The Effect of Chemicals on Mitosis Studied in *Tradescantia* Cells *in vivo*. II. Hydrazino-tropone compounds^{1,2}

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In the present paper the action of two tropone compounds, 2-hydrazino-tropone and 7-bromo-2-hydrazino-tropone, on the mitotic cell was studied by means of the *Tradescantia* test *in vivo*. These compounds recently were synthesized by Nozoe and his coworkers (Nozoe et al. 1952), and provided through the kind favour of Prof. Nozoe of Tôhoku University (Sendai, Japan)³.

Material and method

The investigations were carried on in staminal hair cells of *Tradescantia* sp. (tetraploid species) by the same method which was described in detail in the author's recent paper on the action of tropolone compounds on the mitotic cells of *Tradescantia* (Wada 1952). Therefore it would be superfluous to repeat the *Tradescantia* test *in vivo* by means of the agar plate method. The concentrations of the tropone compounds contained in the agar medium were 0.1, 0.0133, 0.01, and 0.001 per cent. The 0.2 gramm of 2-hydrazino-tropone was completely soluble in 100 cc distilled water, which was colored yellowish but the 0.2 per cent aqueous solution of 7-bromo-2-hydrazino-tropone contained a considerable quantity of insoluble crystals. The complete solution of this bromo-hydrazino-tropone was obtained in a concentration of 0.002 per cent, which was colored yellowish.

Observation

2-Hydrazino-tropone

In an agar plate containing 0.1 per cent solution of this compound, changes in the cell contents appeared as early as 20 minutes after the treatment. The cytoplasm changed to a hyaline state by swelling

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³ The author wishes to express his deep gratitude to both Prof. Nozoe and his coworkers.

and the nucleus also showed swelling. Meanwhile the prophase nuclei also became swollen thus causing their chromonema structure to fade. In general any slight change introduced in the prophase nuclei, especially in their early stages, is injurious enough to suppress further mitotic processes and to make the prophase nuclei revert into the resting nuclear state. In the case of tropone compounds, however, the swollen prophase nuclei were able to continue their mitotic processes and in a certain range of concentrations finally to complete the mitosis.

In this medium it was found that 3 cells could finish mitosis, but 2 of them failed to make the cell plate and became binucleate cells (Figs. 1-2). Three hours after the treatment the toxicity of this

Figs. 1-4. Necrobiotic changes of *Tradescantia* cells treated with a 0.1 per cent solution of 2-hydrazino-tropone. The cell embedded at 10:27⁴, May 20, 1952. **1-2**, a binucleate cell. The cell was mid-anaphase, when observed for the first time in the agar plate. **1**, twenty-seven minutes after the treatment. Early telophase. The development of the phragmoplast and cell plate were suppressed. **2**, twenty-three hours after the treatment. The binucleate cell already coagulated by demixing of the protoplasm. *m* a clump of fluid plasma. **3-4**, development of prophase nuclei and necrobiotic changes. The cells were able to develop in the first three hours from very early prophase to advanced stages before the toxic effect of this compound suppressed the mitotic activity. Photographed twenty-three hours after the treatment. **3**, the cell (the upper one) in the spiral stage. **4**, the cell in the late prophase.

compound appeared in nearly all of the cells, which showed further swelling of the cytoplasm. Then the cytoplasmic streaming began to stop where granules in the cytoplasm were found in Brownian movement. Consequently all the mitotic cells in various stages were hindered from further development of the stages and the mitotic figures gradually entered into a gelatinized state.

⁴ 10:27 means 27 minutes after 10 o'clock: the words "minutes" and "o'clock" are in the following figures also omitted.

Six hours after treatment, protoplasmic streaming was found only in cells of 3 staminal hairs out of 44. The nuclei in these surviving cells showed a swollen chromonema structure with a faint nuclear boundary. Twenty-two hours after treatment, cells in all the hairs excepting the above mentioned 3 hairs were coagulated by demixing of both the swollen cytoplasm and nuclear substances (Figs. 3-4). On the third day of the cells in the experiment the surviving three hairs also died by coagulation.

In a 0.0133 per cent solution of this tropone compound, all the mitotic cells including early prophase cells could continue their mitotic stages and finish mitosis, while only 2 cells, out of 24 embryonal ones were able to enter into mitosis *de novo*. In a 0.01 per cent solution of this compound, as in a control medium, the cells could enter into *de novo* mitosis which, however, often showed binucleate cells. In general the cell contents used to show swelling in this medium and were not completely free from the toxic effects of the hydrazino-tropone. The cell could survive several days in the necrobiotic state. In a solution of less than 0.01 per cent of this compound, cells exerted their metabolism in the normal way and mitoses occurred *de novo* nearly with the same frequency as found in the control medium. They could survive even for weeks.

