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Effects of chemicals upon the configuration of spiral structure in chromosomes

By

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(With 3 plates, 3 graphs and 7 tables)

Since the discovery of the spiral structure in chromosomes various methods have been introduced to demonstrate it. In the present investigation, the writer utilized some series of chemicals as pretreatment agents and examined their influence upon the configuration of the demonstrated spiral structure.

The materials were the PMCs of *Trillium kamtschaticum* gathered from the natural population at Shizunai, Hidaka province, Hokkaido. Moderate heat treatment was employed to securely attainment of the 1st meiotic metaphase of the PMCs in early January. The chromosomes of such material presented more favourable conditions for this investigation than those of plants in field condition.

The method was the same as the water-pretreatment-method (MATSUURA '38) except that, in place of water, aqua solutions of salts were used. The time of the pretreatment to obtain a fine spiral figure of chromosomes can be determined with accuracy when the procedures of the pretreatment were carried out properly. Special care must be taken to perform identically the next steps of the procedures in the pretreatment: a constant and small volume of the anther content is pushed out on the glass slide, a constant and small volume of the pretreatment agent is immediately added on it, and the time of the beginning of the pretreatment is recorded; the sample is kept in *status quo* for a certain while without any disturbance; as soon as the excess of the agent is removed with a piece of blotting paper, a drop of aceto-carmine is added and the time of the end of pretreatment is recorded.

Observations were made with freshly made aceto-carmine preparations.

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I. The action of irons on the demonstration of special structure in chromosome

First, PMCs were pretreated with hypertonic aqua solutions of monovalent-ion-salts. The name, the concentration of each agent and the time of the pretreatment are shown in Table 1 and Graph 1. When anions of the agents are common, the relationship between the time and the concentration are nearely the same irrespective of the partnered cations of the agents. The smaller the mobility of the anion the more the time of the pretreatment is prolonged. The order: KCl, NaCl, NH_4Cl<KNO_3, $NaNO_3$, NH_4NO_3<KSCN, NaSCN, NH_4SCN, is seen as to the time necessary for the demonstration of spiral structure pretreated with solutions of one and the same concentration of these agents.

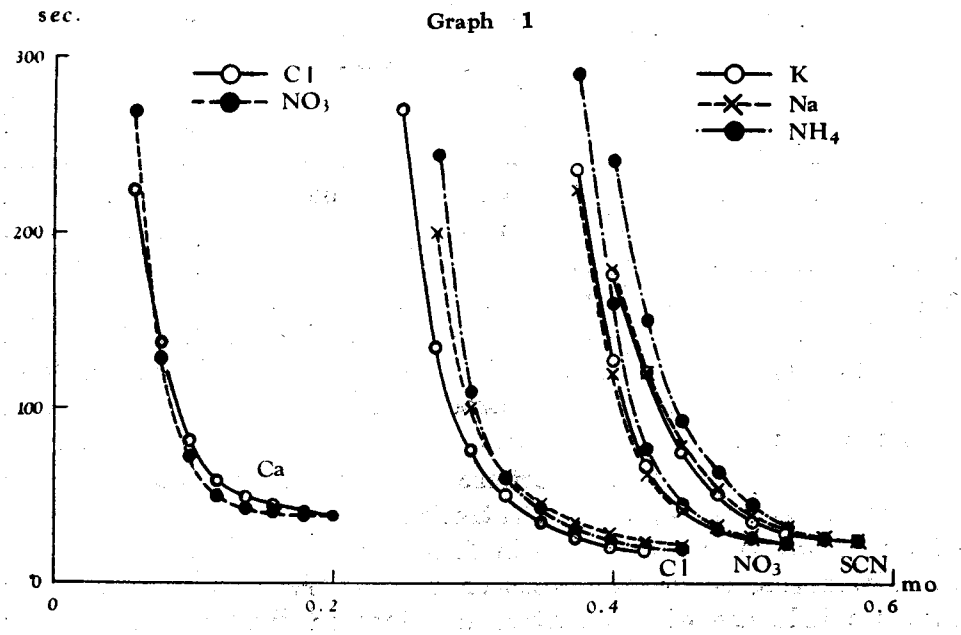
Next, same series of hypertonic solutions of bivalent-cation-salts was used. The name, the concentration of each agent and the time of the pretreatment are shown in Table 2 and Graph 1. In this case the discharging action of cations on chromonemata is so conspicuous that the influence of the partnered anions on the demonstration is almost hidden.

In both cases with solutions hypertonic to the PMCs the time of pretreatment is prolonged in accordance with the decrease of the concentration of the solution.

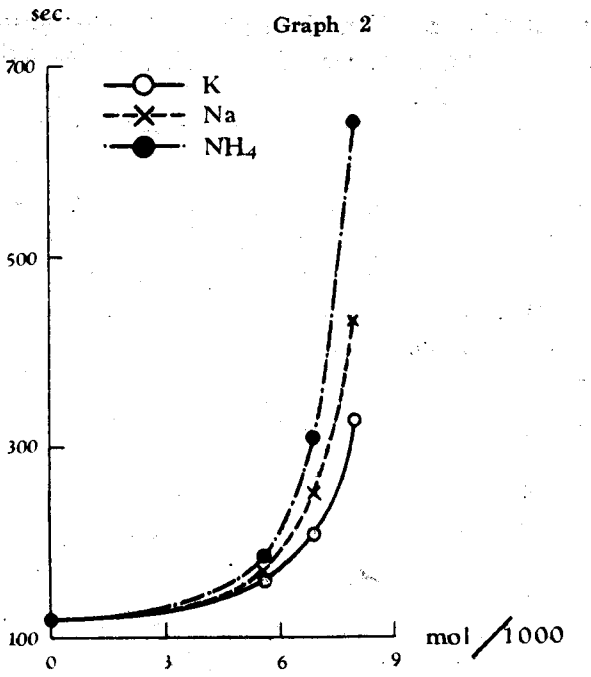
At last, PMCs were pretreated with hypotonic aqua solutions of monovalent-ion-salts. The results with KNO_3, $NaNO_3$ and NH_4NO_3 are shown in Table 3 and Graph 2. Here, it is most probable that the water molecules play a leading part in the manifestation of the spiral structure. The fact that the time allowed for the pre-treatment to act upon the material is prolonged according to the increase of the concentration, is consistently understandable from this point of view.

With aqua solutions of monovalent-ion-salts hypertonic to the PMCs, the demonstration of spiral structure is done under the determinant influence of the anions in the agents. The fine exemplification of lyotropic series on the reaction proves that the absorption of the ions by the chromosome occurs after the manner of GIBBS' law. Now let be assumed that, in the demonstration, a certain part of the ionizing radicals in chromosome is masked with a quantity of ions, the time necessary for the pretreatment may naturally be the function of two variables; the concentration of the agent and the mobility of the ion.

