrations of 0.005% of MCP potassium salt and in these of 0.01% of 2, 4-D, 2, 4, 5-T, 2, 5-D and MCP sodium salt solutions, the mitotic cells could proceed from early prophase to further stages.

With regard to the suppression of the mitosis, it reveals from the results of these experiments that the effective concentrations of the hormonic herbicides tested are lower in *Vicia* cells than in *Tradescantia* hair cells. This result explains cytologically one of the reasons why some chemicals can act to kill selectively dicotyledonous plants.

3. The abnormal behaviour of the mitotic cell

By treating with salt solutions of the hormonic herbicides, various abnormalities were found *in vivo* in the hair cell of *Tradescantia*, the
stipular cell of *Vicia* and the petal cell of *Allium fistulosum*. Among the mitotic abnormalities the retardation of chromosome movements was observed frequently in anaphase. This may be due to the stickiness of the separating chromosomes in various degrees and to the formation of the chromosome...
bridges in anaphase. The chromosome bridges were pulled by the contraction of two daughter chromosome arms at both poles and broken down. Sometimes the bridges were bisected by the growth of cell plate in telophase. Later, the separated portions of the bridges were dragged into the daughter chromosome groups. This behaviour of the chromosome bridges is generally observed in vivo in the abnormal mitosis induced by many farm drugs. When many chromosome bridges appeared in anaphase, some of them were arrested by the cell plate and remained temporarily in the phragmoplast as chromosome fragments lying along the cell plate. They disappeared later into the cytoplasm with the lapse of time (Figs. 7-9). Some lagging chromosomes were delayed in the reconstruction of chromonemata and formed micronuclei or attaching knobs on the surface of the daughter nuclei (Fig. 10). However, the chromosome bridges seemed occasionally to prevent the growth of the cell plate and the cell formed a binucleate one. In this case of binucleate cell formation, neither the development of the phragmoplast nor that of the cell plate was found in the equatorial region of the cell in telophase (Figs. 11-13).

By the treatment with 0.05% solution of 2, 4, 5-T amine salt, the multi-nucleate cell with multi-septa was formed in the mitosis of Tradescantia hair cells and appeared de novo on the second day of the treatment. By the suppression of metaphase plate formation and poleward movement of chromosomes, they could move at random but they formed finally several clumps in the anaphase spindle. During the transformation of the anaphase

Figs. 14-16. Formation of multi-nucleate cell with multi-septa in staminal hair cell of Tradescantia treated with 0.05% of 2, 4, 5-T amine salt. ca. 1050. 14, random clumping of chromosomes without forming metaphase plate. Photo. 12:21. 15, cell plates appear among chromosome clumps. cp, cell plate. Photo. 12:34. 16, multi-nucleate cells with multi-septa. Nuclei in various sizes. n, daughter nucleus. cw, cell wall. Photo. 15:06.
Figs. 17-19. Various types of the mitotic abnormality in staminal hair cells of *Tradescantia*. n, daughter nucleus. cw, cell wall. 17, multi-nucleate cell with six nuclei in various sizes and incomplete cell walls. Treated with 0.005% of 2, 5-D sodium salt. Photographed on the third day of treatment. ca. ×1230. 18, a binucleate cell: upper with nuclei in unequal size and cell lower with septum at abnormal position. Treated with 0.01% of SES sodium salt. Photo. on the second day of treatment. ca. ×880. 19, cell treated with 0.03% of 2, 4-D amine salt, shows two daughter nuclei remarkably unequal in size and an inclined cell wall. Photo. on the second day of the experiment. ca. ×1050.

