Influence of extreme temperatures on mitosis in vivo I. *Hymenophyllum*

by

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Introduction

In this paper further experiments on the influence of shock temperatures on the different stages of mitosis in *Hymenophyllum* in vivo are reported. In the previous paper (B a j e r and M o l è - B a j e r 1952) the authors were interested in the influence of a low and a high temperature applied in violent changes. It appeared that both a low and a high temperature cause numerous effects on cell division. These were described in detail. It was noticed, that if a shock from room temperature to 42^0 — 45^0C was applied in late anaphase, the distance between anaphase chromosome groups increased i. e. the distance traveled by anaphase chromosomes is longer than the spindle length in metaphase (sometimes twice as long). In that work also the course of normal division was carefully observed. From measurements it can be seen that the separation of chromosome groups in anaphase is approximately equal the spindle length in metaphase. The mitotic spindle in living *Hymenophyllum* cells is well visible, and it seems probable that it is enveloped by a membrane similar to that observed by W a d a (1941, 1950) in *Osmunda japonica* and staminal hairs of *Tradescantia*.

In our previous paper the temperature was often changed during shocks, i. e. it was raised or lowered. Exact temperature limits within which these effects might be observed were not determined.

The present paper deals mainly with high temperature shocks and an attempt at establishing the temperature limits within which the anaphase way of chromosomes is elongated. Special notice was given to the influence of the rapidness with which temperature changes, and the time high temperature acted.

Material and methods

As material young *Hymenophyllum tunbridgense* Sn. leaves were used. Methods of handling material are described by Heitz (1942). Apparatuses and methods of applying shock temperatures, were described in detail in our previous work (Bajer and Molè-Bajer 1952). The same methods were used here. Observations were done in liquid paraffine (there is no difference between the effects observed in liquid paraffine and tap water) in Gautheret hanging drop on a special microscope table. The temperature of the drop during shocks was 30⁰—62⁰C, and at 45⁰C was controled with a thermocouple. The difference in temperature between the drop and readings of the thermometer were less than 0,5⁰C just after the shock started and steadied at 0,1⁰C after several minutes. In most cases the temperature was raised within 1—1,5 minutes.

Observations

Influences of shock temperatures (30⁰—62⁰C) on all stages of mitosis were examined. Temperatures up to 35⁰C are not harmful in metaphase and the earlier stages. The influence on metaphase of a temperature of 44⁰—47⁰C was previously described (Bajer and Molè-Bajer 1952). Such temperatures cause an elongation and a more pointed ending of half spindles. Higher temperature (up to 55⁰C) cause either similar effects or, a coagulation of cells. In temperatures above 55⁰C, usually after several minutes already, the spindle coagulates. The higher the temperature the quicker the coagulation, though in some cells similar effects as at 45⁰C are observed.

In metaphase and earlier stages, when shock temperatures are not too high and do not last too long, the cells recover, and the course of mitosis is either continued or restitution nuclei are formed. The cell may also recover if even a very high temperature is applied for a short time. Recoveries were observed in some cases after a 5 minutes action of a temperature of 55⁰C. There is no satisfactory explanation for the continuation of cell division in some cells and the formation of restitution nuclei in others (in numerous cases neighbouring cells from one leaf) under the same experimental conditions. Is seems probable that the stage of mitosis has here much influence on the further behaviour of the cell. It was also noticed that at a given stage each cell has a different degree of resistance to high temperatures.

Neither metaphase nor the earlier stages but anaphase was that stage in which the authors were most interested in. Anaphase was observed at room temperature (18⁰—22⁰C) in more than a hundred cells. Shock changes of temperature from 30⁰ to 62⁰C were applied in late anaphase. Chromosomes were than separated by 3—6 μ less than the spindle length. The distance of chromosome separation after shock action was carefully observed. The duration of shock action was different, and in most cases it lasted till the maximal separation of anaphase chromosome groups (in telophase chromosome groups move often towards the middle of the cell, and in consequence it is necessary to introduce the term „maximal separation").