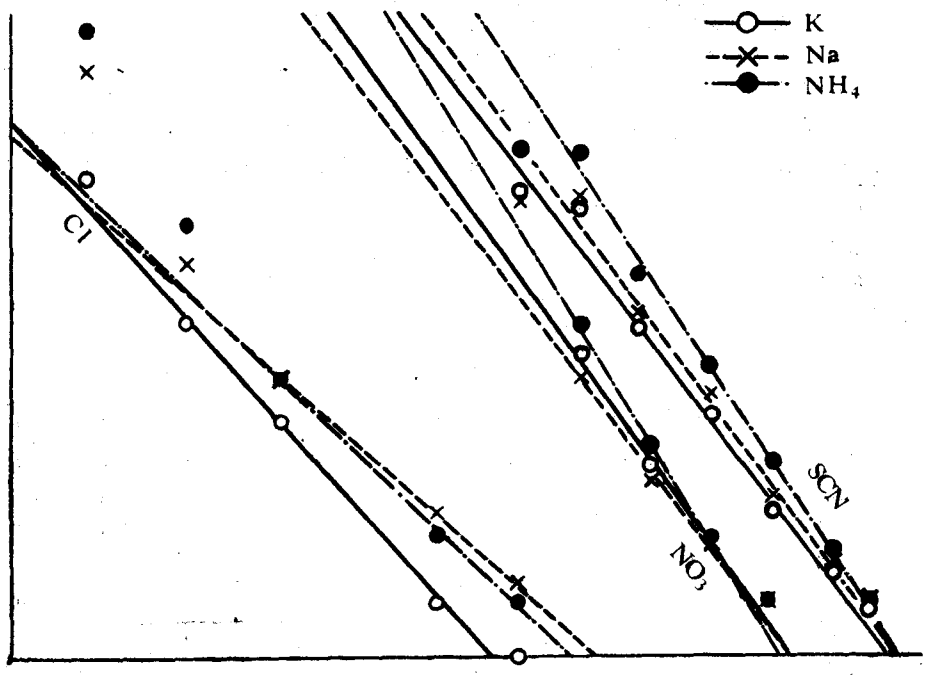
Graph 1



Graph 2



Graph 3



Expanation of Graphs 1, 2 and 3. The abscissa represents the concentration in mol in Graphs 1, 2, and log (mol $\times 100$) in Graph 3. The ordinate represents the time of pretreatment in sec. in Graphs, 1, 2 and log (sec.) in Graph 3.

Plotting the data with these agents against the exponential graph, linear relation is found in the middle concentration of each agent (Graph 3). In these concentrations, the degree of the swelling of chromosomes at that time of the demonstration was proportionate to the capacity of the swelling of anions in the positive colloid (see the second experiment).

With bivalent-cation-salts the demonstration is done under the determinant influence of the cations, the chromonemata being discharged by the ions. The matrix is hydrated under the influence of anions in this case also as in that above described, and so the volume of chromosomes is smaller with $CaCl_2$ than with $Ca(NO_3)_2$ (see Plate XIV, Figs. 9 and 10).

With hypotonic solutions the quantity of ions entering into the PMCs is definitely small, so that the masking of chromosomes is done chiefly by the water having entered or pre-existing in the PMCs.

The experiments carried out above make it clear that the mecha-

TABLE 1

The concentration (mol) and the time of pretreatment (sec). for the demonstration of spiral structure in chromosomes with aqua solutions of monovalent-cation-salts hypertonic to the PMCs

No. of Plant*	1	1	1	2	2	3	1	4	4
Agent	KSCN	NaSCN	NH_4SCN	KNO_3	$NaNO_3$	NH_4NO_3	KCl	NaCl	NH_4Cl
Concentration	Time of Pretreatment								
0.575	22	22	23						
0.550	24	25	25						
0.525	28	29	30	23	23	24			
0.500	35	37	43	23	23	24			
0.475	52	55	62	25	25	25			
0.450	75	78	91	31	30	31		21	20
0.425	118	120	150	43	41	47	18	23	20
0.400	175	180	240	65	61	75	20	27	25
0.375				125	120	148	25	34	32
0.350				235	225	290	35	45	46
0.325							50	62	60
0.300							76	100	110
0.275							135	200	240
0.250							270		

TABLE 2

The concentration (mol) and the time of pretreatment(sec.) for the demonstration of spiral structure in chromosomes with aqua solutions of bivalent-cation-salts hypertonic to the PMCs

No. of Plant*	5	5
Agent	$Ca(NO_3)_2$	$CaCl_2$
Concentration	Time of Pretreatment	
0.20	40	40
0.18	42	40
0.16	45	42
0.14	50	45
0.12	60	52
0.10	83	75
0.08	115	135
0.06	225	270

TABLE 3

The concentration (mol) and the time of pretreatment (sec.) for the demonstration of spiral structure in chromosomes with aqua solutions of monovalent-cation-salts hypotonic to the PMCs.

No. of Plant*	3	3	3
Agent	KNO_3	$NaNO_3$	NH_4NO_3
Concentration	Time of Pretreatment		
0.0000	120	120	120
0.0056	155	165	185
0.0069	210	250	310
0.0080	325	430	640

* (Table 1, 2 and 3) Five plants, No's. 1, 2, 3, 4 and 5 had been kept in identical environmental condition until MI was attained so that the individual difference is assumed to be small.

nism of demonstration of spiral structure in chromosomes is to loosen or to mask the cohesional bindings in chromosome micell and to give suitable swelling to it. The substance which acts as the determinant factor on the masking or the swelling is changed with each pretreatment agent. This is because the course of the demonstration is altered by the mode of the reaction between chromosomes and the agent: *i.e.*, by the nature of the substance which reacts most effectively with chromosomes, and by the nature of the bindings which are affected by each substance respectively.

II. The influence of pretreatment agents upon the swelling of chromosomes

SALT SOLUTIONS. Salt-solutions which do not exert any marked change of hydrogen ion concentration upon the PMCs were prepared. The names and concentrations in mol are given in Table 4. The concentrations of the solutions were so adjusted as to make the spiral structure in chromosome attain the finest demonstration after the pretreatment of about 50 sec. with each solution. The distinctive features of chromosomes represented by the ions in each solution can be observed most obviously when the spiral structure attains to the finest demonstration (Plate XIV, Figs. 1–10). The swelling of major matrix was remarkably influenced by the anions and the volume of chromosomes varied by the series of anions: $SCN > NO_3 > Cl > CH_3COO$. The influence of cations was not so conspicuous as that of anions. The chromosomes pretreated with sodium salts were a little smaller than those with potassium salts. The lengh and the diameter of the major spiral thread* varied in the same manner as that of the major matrix, but the difference with each agent was not so conspicuous as in the latter.

The minor coils were represented more finely with potassium salts than with sodium salts (Figs. 1–6) indicating that the chromonema is negatively charged. The much more contracted apperance of major spiral thread represented with calcium salts and the smaller concentration of their solutions to attain the demonstration of spiral structure after 50 sec. pretreatment than those with alkali and ammonium salts (see Table 4), is due to the bivalency of calcium ions, resulting in effective discharge of the negatively ionizing centers in chromonemata.

* This term is used for the thread which is composed of minor matrix and chromonemata and spiraling into major coils.