Figs. 20-23. Petal cells of *Allium fistulosum* embedded in 0.03% of 2, 4-D sodium salt at 9:53. ca. ×1400. 20, mid-prophase. Photo. 10:28. 21, metaphase seems to be normal. Photo. 14:01. 22, arrow indicates poleward movement of some chromosome masses showing no cell plate development after long duration of anaphase. Photo. 16:28. 23, binucleate cell with daughter nuclei unequal in size. n, daughter nucleus. Photo. on the second day of treatment.
spindle into the phragmoplast, the phragmoplast substance occupied the spaces among chromosome clumps, and in the middle of each mass of phragmoplast substance a cell plate appeared. Thus, a multi-nucleate cell with multi-septa was formed (Figs. 14–16). This abnormality of mitosis appeared only when the cell was treated in a resting state or in early prophase. In the former case, abnormal cells were found in the de novo mitosis on the second day after the treatment. The formation of the multi-nucleate cell with multi-septa was reported by Niitsu (1958); he obtained this abnormality in the cells treated with some troponoid compounds. Similar mitotic figures of the multi-polar spindles were also reported by Croker (1955) who studied the effect of 2, 4-D and 2, 4, 5-T on mitoses of root tip cells in Allium cepa. According to the explanation proposed by Wada (1950) and Wada and Satô (1958), the cause of the appearance of multi-nucleate cell with multi-septa may seem to be due to the suppression of the differentiation of the chromosomal fibers during the development of the spindle. The relationship between the fine structure of the chromosomal fibers and the unit fibrils of the atractoplasm has been demonstrated clearly in the electron microscope studies by Satô (1958, 1960).

Among the mitotic aberrations found on the second day after the treatment with 0.005% solution of 2, 5-D sodium salt, one cell showed six

Figs. 24–27. Stipular cells of Vicia fava embedded in 0.05% of 2, 4, 5-T amine salt at 13:32. Cell was in mid-prophase at the beginning of treatment. ca. ×1050. 24, late prophase. Photo. 14:27. 25, chromosomes swollen markedly. Photo. 14:48. 26, cell plate appears temporarily in telophase. cp, cell plate. Photo. 15:37. 27, cell plate disappears by gradual degeneration of phragmoplast substances. Photo. 15:59. Cell coagulated entirely at 17:05.
nuclei in various sizes and each of them was surrounded by a cell wall respectively (Fig. 17). Although such complicated multi-nucleate cells could survive for a long time, they grew only rather slightly. In the treatments with low concentrations of the hormonic herbicides, binucleate cells occurred with daughter nuclei unequal in size (Fig. 18) or daughter cells showed a remarkable difference in size. In the staminal hair cells of Tradescantia, various types of the abnormal cytokinesis, such as incomplete cell walls, irregular septal position and formation of the inclined cell wall, were found (Fig. 19).

In the mitosis of petal cells in Allium fistulosum treated with 0.03% solution of 2, 4-D sodium salt, some of the chromosomes could finish longitudinal splitting and move toward the poles (Figs. 20–22), but the majority of them changed into chromonemata of the resting nucleus lying at their incipient position. Thus, no development of the cell plate occurred and a binucleate cell with nuclei unequal in size was formed (Fig. 23).

Figs. 28–29. Root tip cells of Triticum embedded in 0.002% solution of 2, 4-D amine salt and fixed 210 minutes later. ca. $\times830$. 28, disturbance of chromosome arrangement in anaphase. 29, clumping of anaphase chromosomes.

In the stipular cells of Vicia treated with 0.05% solution of the hormonic herbicides except SES, the mitoses showed abnormality in a necrotic state of the cell, and the degree of the necrotic effect corresponded to the increasing penetration of the chemicals. After the treatment with 0.05% solution of 2, 4, 5-T amine salt, the mitotic cell in late prophase could continue its activity although the chromosomes swoll markedly (Figs. 24–25). In the beginning of telophase, the phragmoplast appeared and the cell plate developed slightly (Fig. 26). A little later, however, the proceeding of the mitotic stage became sluggish and the cell plate disappeared first and then the phragmoplast substance (Fig. 27). Finally, the cell coagulated. The 0.05% solution of each hormonic herbicide except SES indicated the lowest concentration of which Vicia cells suffered from coagulation and died.

In experiments of cytological studies observed in fixed preparations, the importance to determine the fatal threshold concentrations of chemicals becomes very difficult for the discussion on the chemical effects. Wada (1952a) stated that the necrobiotic changes of mitotic figures studied by means of
fixed preparations are apt to be considered as abnormal mitoses still in a living state under chemical reaction. In practice, as demonstrated in the above experiment, it proved to be very difficult to distinguish mitotic aberrations in fixed preparations, whether they appeared in a necrotic state or still in a living cell.

The root tip cells of *Triticum* treated with 0.002% solution of 2, 4-D amine salt, were observed in aceto-orcein preparations. In this material, mitotic figures in anaphase showed irregular behaviour of the chromosomes, such as chromosome lagging or irregular distribution in the spindle (Figs. 28-29). All these abnormal behaviours of chromosomes were found by *in vivo* observations in the experiments mentioned above.