If a temperature of 30⁰—35⁰C acts in anaphase the further course of mitosis is normal and without disturbances. The shape of the spindle and the formation of middle lamella is the same as at room temperature. Only the maximal separation of chromosomes seems to be somewhat longer than the spindle length.

The action of a temperature of 40⁰C on late anaphase has similar influence as that of 30⁰—35⁰C. Usually however, the formation of cell walls is disturbed, and begins some hours after the return of the cell to normal conditions.

In shock temperatures above 41⁰—42⁰C acting in late anaphase, the maximal separation of chromosomes is greatly elongated. Such elongation is maximal at 44⁰—46⁰C. At such temperatures the separation of chromosomes in anaphase is often twice the length of the spindle in metaphase. Outer limits of temperature in which tle elongation of anaphase chromosome separation occurs are: 42⁰C and 47⁰C, — which makes a difference of elongating temperatures only approximately 5⁰C. Mean values of chromosome separation in higher temperatures decrease, though different values in individual cases are found. If the temperature of the shocks is raised more and more, the maximal separations shorten quickly at first and more slowly as temperatures become higher. The graph of chromosome movement at room and higher temperatures are given in Fig. 1 and numerical values in Table I.

The acceleration of chromosome movement does not begin immediately after high temperature action starts, but several minutes later (5—12). The time before acceleration begins varies in different temperatures and it seems that it shortens as the shock temperature rises.

Beginning at 41⁰C the half spindles become more pointed and lengthen. This process is much quicker in higher than in lower temperatures, and at 41⁰C it lasts 20—26 mins., whereas at 50⁰C 10 mins. are sufficient.

There is a close relation between the continuation of cell division and the time the high temperature acts. Although there are differences in individual cells, mitosis is usually continued in temperatures of: 42⁰C, 45⁰C, 50⁰C, acting for not more than 1 h 30 mins., 30 mins., and 5 mins. (not in all cases) respectively. In temperature above 55⁰C, most cells coagulate irreversibly after 5—15 mins. (some survive after 5 mins. of 55⁰C).

TABLE I

Influence of high temperature action *

Stage of cell division in which shock was applied	shock temperature in °C	spindle length in metaphase in μ	Distance of max. seperation of chromosomes in μ	p
A late (15)	30	21	18	— 14
A late (23)	30	27,5	28	1,8
A late (17)	30	22	23	4
A late (18)	30	25	26	4
A late (17)	30	21	22	4,7
A late (13)	30	21	22	4,7
A late	30	20	21	5
A late (20)	30	20	24	20
A late (20)	30	22	27	22
A late (17)	35	21	21	0
A late	35	20	21	5
A late	35	17	18	5,8
A late (18)	35	20	22	10
A late (15)	40	17	16	— 5,8
A late (15)	40	22	21	— 4,5
A late (15)	40	18	19	5,5
A late	40	21	24,5	16
A late (18)	40	18	22	22
A late (18)	41	15	15	0
A late	41	21	26	24
A late (21)	41	19	26	37
A late (17,5)	41	18	28	55
A late (19)	41	19	30	57
A late (18)	42	17	15	— 11
A late (18)	42	15	16	7
A late (17)	42	22	25	14
A late	42	19	23	21
A late (18)	42	15,5	20	30
A late (19)	42	19	25	31
A late (23)	42	16	21	31
A late (20)	42	18	24	33
A late (20)	42	16	24	50
A late	43	15	22	46
A late (14)	45	21,5	24	17
A late (18)	45	22	28	27
A late (14)	45	19	25	29
A late	45	19	25	31
A late (15)	45	22	29	32
A late (25)	45	22	31	41
A late (18)	45	17	37	58
A late (19)	46	18,5	40	116
A late	47	19	22,5	18
A late (27)	47	18	23	27

* p—explanation in text;

A—anaphase; time from anaphase beginning in mins. given in brackets; 88 cells from 211 observed.