TABLE 4

Chemicals prepared for the 50 sec. pretreatment. The numerals written under the names of the chemical are the concentration in mol

Anion / Cation	SCN	NO_3	Cl	CH_3COO
Na		$NaNO_3$ 0.353	NaCl 0.339	
K	KSCN 0.376	KNO_3 0.365	KCl 0.333	CH_3COOK 0.376
NH_4		NH_4NO_3 0.368	NH_4Cl 0.350	
Ca		$Ca(NO_3)_2$ 0.080	$CaCl_2$ 0.075	

HYDROGEN ION CONCENTRATION. To examine the influence of induced changes of hydrogen ion concentration upon chromosomes, the next three agents were prepared: a) NH_4OH 0.353N + KOH 0.031N., b) NH_4OH 0.519N and c) NH_4OH 0.353N. + HCl 0.031N. The time of pretreatment was 40 sec. with (a), 50 sec. with (b) and 65 sec. with (c) respectively. The most pronounced relaxation of spirals after the shortest pretreatment with (a) (Plate XV, Fig. 1) is due to the rapid alkaline reaction in the PMCs. The positive charge of matrix should be minimized in the decrease of hydrogen ion concentration and the cohesions between it and the chromonemata should be weakened allowing free expansion of chromonemata whose elasticity is increased by the greater dissociation of the negatively charged ionizing centers. The reverse is the case when pretreatment is with (c) (Plate XV, Fig. 3), where minor coils are slightly relaxed and the hydration of chromosome is attained after the longest pretreatment.

OXIDO-REDUCTION. The first test for oxidizing agents was carried out with hydrogen-peroxide. Commercially made hydrogen-peroxide (3% aqua sol.) was diluted from fifteen to thirty times with re-distilled water. Pretreated with such diluted hydrogen-peroxide for 50 to 60 sec. the major spiral thread represented itself as a thread with more compact packing and sharper outline than that pretreated with distilled water. To represent the contraction of major spiral thread more exaggeratedly, a good swelling was given to the chromosomes pretreating them with 0.39 mol KSCN for 50 sec. (Plate XV, Fig. 4). Then a drop of 0.15% H_2O_2 was added successfully to the material and it was kept in the condition for 7 sec. further (Plate XV, Fig. 5). After the addition

of hydrogenperoxide the swelling of matrix is hampered by the oxidizing action resulting in the recontraction of chromosomes. This contration proceeds to much greater extent in major spiral threads than in the major matrix. Thus the reduction of the number of major coils results.

Now it was found that oxidation contracts chromosomes. Next we must see if reduction can loosen them.

Nitrites were chosen as the reducing agent. As had been expected nitrites caused so severe a relaxation of minor coils that chromonemata were apt to be destroyed before the attraction between major coils and major matrix was enough relaxed. For the purpose of obtaining suitable figures for photographs, rhodanites were added to weaken the action of nitrites. The pretreatment agents prepared were as follows: KSCN 0·136 mol+KNO_3 0.227 mol, KSCN 0.136 mol+KNO_2 0.227 mol, NaSCN 0.136 mol+$NaNO_3$ 0.227 mol, NaSCN 0.136 mol+$NaNO_2$ 0.227 mol. The time of pretreatment was about 50 sec. in each trial. In the figures obtained with nitrite-mixtures a clear aspect of chromonemata can be seen (Plate XV, Figs. 7, 9). The unravelling of minor coils is greater and the expansion of major coils is smaller than those observed in the alkaline reaction (see Plate XV, Fig. 1).

III. The Change of chromosome configurations as observed in anaerobic conditions.

The pretreatment method hitherto employed in this investigation includes the procedure of pushing out the anther contents on the glass slide. The PMCs are, then, placed in more aerobic condition than they are in the anthers. There must accompany some changes, which had universally occurred, to some extent, in every pretreatment carried out before. If one of the agents is allowed to manifest its action upon the PMCs in the anther, accompanying changes can be avoided and the mode of the reaction with chromosomes may differ from that manifested on the glass slide.

To examine such circumstances, the PMCs were pretreated in the anther. One whole anther was used for each test. After the pretreatment it was put into acetocarmine and the contents were pushed out directly into the fixative. The penetration of pretreatment agents through the anther wall is so difficult that most of the pretreatments were carried out under low pressure. "Thunberg-rohl*" and an air-

* "Thunberg-rohr" is called in the following description the "tube".

pump were used as the instruments to keep the samples under low pressure, in which the agents penetrate into the PMCs and attain enough concentration to manifest markedly the action upon chromosomes.

At first an anther was kept in re-distilled water and the air of the tube was sucked out with the air-pump for ten to sixty minutes. After ten to thirty minutes some swelling of minor coils occurred and the diameter of the major spiral thread became a little wider. But no change was observed in the major matrix except that it lost a little affinity to the dye. Further continuation of this treatment brought about contraction of chromosomes and the spiral structure was not demonstrated. Next, an anther was put into the tube containing 10 c.c. of 0.75 N. NH_4OH and the air of the tube was drawn out for 10 to 30 minutes. The swelling of chromosomes was brought about, but cohesions of the major matrix were not so sufficiently relaxed as to make a fine demonstration of spiral structure possible. Even with longer duration of the driving of the pump, the major matrix could not be fully relaxed and it caused a damage to the chromonemata whose fine structure changed into an irregularly vacuolised from in the swollen matrix. Finally, there came a complete destruction of chromosomes leaving only faintly stained cloud like remnants. The unravelling of spirals seen in the longer pretreatment with ammonium hydroxide on the glass slide was not observed.

A fine demonstration was successfully obtained at last by the following procedure: an anther was put into 10 cc. of 0.75 N. NH_4OH, the air of the tube was drawn out for 15 minutes and then it was kept in the tube continuously for 15 minutes longer. During the latter 15 minutes the material was gently heated by grasping the tube with the hand. On the driving of the pump, due to the continuous removal of NH_3 from the solution and to the cooling of the solution, being deprived of the evaporation heat, the sample was in unsaturated condition with ammonia. When the pump stopped moving and the tube was heated, an oversaturation was brought about which increased the chances for the entrance of ammonia into the PMCs. The configuration of chromosomes obtained here has quite characteristic features. The chromosomes in Plate XVI, Fig. 8, represent an incompletely demonstrated spiral structure. The cohesion of the major matrix still remains resisting the expansion of major coils in which minor coils have been already liberated from the restraint of their matrical substance. In Plate XVI, Fig. 9, a

fine demonstration is attained.

When an anther was put in 0.75 N. NH_4OH under 1 atm. for about 40 minutes, there could, rarely and in only a few PMCs in the anther, be demonstrated a spiral structure which differed much not only from that obtained in the low pressure but also from that obtained by the pretreatment with ammonia on the glass slide (see Plate XVI, Figs. 7, 8, 9 and Plate XV, Fig. 2).

In aerobic condition under 1 atm., the swelling of the major matrix was more easily attained than in anaerobic conditions under low pressure. One of the factors responsible for this difference is the quantity of electrolytes penetrating into the PMCs. The penetration of the electrolytes is more rapid into the PMCs pushed out on the glass slide than into the anther wall. Accordingly the swelling of the major matrix occurrs more rapidly in the former. The other is the increase of gel rigidity of plasm in the decrease of pressure. The reversible sol-gel-transformations by changes of pressure affect the surface tension and the viscosity of matrix (PEACE '46). The delayed and decreased swelling of the major matrix under low pressure is largely due to its increased rigidity.