**Discussion**

From her experimental results, Croker (1953) concluded that 2, 4-D and 2, 4, 5-T bring stickiness or condensation of chromosomes and delay of spindle formation as physiological effects and cause breaks of chromatid or chromosome as structural effects. She classified abnormal mitotic figures in anaphase into four types: (a) normal separation of the daughter chromosomes, (b) figures with temporary bridges resulting from stickiness of the chromosome ends, and (c) anaphase bridges resulting from the fusion of broken chromosome ends and accompanied by acentric fragments. These were often accompanied by sticky bridges. (d) As a few irregular cases in anaphase, it was indicated that the chromosomes were spread from equator to poles, or the poles became diffuse and appeared plate-like. A few spindles were multipolar. Swanson and others (1949) reported abortions of ovules in *Tradescantia* caused by 2, 4-D. The occurrence of malformations induced by 2, 4-D were reported by Olsen (1950) and many workers on various cultivated plants. Yokoyama and Takahashi (1953) investigated the susceptibility of flax to 2, 4-D and MCP, and pointed out that concerning the malformation in flax, the action of 2, 4-D is more effective than MCP. A similar result was obtained by Takematsu (1955) in the culture of potatoes treated with 2, 4-D and MCP.

With regard to the effect of the hormoneic herbicides on the living cells traced by *in vivo* observations, the author classified the action of the drugs and the behaviour of the mitotic cells into the following four types: (1) The chromosome stickiness in anaphase movement as a common reaction of the herbicides, (2) the appearance of chromosome bridges or chromosome fragments and the retardation of chromosome movement inducing frequently daughter nuclei with a knob or isolated micronuclei, (3) the degeneration of the phragmoplast substance and binucleate cells with sticky chromosome bridges, and (4) multi-nucleate cells with multi-septa and daughter nuclei remarkably unequal in size. This type of mitotic aberrations occurred as a result of partial impediment of the spindle mechanism inducing incomplete
cell walls and their irregular positions.

In author's experiments studied by means of in vivo observations, the occurrence of malformations appeared less in the treatment with MCP than the cases of with 2, 4-D. The effect of MCP sodium salt was concluded to be rather weaker than that of 2, 4-D sodium salt with regard to killing the cells as well as to the malformation of cells. However, the effect of MCP potassium salt was recognized to be stronger than that of 2, 4-D sodium salt with regard to the suppression of de novo mitoses and to the formation of various abnormal mitoses.

From these facts, it becomes clear that the toxic activity of the chemicals on the living cells appears strikingly in different types according to the difference of salts combined the herbicides. Moreover, it is demonstrated that the hormonic herbicides are effective on making malformations of plants. Therefore, as composite result of the hormonic herbicides it is concluded at the cellular level that the hormonic herbicides induce at first various types of abnormal mitoses which appear differently by the difference of salts and secondarily abnormal tissue development making malformations of plants and their degeneration.

Summary

By means of the in vivo observation, the effect of various herbicides on cells in the metabolic as well as in the mitotic state was studied in the staminal hair cells of Tradescantia, the stipular cells of Vicia faba, the petal cells of Allium fistulosum and Allium cepa, the isolated cells of Triticum vulgare root tips and the cells of pollen grains in Tradescantia.

The effect of the five hormonic herbicides, 2, 4-D, 2, 4, 5-T, 2, 5-D, MCP and SES, was summarized in Table 1. The abnormal mitoses induced by these chemicals, showed chromosome bridges due to stickiness, a retardation of chromosome movement in anaphase, a binucleate cell, a multinucleate cell with multi-septa, a formation of the incomplete cell wall and an impediment in the differentiation of meristematic tissues. These effects of the herbicides on the mitotic cells revealed intimate connections with the herbicidal activity to weeds and with malformations to cultivated plants.

The different resistance of cells to the herbicides between monocotyledonous plants and dicotyledonous ones was discussed at the cellular level from the results of their different behaviour with regard to the occurrence of abnormal mitoses and the survival of cells.

Acknowledgment

The author wishes to express his hearty thanks to Professor B. Wada, Biological Institute, Faculty of Liberal Arts and Sciences, Shizuoka University, for his valuable advice and encouragement through this work.
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