TABLE I (continued)

Stage of cell division in which shock was applied	shock temperature in °C	spindle length in metaphase in μ	Distance of max. seperation of chromosomes in μ	p
A late (15)	47	17	23	29
A late (17)	47	16	22,5	34
A late	47	18	26	44
A late	47	18	28	55
A late	47	17	28	64
A middle (13)	48	20	24	20
A late (20)	48	19	23	21
A middle (12)	48	21	26	23,8
A late (15)	48	20	25	25
A late (18)	48	17	22,5	32
A late	48	22	30	36
A late (32)	49	18	25	33
A late (19)	49	23	40	76
A late (21)	50	17	16	— 5
A late (22)	50	20	19,5	— 2
A late (27)	50	17	17	0
A late (19)	50	15	15	0
A late (20)	50	16,5	17	3
A late (20)	50	20	23	15
A late (17)	50	17	21	23
A late	50	25	37	48
A late (18)	50	18	27	50
A late	51,5	20	25	25
A late (20)	52	15	20	33
A late	52	20	27	35
A late (23)	53	19	16	15
A late	53	17	17	0
A late (22)	53	16	19	18
A late	53	14	17	21
A late (22)	53	19	27	31
A middle (13)	53	21	30	42
A late	54	19	21	10
A late (23)	54	18	24	33
A middle (13)	56	17	14	— 17
A late (25)	56	16	14	— 12
A late (18)	56	19	16	— 15
A late (20)	56	14	13	— 7
A late (26)	56	17	17	0
A late	59	19	17	— 10
A late (21)	59	20	20	0
A late	59	17,5	18	2
A late (19)	59	19,5	21	7
A late (22)	60	16	19	24
A late	61	18	17	— 5
A late (23)	61	19	19	0

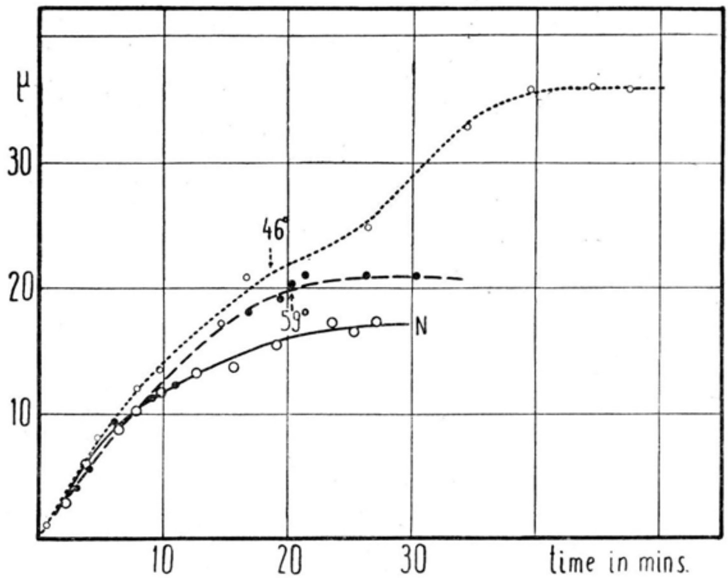

Fig. 1. Graphs of chromosome movement. Distance between anaphase chromosome groups plotted against time. Arrows indicate beginning of high temperature action. N — normal division at 20°C.

In cases when a cell recovers cell division is not continued immediately, but after several hours (in some cases 7—12 hrs.).

In numerous cells high temperature causes distribances in cell wall formation, and often after long acting temperatures above 47°C, cell walls do not form.

Discussion

In *Hymenophyllum* cells, in which the way of anaphase chromosomes is elongated as a result of high temperature action, both the course of anaphase and the graphs plotted for chromosome movement are similar to the anaphase and the graphs in animal cells. In animals the half spindles shorten in anaphase, and also in most cases there is an activity of the spindle parts between chromosome groups i. e. pushing body („Stemmkörper" of Bělař 1929). These two processes may be either separate in time or simultaneous.