IV. The change of chromosome configurations as observed in aerobic conditions

As mentioned before the PMCs pushed out on the glass slide are placed in different codnitions from those in the anther. The equilibrium having existed in the anther changes, passing through various transitional alterations, to another one. The fixations are made at a certain period in the course of this transition. By addition of various pretreatment agents the modes of the alteration is transformed. This is visualized as changes in the chromosome configurations reflecting the qualitative and quantitative characters of the agents. It must not be forgotten, however, that, other changes of conditions in the PMCs, being pushed out of the anther and placed in aerobic conditions, cooperate on the manifestation of the resultant figures. The influence of these factors is conspicuous if the pretreatment agent induces only small alteration on the transformation.

In this experiment an attempt was made to examine the influence of these factors based on the results with re-distilled water and glucose solutions.

For convenience' sake, the configuration of chromosomes represented by the pretreatments was distinguished into seven states: A, B, C, D, E, F andG. A is the states of the chromosomes fixed without pretreatment. Chromosomes are observed in the "bulk stained" condition. In B state minor coils expand revealing their minute structure. The minor matrix, losing stainability, swells to give a wide diameter to minor coils. Major coils are more or less enlarged in width but not in lengh. The swelling of the major matrix is small and it faintly retains stainability. In C state minor coils can hardly be seen being embedded in the deeply stained and contracted minor matrix. Major coils represent themselves in a orderly spiral form. Major matrix swells in width and lengh and becomes unstained. In early D state minor coils are relaxed to reveal themselves as fine threads. Both minor and major matrix swell and become completely unstained. Major coils are unravelled to some extent. The spiral structure attains to the finest demonstration at the end of this state. But very soon minor coils lose their clearness. In late D state minor coils become obscure in their outlines. Major coils are unravelled and deformed more or less in compliance with the degree of the swelling of major matrix. In E state minor coils become indistinct being packed again in the re-contracted minor matrix, which recovers stainability. Major coils modify to retain the orderly spiral form. The major matrix shrinks again and recovers stainability a little. In F state minor coils are destroyed and remain as irregular zigzag lines in minor matrix which swells once more and becomes unstainable. The major matrix swells again and loses stainability. In G state both major and minor coils are destroyed and chromosomes are damaged leaving irregularly collapsed chromatin masses (Plate XVI, Figs. 1–6). In Table 5, the chromosome configurations demonstrated with the agents are summarized according to the seven states as described above.

In 30 percent of the PMCs in the preparation with re-distilled water for 30 sec. chromosomes attained to B state. According to SAKAMURA ('27) the anther juice of *Tradescantia* is an alkaline solution with remarkable buffer action. This is also the case of *Trillium* and the pH is about 7.4. The pH of the PMCs of several plants was measured by YAMAHA ('35) and the values found to be much smaller than this (for example, the pH of the PMCs at 1st meiotic metaphase of *Tradescantia* varies from 4.2 to 4.6). Such a difference of hydrogen ion concentration between the PMCs and the surrounding medium can be retained in living state in anthers, but on the glass slide it may not. The hydrogen

ion concentration in the PMCs must, then be decreased. So the alkaline reactions occurring in the PMCs on the glass slide is one of the causes for the manifestation of B state. On the other hand the change of redoxpotential is another cause for it, because there is difference between the results obtained with glucose solutions and re-distilled water (as to the further evidence for this, see the next chapter).

After 60 sec. pretreatment with re-distilled water, 70 percent of the PMCs were in C state. The contraction of minor coils is brought about by the oxidation due to the oxygen in the air. With solutions having reducing action such as nitrites and glucose, and in anaerobic conditions (see the previous and next chapter), C state could not be brought about.

TABLE 5

The state of chromosome configuration demonstrated with re-distilled water and glucose solutions

Pretreatment Agent	Concentration in Mol	Time of Pretreatment in Sec.					
		30	60	120	240	480	960
Re-distilled Water	0.00	A(B)*	C(A)	D	E	F	G
Glucose	0.01	B(A)	B(A)	C(B)	D	D	FG**
	0.04	B(A)	B(A)	D(B)	E	F	E
	0.10	B(A)	B(A)	D	D	D	E
	0.40	B	D	D	D	D	E

* When a larger part of the PMCs in a preparation had chromosomes in one state and a smaller part of them had those in another, the capital letter for the latter is put in parentheses.

** When nearly equal proportions of the PMCs in a preparation had chromosomes in several different states, the capital letters for them were written alined.

After two minutes treatment with re-distilled water, D state was attained in all the PMCs in the preparation. At the beginning of this state, detailed spiral structure is demonstrated. But very soon minor coils become obscure and chromosomes undergo great swelling which is caused by hydration. With glucose solutions more concenfrated than 0.02 mol, such swelling of chromosomes does not occur and they stay in D state until they shift to E state (see below).

Pretreated with re-distilled water for four minutes E state was attained by the chromosomes. Major coils retain the orderly spiral form, but the fine structure of minor coils is no longer seen. Matrices

re-contract and recover their stainability. The contraction is brought about not only by the oxidation in aerobic condition but also by some other unknown factors, because E state was also attained in anaerobic conditions (see description of first and the third experiments performed in anaerobic conditions). In this connection it may be noted that E state is seen only when the pretreatment agents contain few electrolytes. With hypertonic glucose solutions this state is attained the easier the greater the concentrations are. With concentrated salt solutions and with ammonia, the destruction of chromosomes occurs so soon that E state cannot be reached.

After eight minutes treatment with re-distilled water, F state was attained. Minor coils are destroyed, but major coils retain the orderly spiral form. The swelling of chromosomes is due to the hydration of matrices which become again unstained. With glucose solutions more concentrated than 0.02 mol the transition to this state from E state could not be observed even after long duration of pretreatment. In such media lacking in electrolyte, the quantity of free water in cytoplasm seems to determine the degree of the swelling of chromosomes.

After sixteen minutes with re-distilled water, more than half of the PMCs had chromosomes in G state. There occurs a destruction of chromosomes due to overhydration and coagulation. With glucose solutions more concentrated than 0.02 mol this state was attained after a very long pretreatment succeeding to E state. The chromosomes were then contractedly collapsing.

During every pretreatment made on the glass slide in the previous experiments such changes in chromosome as mentioned above associated with those due to the factors which were chosen to be investigated exerted a combined influence upon the transformations of chromosome configurations. However, these factors were sufficient determinants for the reactions induced, because they were so conspicuous as to cover the contaminating reactions.

V. Examination of the Alkaline and Reducing reaction in minor coils

As mentioned above, B state is revealed by the alkaline and reducing reaction. If so, the disturbance of these reactions must result in acceleration or suppression of the manifestation of B state. Several tests were carried out to examine how the manifestation of B state

is disturbed by additional factors.

The acceleration and suppression of alkaline reaction were induced with ammonium hydroxide and acetic acid respectively. The results are shown in Table 6. The concentration of these agents was 0.04 N. With ammonium hydroxide the chromosomes in more than half of the PMCs were in B state after 30 sec. pretreatment. With acetic acid, even after 6 minutes the chromosomes were still in A state.