7-Bromo-2-hydrazino tropone

In a 0.01 per cent solution of this compound, a saturated solution, the mitotic cells could maintain their normal state for a while. A late prophase nucleus, which began mitosis *de novo* in this medium, showed precocious longitudinal splitting of chromosomes and this sometimes could be confused with the mitotic figure of the mid-anaphase in the living state (Fig. 5). However, the shape of the nuclear cavity of the former was not so obvious as the spindle body of the latter. The split chromosomes were carried on through the metaphase configuration towards the spindle poles. However, the cell finished karyokinesis without being followed by cytokinesis and became a binucleate cell (Fig. 6). Figs. 7-8 show another example. Thirty minutes after treatment, the chromosomes in a metaphase cell swoll and the outline of each chromosome individual became faint, while the spindle body itself exerted its force upon the swollen chromosomes. As a result of the chromosome swelling, the chromosome surface became considerably sticky, and thus in the telophase, chromosome bridges were found across the cell plate (Fig. 7). In the case of Figs. 7-8 the cell at the beginning of the experiment was already in the metaphase and entered into the telophase within an hour. In the lapse of this short time the toxic effect of this compound did not appear strong enough to suppress the develop-

ment of the phragmoplast, so the cell completed the mitosis (Fig. 8). This temporary stickiness of chromosome individuals sometimes introduced unequal distribution of daughter chromosomes and as a consequence making a pair of daughter nuclei of unequal size.

Figs. 5-12. Effects of a 0.01 per cent solution of 7-bromo-2-hydrazino-tropone [on the *Tradescantia* cells. The cells embedded in the agar plate at 11:00, May 15, 1952. **5-6**, a precocious longitudinal splitting of chromosomes. **5**, two hours after the treatment. Late prophase showing precocious longitudinal splitting of the chromosomes. **6**, six hours after the treatment. A binucleate cell already gelatinized. **7-8**, the chromosome bridge. **7**, fifty-five minutes after the treatment. Mid-telophase. A chromosome bridge caused by stickiness of the swollen chromosomes in the figure. **8**, twenty-three hours after the treatment. The cell in a necrobiotic state, but still continued to show weak local protoplasmic streamings. *b* chromosome bridge. **9-12**, development of a binucleate cell. **9**, two hours and twenty minutes after the treatment. Beginning of the late anaphase. **10**, three hours and eighteen minutes after the treatment. Early telophase. Disintegrated phragmoplast substances appeared as a clump of fluid plasm with abundant granules in Brownian movement. **11**, twenty-six minutes later than fig. 10. Mid-telophase. **12**, Twenty-four hours after the treatment. Binucleate cell. The cell as a whole was slightly gelatinized. *m* a mass of fluid plasm.

From the results of these experiments it is concluded, that the toxic effect of this compound appeared rather slowly both on the nucleus and the cytoplasm. Therefore some cells complete a whole

cycle of the mitosis, but others are affected in a later stage of the cycle and very often the development of the phragmoplast as well as of the cell plate is partly or completely suppressed with the result that thus binucleate cells or cells with incomplete cell walls appear (Figs. 9-12). When the development of the phragmoplast is suppressed completely a clump of fluid plasm containing abundant granules appears between the two daughter chromosome groups and the granules show active Brownian movement. Continuous observations on the morphogenesis of this plasm mass *in vivo*, leave no doubt that it occurs by the disintegration of the phragmoplast substances.

On the third day of the experiment, cells in 17 hairs out of 40 showed protoplasmic streaming, but the others were gelatinized or coagulated by demixing of the swollen cytoplasm and showed enlargement of vacuoles and the coarse structure of the nucleus. On the fifth day of the experiment, all the cells except those in one staminal hairs died by coagulation.

In a 0.001 per cent solution of this compound any toxic action on the mitotic cells was hardly found, so the mitosis occurred *de novo* and cells continued growth and metabolism in the normal way and survived for weeks.

Considerations

In the working hypothesis of the mitosis mechanism based on studies of the submicroscopic structure and of the living state of the *Tradescantia* cell, the author expressed the opinion that mitotic action may be commenced by the swelling of the embryonal nucleus, in which the spiralisation of chromonemata to chromosome and differentiation of chromosome matrix and of atractoplasm from the karyolymph are carried on (Wada 1950). A series of these actions in the prophase nuclei is also continued in the swollen nuclei treated with tropone compounds. The development of the prophase nuclei in the experimentally induced circumstances is rather a rare case and has been reported on the mitotic cells treated with ammonium vapour (Wada 1937, 1939).

It is a generally accepted fact that the morphological changes in the telophase appear to be prophasic processes in reverse. Therefore it is thought that any chemical effects which are favourable for carrying on the prophase processes should be obstructive to finishing the mitosis. Through the foregoing investigations, these physiological relationships between the beginning and the ending of one mitotic cycle are understood: the environment in which these tropone compounds are regarded as favourable for the mitotic processes in the prophase makes

for the cell in the telophase a difficulty, impeding the completion of the cytokinesis and often introducing a binucleate cell.

The fact that the tropone compounds which are inactive against the atractoplasm act injuriously to the phragmoplast substances does not always mean that there is an intrinsic difference between the atractoplasm and the phragmoplast substances, but from the results of other experiments it is clear that the atractoplasm may be altered into phragmoplast substances by hydration. Therefore it may be concluded that the tropone compounds become active, when the atractoplasm is hydrated. With the treatment by the tropone compounds the phragmoplast substances as well as cell plate gradually disorganize themselves and become converted into a cytoplasmic state, appearing as a mass of fluid protoplasm with abundant granules in Brownian movement.