In *Hymenophyllum*, in lower temperatures (up to 44°C), the half spindles shorten and the distance between the half spindles increases. In higher temperature the action of half spindles ceases. The mechanism of the increasing of chromosome separation is not fully understood. The photographs given in our previous work (Bajer and Molè-Bajer **1953**, flattening of chromosome groups in late anaphase) indicate that the elongation of chromosome way is due to a force acting towards the poles. Similar facts were observed by H e i t z (1943) in root tips and explained by him as the consequence of „Stemmkörper" action. However the pushing body action in plants is not fully known and was questioned (cf. R i s 1943) as most evidence in its favour is not convincing. However in some cases there is no doubt that strong pushing body action exists in plants, e. g. in *Haemanthus* endosperm where spindles and cells elongate in anaphase (B a j e r 1953 a, b). In *Haemathus* active pushing body is liquified in comparison to the half spindle (viscosity in poises: pushing body — 0,12, half spindle in metaphase — 0,26, and much higher during anaphase; measurements parallel to spindle length). Low viscosity of active pushing body was also confirmed by C a r l s o n's (1952) micrurgical experiments in neuroblast of grasshopper. There arises the question whether in *Hymenophyllum* the elongation of maximal separation in anaphase is comparable to pushing body action, and what is its mechanism. As such elongation begins within narrow limits of temperature (41⁰—42⁰C) this indicates that such temperature liberates factors, which are not understood sofar. E. g. it seeems that if the liberated factors were corelated with enzymes then the shape of the curve in Fig. 2 would be different. If the viscosity between the parts of chromosome groups could be measured in *Hymenophyllum* it would be possible to say whether the mechanism is here similar to pushing body action in other objects.

It is intersting to examine the dependence between the values of shock temperatures and the elongation of anaphase separation (Fig. 2, Table I). Observations show that elongation is dependent on: 1) temperature of shocks; 2) cell length, and mechanical conditions in the cells; 3) stage of anaphase (elongations occur in most cases if shocks are applied in late but not too late anaphase); 4) the time during which the temperature changes i. e. a high temperature (above 55⁰C) may have a similar effect as lower temperatures (e. g. 45⁰C) if it rises from room temperature within several minutes; 5) spindle length. To eliminate the 3-rd and the 5-th factor, only those cells, on Fig. 2 on which the shock temperature acts in similar stages, are considered. The elongation is expressed in percentage, i. e. the difference between the chromosome separation and the spindle length as the percentages (p) of spindle length (s) is obtained from the formula:

$$p = \frac{d - s}{s} 100$$

where:

d = maximal separation of chromosomes
s = spindle length in metaphase

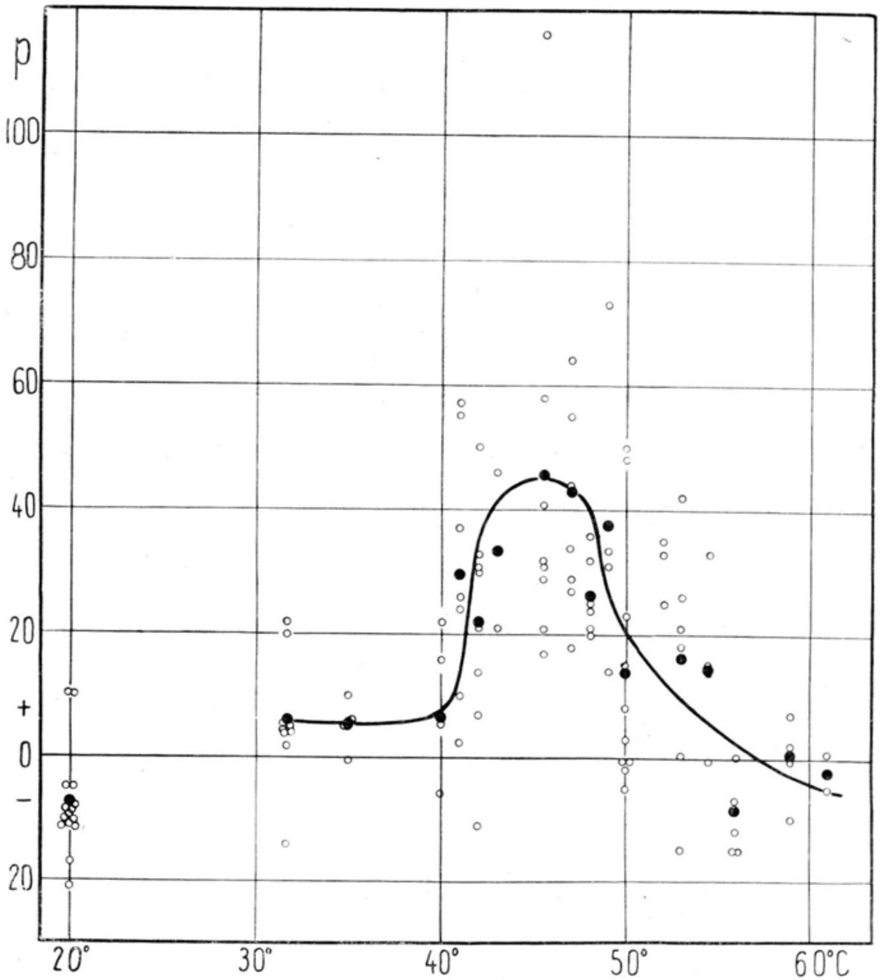

Fig. 2. Elongation of anaphase chromosome separation in dependence on temperature. White small circles — individual measurements, large black — mean values. In all cases temperature was raised in less than 1,5 min. Explanation in text.

The value of p is positive if separation is longer than the metaphase spindle, is zero if the separation is the same as the length of metaphase spindle, and is negative if the separation is shorter than the spindle length. On Fig. 2 the factor, which causes the great dispersion of individual measurements, i. e. the cell length, is not eliminated as its action is the same in all temperatures where such elongations are observed. The curve on Fig. 2 is plotted according to mean values. From the shape of this curve the following 4 conclusions may be drawn. 1) Temperatures 30^0—40^0C cause similar elongation in comparison to separation at room temperature. 2) Chromosomes begin to separate more than the spindle length in very narrow limits of temperature (no more than 2⁰C). This seems to indicate that changes referring most probably to submicroscopical level of live proteins begin at this temperature. 3) The greatest maximal separation is attained within limits of about 5⁰C only. 4) The elongation ceases when the temperature is increased. The curve of elongation diminishment in high temperatures (right part of the curve in Fig. 2) sinks more slowly than it rises in temperatures 40⁰—43⁰C. The shape of this curve is most probably influenced greatly by coagulation of proteins which depends on temperature.

The authors think that the present data are unsufficient to explain the shape of this curve and the mechanism of elongation of chromosome separation. Probably changes in live proteins are here of importance, but so far the properties of live proteins are not known sufficiently. It is known that viscosity of proteins decreases in a consequence of hydration caused by high temperature action (H a u r o w i t z 1950) but it is not known whether this occurs also in live cells.

A k n o w l e d g e m e n t s

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S U M M A R Y

Influence of shock temperatures (30—62⁰C) on different stages of mitosis in *Hymenophyllum* was observed. Influence on late anaphase was most exactly examined. It appeared that if temperature shocks are applied in late anaphase:

1. In temperature 30—40⁰C anaphase chromosome separation lengthens only slightly in comparison to anaphase in lower temperatures.
2. In 41—43⁰C separation of anaphase chromosome increases. The maximal greatest separation is observed within temperatures 42—47,5⁰C.
3. In temperatures above 47⁰C the maximal separation decreases with the increase of temperature and the higher the temperature the smaller the chromosome separation.
4. In temperature above 55⁰C cells begin to coagulate after 5—15 minutes.

The resistance of different cells to high temperature action differed and observations were made on the return to the normal stage of cells in mitosis after different time of high temperature action.

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