The acceleration and suppression of reducing reaction were induced with potassium nitrite, potassium nitrate, and hydrogen-peroxide. With 0.04 mol potassium nitrate, the chromosomes were in A state for as long as 4 minutes continuation of the pretreatment, then C state was attained. With 0.04 mol potassium nitrite, B state was attained after 30 sec. The addition of one drop of 3% H_2O_2 in 10 cc. of re-distilled water was enough to suppress the manifestation of B state. When the solution was diluted twice with re-distilled water, C state was attained in half of the PMCs in the preparation, omitting B state, after one minutes pretreatment (Table 6).

TABLE 6

Configuration of chromosomes* obtained after pretreatment of 30, 60, 120 and 240 sec. The test with NH_4OH and CH_3COOH, and that with KNO_3, KNO_2, oxyfull and re-distilled water were carried out with the PMCs of one and the same plant respectively

Agent	Concentration	Time of Pretreatment in Sec.			
		30	60	120	240
NH_4OH	0.04 N	B(A)	D(C)	D	G
CH_3COOH	0.04 N	A	A	A	A
KNO_3	0.04 mol	A	A	A	C
KNO_2	0.04 mol	B(A)	C(A)	CD	E
One Drop of H_2O_2 Added in 10 c.c. of Re-distilled Water		A	A	A	A
Re-distilled Water		A(B)	A(BC)	AC	CD

* The seven stages division of the states of chromosome configurations: A, B, C, D, E, F and G, made in the previous chapter is also applied here.

As indicated in the foregoing experiment with hypo- and hypertonic salt solutions C state is attained, omitting B state, directly after A state. When the concentration of a salt-solution was greater than a certain value, the transformation of the chromosome configuration for the demonstration of spiral structure was suddenly suppressed and the

chromosomes remained in A state until they gradually convert themselves into C state, omitting B state. The marginal or the minimum concentration to cause the omission of B state was searched for with various chemicals.

Pretreated with 0.02 mol glucose for 60 sec., the PMCs of one plant had chromosomes in B state. Utilizing the PMCs of this plant, the minimum concentrations were measured with seven chlorine compounds dissolved with 0.02 mol glucose solution (Table 7). Pretreated with 0.01 mol glucose solution, the PMCs of another plant had chromosomes in B state. With three sodium-compounds dissolved in 0.01 mol glucose solution, the minium concentrations were searched for in the latter material (Table 7).

TABLE 7

Minimum concentration for the omission of B state with metal-salts. The relative value of the concentration and the standard single elctrode potentials of the metals mesured against the standard hydrogen electrode are written in the third and the fourth columns respectively

Name of Chemical	Minimum Concentration in Mol	Relative Value of the Concentration	Single Electrod Potential (V)
KCl	0.036 — 0.037	100	− 2.9224
NaCl	0.027 — 0.028	76	− 2.7125
$CuCl_2$	0.0023 — 0.0025	6.6	+ 0.3448
$HgCl_2$	0.0023 — 0.0025	6.6	+ 0.807
$CaCl_2$	0.0014 — 0.0016	4.1	− 2.81
$AlCl_3$	0.0014 — 0.0016	4.1	?*
HCl	0.00022— 0.00024	0.63	0.0
NaCl	0.033 — 0.034	100	
Na_2CO_3	0.020 — 0.021	60	
$Na\,SO_4$	0.017 — 0.018	53	

* The S. E. P. of Aluminium against the H-electrode is not known because of the small difference.

What is the quality of these agents responsible for the omission of B state ? The difference of hydrogen ion concentration among the agents is one of the causes of the omission. The minimum concentration of hydrochloric acid is 0.63/100 of that of potassium chloride. Both calcium and aluminium chloride give slight acidic reaction to the PMCs, because the alkaline buffer action of anther juice may suppress the dissociation of $Ca(OH)_2$ and $Al(OH)_3$. So that the difference of the

minimum concentration between KCl, NaCl and $CaCl_2$, $AlCl_3$, without any correspondence to the potentials of these cations (see the fourth column in Table 7), may be regarded as responsible for the acidic reaction by the latter upon the PMCs. Why the effects of the minimum concentration differ conspicuously between KCl, NaCl and $CuCl_2$, $HgCl_2$, is attributable to the difference in the combining affinity among these cations with the electron (*cf.* note on the standard single electrode potentials of these cations written in Table 7).

With three sodium salts it was found that the anions in the agents have no effect on the omission of B state. The ratio of the minimum concentration of NaCl, Na_2CO_3 and Na_2SO_4 was about 2 : 1 : 1, indicating that the effects are determined by the activity of the cations, with which the chromonemata interact.

Discussion

The morphological investigation of spiral structure in chromosome has been one of the most interesting problems in cytology. Many investigators have agreed that chromosomes in division stage comprise chromonemata coiling into spirals of several orders and matrices embedding the former. As for the mechanism of spiralization, several explanations have been offered presuming various mechanical forces, some of which are working from chromonemata and others from matrices, canceling and balancing each other to maintain the regular spirals (KUWADA '35, '39, MATSUURA '40, '41, etc.). These interpretations are made for the purpose of explaining the cytological behavior of chromosomes. DARLINGTON introducing in his earlier work the "molecular spiral" theory ('35), attempted to explain the spiralization cycle in chromosome at the molecular level (DARLINGTON and MATHER '49). Several other investigators also gave models of molecular patterns in chromosomes (WRINCH '36, WADDINGTON '39, etc.). Such tentative explanations, though hypothetical, are of value connecting the molecular and cytological studies about the chromosome structure. However, to attain complete understanding of the matter, much more must be learned about the cyto-genetical behavior, physio-chemical structure and biochemical function of chromosomes.

In the present study, an attempt was made to obtain some knowlege about physico-chemical forces which may play in the retainment of the spiral structure of chromosomes. The matter is concerned chiefly with

the attracting forces among the microscopical constituents of chromosomes, *i.e.*, the minor and major spiral threads and the matrices of them.

According to the FREY-WYSSLING's "Haftpunkttheorie", the chains of protein molecules in protoplasm are connected with each other at the "haftpunkt" where the free side chains of them enter in cohesional bindings (FREY-WYSSLING '37, pp. 118–121). In chromosome micell there are free side chains of nucleoproteins and other high molecular substances. Among these side chains the cohesional bindings are formed, which are contracted or released, in the above experiment, due to the changes of conditions induced by the pretreatment. The transformation of chromosome configuration observed during and after these changes, however, is not due to the alteration in side chains alone, but to the total result of the reactions of the chromosomes to the induced changes. So that if it is desired to compare the degree of the contraction or relaxation of the cohesinal bindings at the side chains, each set of tests must be carried out in a comparative way choosing suitable determinant factors respectively.

In the first experiment, the influence of aqua solutions of salts upon the time of pretreatment for the demonstration of spiral structure in chromosome was investigated. Here we are certainly concerned with the heteropolar cohesion bindings in chromosomes, the dipole radicals employed in the bindings being masked by the ions in the agents. What constitutes the determinant influence upon the masking and how it occurrs can be judged from the mode of the alterations in the time of pretreatment for the demonstration with each series of the agents employed. The results obtained here, together with the mode of the swelling of chromosomes observed in the experiment with salt solutions indicate that there is a clear difference in the distribution of dipole radicals among the constituents of chromosome micell. The spiral thread, or chromonema, seems to have excess of negative electric charges whilst the substance embedding it or matrix, is charged positively. The negative charges of chromonema should be attributable to the dissociations of deoxiribonucleic acids which become deposits upon chromonema at prophase. It may be a natural sequence that positively charged substance becomes attached around chromonema to form matrix.