The stickiness of the chromosomes caused by the tropone compounds may introduce various kinds of chromosome aberrations in appearance only; neither the dicentric chromosomes nor chromosome fragments are found in the telophase, but the equal distribution of the daughter chromosomes in the sister cells may be often disturbed. Therefore these chemicals have in them the possibility of being available as mutagenic substances. In short when the tropone compounds introduce any swelling or homogeneity on the nuclear substances, similar changes would occur on the cytoplasm which become swollen and hyaline and in a certain range of concentrations the cells undergo gradual necrobiotic changes even though the cells are able to carry on mitosis *de novo*. It is, however, not clear whether these tropone compounds are to be regarded as acting selectively or generally on the biocolloid of the mitotic cells or acting on part of the general enzyme systems of the cell.

In conclusion a brief note on the cell biological significance of the tropone compounds will be added. According to the author's experience on the mitotic cells tested in *Tradescantia* cell *in vivo*, early prophase nuclei show a high susceptibility for physical as well as chemical treatments with the cells discontinuing further mitotic activity and reverting into the resting nuclear state. However, these tropone compounds showed an exceptional to the above.

When the reaction types of these tropone compounds on mitotic cells are arranged on the ordinate corresponding to concentrations on the abscissa, the results of the above experiments can be shown as in the diagram given in Fig. 13. A glance at the diagram will show that in a 0.1 per cent of hydrazino-tropone the cells which already have entered into the first step of the mitosis can go on to advance mitotic stages, even though no resting nucleus can commence the mitosis *de*

novo. On the contrary in a 0.0133 per cent solution of hydrazino-tropone and in a 0.01 per cent solution of bromo-hydrazino-tropone, mitosis used to occur *de novo* even in the necrobiotic state of the cell and was accompanied sometimes by abnormal mitotic figures as mentioned above, although no indication had been in evidence to promote the mitotic activity.

With respect to the mitotic activity in animal cells Bullough and Johnson (1951) have expressed the opinion that "once a division begins

NO MITOSIS OCCUR <i>de novo</i>	Cells die within 72 hours	FIXING ACTION				
	Cells die within 72 hours	EARLY PROPHASE NUCLEI go on				0.1 %
MITOSIS OCCUR <i>de novo</i>	Cells die within 2-4 days	Showing effect in SPINDLE MECHANISM (Atractoplasm) Phragmoplast CHROMOSOME ABERRATION (Fragment, Bridge) Sickness HYPERTROPHIC GROWTH (c-Tumor)		0.01 % (sat. sol.)	0.0133 %	
	Cells survive	NO VISIBLE CHANGE	0.001 %	0.01 %		

Fig. 13. A diagram showing the distribution of reaction types in Tradescantia cells treated with hydrazino-tropone and bromo-hydrazino-tropone in various concentrations. The reaction areas enclosed with heavy lines indicate 2-hydrazino-tropone and areas with broken lines indicate 7-bromo-2-hydrazino-tropone. Letters placed in brackets in the third level of the ordinate are not introduced directly by these tropone compounds.

it can continue to completion in almost any circumstances short of the death of the cell". The inconsistency of the susceptibility of the prophase nuclei in Tradescantia cells, the author believes, may be due partly to the difference of the material tested and partly to the different point of observation taken. In Tradescantia cells the author's attention has been focussed not upon the behaviour of the chromosomes but upon that of the chromonemata in the prophase. The characteristic action of the tropone compounds is this that it shows very little influences upon the development of chromonemata into chromosomes in the early prophase. During this period the despolarization of chromonemata in the preceeding telophase, the longitudinal splitting of chromonemata including the duplication of genes, the spiralization of new chromonemata, the development of chromosome matrix, and the coiling of chromosomes accompanied by a condensation upon them of nucleic acids occur. Indeed, the present author also can say that once chromo-

somes are built up in the prophase nuclei, the mitosis can continue to completion in almost any circumstances.

Summary

1. By means of the agar plate method, the action of 2-hydrazino-tropone and 7-bromo-2-hydrazino-tropone on mitotic cells was investigated in the *Tradescantia* test *in vivo*.

2. In a certain range of concentrations of these tropone compounds, swelling of the nucleus, of the cytoplasm and of the chromosomes occurs. In this state, not only the swollen prophase nuclei can continue the mitotic processes but also mitosis occurs *de novo*; most of the mitotic cells, however, become binucleate cells by the disorganization of the phragmoplast in the telophase.

3. These tropone compounds scarcely act on the atractoplasm, but due to the stickiness of the swollen chromosomes sometimes introduce bridges merely in appearance in the telophase.

4. From the standpoint of the cell biology, the relationships between the swelling of the prophase nuclei and the disorganization of the phragmoplast and the susceptibility of the prophase nuclei are discussed.

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