The existence of matrix has been admitted by many cytologists (KUWADA '39, MATSUURA '41, etc.). Several investigations have been made to indentify it under the microscope (FUJII and YASUI '36, SHINKE and

SHIGENAGE '33, etc.). In the present investigation also its existence was realized as having connection with the mode of the swelling of chromosome. The independence of the swelling in chromonema itself and chromosome as a whole, and the fact of the unravelling of major and minor coils cannot be explained unless one assumes the presence of matrix which combines with chromonema. Very little is known about the physico-chemical nature of matrix. Whether it is possible to distinguish the matrical substance from chromonema by chemical analysis is a problem for the future. However, it seems proper to admit its existence regarding it as a substance forming intimate cohesional bindings with chromonema and being separated from the rest of protoplasm by an interfacial membrane. Its existence is substantiated by the experiments of PEACE ('46).

In the second experiment some series of tests were performed with various pretreatment agents and their influence upon the configuration of spiral structure in chromosome was examined. Here again the changes in chromosome configurations represented clearly the mode of the interactions between the molecules in the agents and the radicals existing in cohesional bindings in chromosome.

In the third, fourth and fifth experiments, critical examinations of the tests carried out in the foregoing experiments were made. It was found that various kinds of cohesional bindings are responsible for the maintenance of the spiral structure. Impossible as it was to show in detail how these bindings distribute in the chromosomes, it seems probable that there are some sort of regular rearrangements of radicals responsible for the cohesional bindings of each kind in the chromosomes.

The assumption that chromosomes are composed of chromonema and matrix which combines with the former by cohesional bindings of various kinds being arranged in some regular manners seems to explain fairly well the micellar structure of chromosomes. However, this assumption affords no exact knowledge upon the molecular structure of them, the chemical reaction which may occur in chromosomes during the pretratments being left unexamined. But the present author's results have superiority for the practical application over the preparation method in cytological observations of meiotic chromosomes in the PMCs. Applying various kinds of chemicals or their combinations as the pretreatment agents for the demonstration of spiral structure, it is possible to change, to some extent, the length and diameter of chromosome and spiral thread, and the number of major and minor coils. This method

can be applicable for the PMCs of every plant to which MATSUURA's water pretreatment method is effective (MATSUURA' 37).

In finishing the paper, the writer wishes to express his cordial thanks to Professor HAJIME MATSUURA. Under his kind guidance and criticisms the present investigation was carried out.

Summary

In the present investigation, the effects of chemicals upon the configuration of spiral structure in chromosomes were examined with 1st meiotic metaphase chromosomes in the PMCs of *Trillium Kamtschaticum* PALL. The obtained results are summarized below.

1. The relationship between the concentration and the time of the pretreatment as influencing the demonstration of spiral structure in chromosomes was investigated utilizing aqua solutions of salts as pretreatment agents.

Pretreated with solutions of monovalent-ion-salts hypertonic to the PMCs, the demonstration can be performed because of the masking of cohesional bindings in matrix by the anions in the agents. With solutions of bivalent-cation-salts it is possible because of the discharge of chromonemata by the cations. Accordingly, in these cases the time of the pretreatment is prolonged with the decrease of the concentration of the agents.

Pretreated with hypotonic solutions the demonstration occurs because of the hydration by the water molecules in the PMCs. The supply of water is decreased with the increase in concentration of the pretreatment agents. Accordingly in this case the time of the pretreatment is prolonged with the increase of the concentration.

2. The influence of various pretreatment agents upon the swelling of chromosomes was examined. With salt solutions the swelling occurred after the lyotropic series. In this case the matrix behaves as the positively charged substance and chromonemata as the negative one. The unravelling of the minor coils was influenced conspicuously by the solutions causing the change of hydrogen ion concentration and the redox potential in the PMCs.

3. Investigations were made upon the change of chromosome configurations as observed in anaerobic conditions. The PMCs were pretreated in the anther. To make the penetration of the agents through the anther wall easy, most of the pretreatments were carried out under

low pressure.

In anaerobic conditions and under low pressure, the swelling of major matrix is inhibited by the decreased supply of electrolytes and the increased rigidity of the matrix. So that a fine demonstration of spiral structure in chromosome is attained there only when special means are taken to make the swelling of major matrix become sufficient in the course of time.

4. The PMCs were pretreated on the glass slide utilizing redistilled water and glucose solutions as the pretreatment agents.

After the PMCs are pushed out on the glass slide the equilibrium having existed in the anther transforms itself, passing through various transitional alterations, to another one. Any change of conditions during the transition exerts influence upon the chromosomes inducing visible changes in their configurations. As the primary event, alkaline and reducing reaction occur chiefly under the influence of anther juice to relax minor coils. Soon afterwards oxygen in the air begins to manifest its oxidizing action, which recontracts minor coils. Later, various irreversible alterations are seen due to denaturation and coagulation of protoplasm. The chromosomes fixed in the course of this transition after the various times of continuations of the pretreatments show respectively characteristic figures which are explicable as synthetic resultants of the inter-actions of chromosome and the surrounding medium.

5. In the fifth experiment, examinations were made about the acceleration and suppression of the relaxation of minor coils with various agents. The relaxation in minor coils was quite sensitively influenced by the hydrogen ion concentration and redox-potential of the pretreatment agent.

Synthesizing the above findings, it was assumed that the metaphase chromosome is composed of chromonemata and matrices, the side chains of the substances composing them forming cohesional bindings of various kinds. The demonstration of spiral structure in chromosomes may be performed due to the masking of these side chains before the fixation of the cytological preparation.

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Explanations of the Plates

Plate XIV.

The figures (Figs. 1–10) represent the effect of the pretreatment with various salt solutions on the chromosomes at MI of the PMCs of *Trillium kamtschaticum*. The solutions used here are as follows:

1.	$NaNO_3$	0.353 mol.	2.	NaCl	0.339 mol.
3.	KSCN	0.376 mol.	4.	KNO_3	0.365 mol.
5.	KCl	0.333 mol.	6.	CH_3COOK	0.376 mol.
7.	NH_4NO_3	0.368 mol.	8.	NH_4Cl	0.350 mol.
9.	$Ca(NO_3)_2$	0.080 mol.	10.	$CaCl_2$	0.073 mol.

In each case, the duration of pretreatment was about 50 sec., and after that the usual aceto-carmine method was employed. Each photograph ($\times$ 1000) represents the "average" figure, namely, that most frequently met with. In order to avoid a possible individual difference in the configuration of chromosomes, these figures were all taken from one and the same plant.

The degree of swelling of chromosomes is in the following order:

$NaNO_3 > NaCl$ (Figs. 1 and 2),
$KSCN > KNO > KCl > CH_3COOK$ (Figs. 3–6),
$NH_4NO_3 > NH_4Cl$ (Figs. 7 and 8),
$Ca(NO_3)_2 > CaCl_2$ (Figs. 9 and 10) and
$NaCl < KCl$ (Figs. 2 and 5).

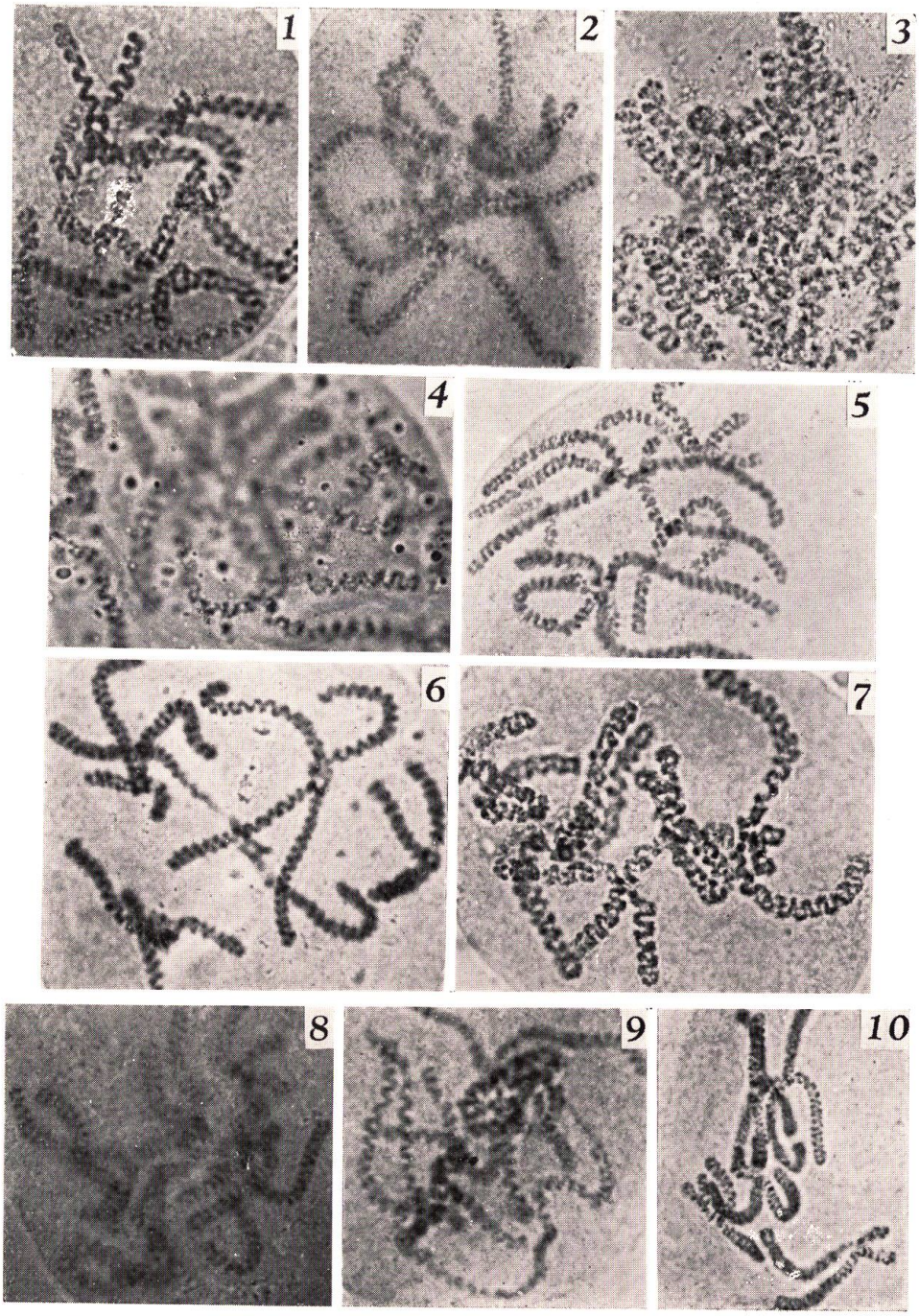
Plate XV.

The PMCs in Figs. 1–3 are taken from one and the same plant pretreated with (1) NH_4OH 0.353 N. + KOH 0.031 N. for 40 sec. (2) NH_4OH 0.519 N. for 50 sec., and (3) NH_4OH 0.353 N. + HCl 0.031 N. for 65 sec. respectively.

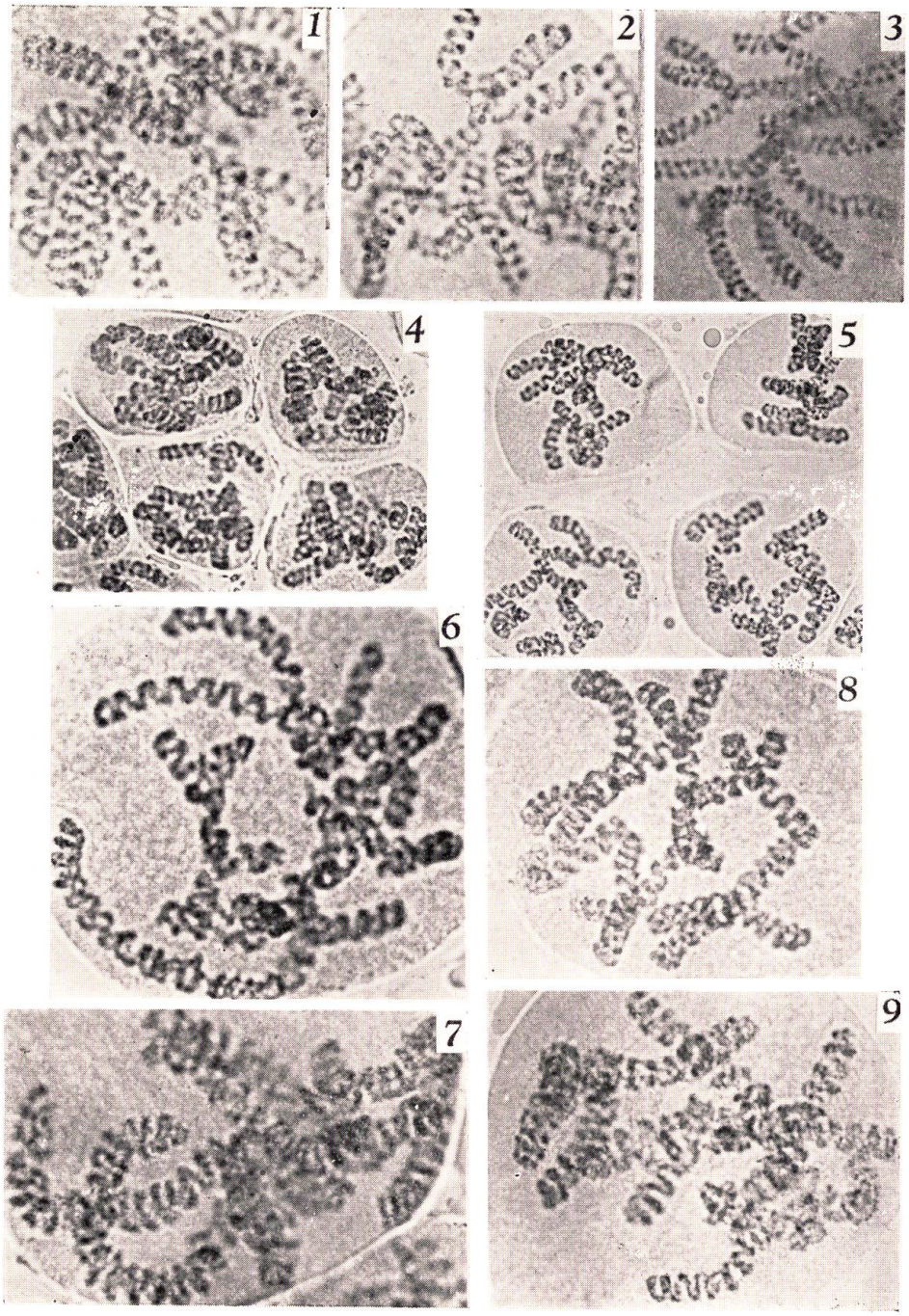
The PMCs in Figs. 4 and 5 are taken from one and the same plant and those in 6–9 from another one. (4) KSCN 0.39 mol, 50 sec. (5) KSCN 0.39 mol, 50 sec. + 1/20 H_2O_2, 7 sec., (6) KSCN 0.136 mol + KNO_3 0.227 mol, (7) KSCN 0.136 mol + KNO_2 0.227 mol, (8) NaSCN 0.136 mol + $NaNO_3$ 0.227 mol, (9) NaSCN 0.136 mol + $NaNO_2$ 0.227 mol. The time of pretreatment was 50 sec. in (6–9). In Figs. 6–9, the volume of chromosomes is larger with potassium salts than with sodium salts, but the chromonemata are more slender with the former than with the latter. (Figs. 1–3, 6–9: $\times$ 1000. 4 and 5: $\times$ 476).

Plate XVI.

The figures represent the effect of the pretreatment carried out in aerobic (Figs. 1–6; × 476) and anaerobic (Figs. 7–9; × 1000) condition. (1) Chromosomes in B state, pretreated with 0.04 mol glucose solution for 30 sec. Unravelled minor coils are seen embedded in faintly stained major matrix. (2) C state with re-distilled water for 60 sec. Minor coils are more compact than those in B state. (3) D state with 0.4 mol glucose for 120 sec. PMC in the left side is at the end of early D state. Minor coils are clearly seen. They are slender being dehydrated by the hypertonic solution. The one in the right side is in late D state. Swelling of chromosomes and deformation of spirals are advanced. (4) E state, with 0.1 mol glucose for 120 sec. Chromosomes contract. Major and minor matrix recover the stainability. (5) F state, with 0.01 mol glucose for 960 sec. Spiral structure is again demonstrated, but minor coils are destroyed. (6) G state, with 0.01 mol glucose for 960 sec. Spiral structure is deformed into irregular zigzag in the left PMC, and at last chromosomes are broken down into vacuolized chromatin masses in the right PMC. (7) PMC kept in 0.75 N NH_4OH for 40 min. under 1 atm. Major matrix is stained faintly and something as the shadow of major coils is seen. The chromosomes are in B state. (8) and (9) PMCs taken from one and the same anther kept in 0.75 N NH_4OH for 15 minutes under low pressure in the "Thumbergrohr" and then kept there for 15 min. further being gently heated. Chromosomes in (8) represent the incompletely demonstrated spiral structure (B state). Expansion and relaxation of minor coils are conspicuous in the swollen major matrix. A fine demonstration is seen in chromosomes in (9).



M. Kurabayashi; Effects of chemicals upon chromosomes



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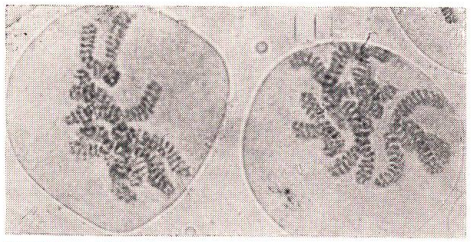

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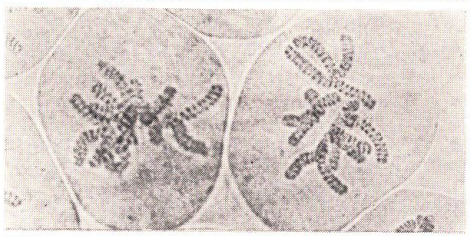

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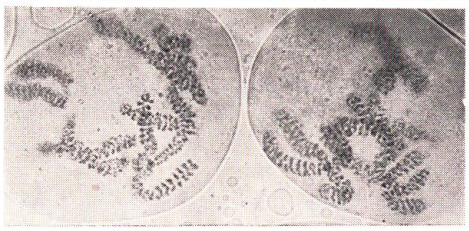

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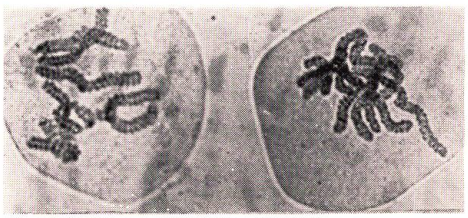

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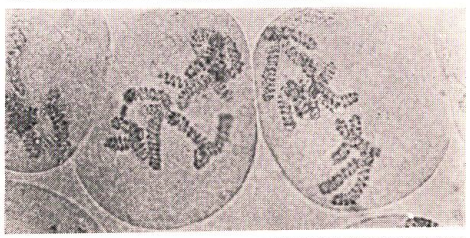

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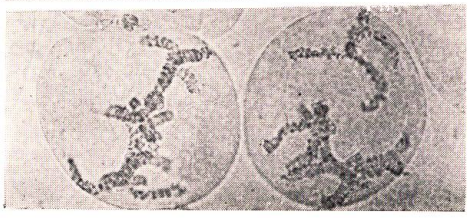

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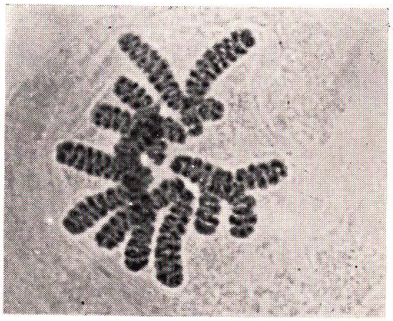

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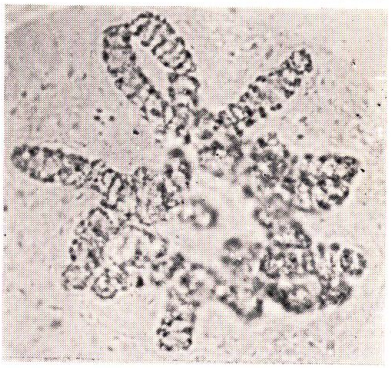

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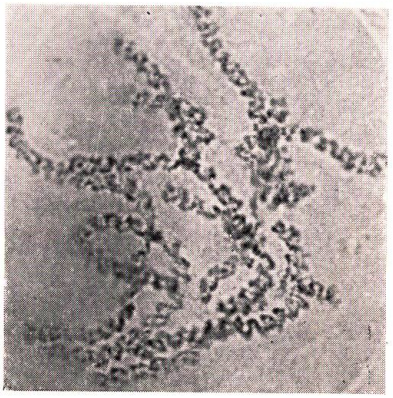

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