Absolute viscosity and living mitotic spindle structure

by

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I n t r o d u c t i o n

The importance of viscosity problems in cell division is indicated by: 1. studies made by different methods which prove the change of viscosity coefficient of cytoplasm in the different stages of mitosis and meiosis (C a r l s o n 1946, H e i l b r u n n and W i l s o n 1948 and others), 2. experimental decreasing of viscosity in dividing cells caused by K ion action, which accelerates the movement of anaphase chromosomes, or if the viscosity is further decreased, the movement is retarded and even stops altogether (M o l è - B a j e r 1951, 1952). Values of viscosity coefficient of the spindle are so far unknown, though most of the experiments seem to show that the spindle is a rigid body. It is also probable that in some objects the spindle is rigid, while in others it is more or less liquefied, which was demonstrated by C h a m b e r s (1925) in his micrurgical studies.

In the course of the present work the writer was interested in changes of absolute viscosity of the spindle and pushing body („Stemmkörper“ of B ě l a ř 1929 a) in metaphase and anaphase and the structure of the spindle.

M a t e r i a l a n d m e t h o d s

As a material endosperm of *Haemanthus Katherineae* Bak. was used. As a medium 0,5 percentage agar in 6—7 percentage saccha-

rose or 3 percentage glucose solution was used (details on the methods of handling material are given in my previous work Bajer 1952).

For observations Zeiss phase contrast equipment was used (oil immersion lens $90\times$, eyepieces: $10\times$ and $20\times$). Heat rays were checked by means of a 20 percentage Mohr salt water solution.

Observations were carried out at 18° — 22°C .

Pekarek's method (1931) for measurements of viscosity coefficient was applied. Viscosity coefficient η in cm, g, sec, system (poise) was calculated from the formula:

$$1) \quad \eta = 0,145 \cdot 10^{-16} \cdot \frac{Tt}{a l^2 n}$$

where:

T = absolute temperature,

t = time in sec,

a = diameter of particle in cm,

l = distance between lines of eyepiece micrometer in cm (in my apparatus $1,08 \cdot 10^{-3}$),

n = number of two sides first passage (doppelsetige Erstpassage); in formula (3) statistical weight.

Explanation of this formula is given in Pekarek (1931) or in Stруггер (1949). In using this method η ought to be the mean of numerous measurements. Particular measurements differ often by more than 100 per cent even in the case of measurements of water viscosity coefficient. To calculate the mean value it is necessary to make numerous measurements of η in the same medium. If a cell in mitosis is considered, it is impossible to make even one measurement in unchanged conditions, as the cell division develops and viscosity coefficient may change. For this reason, measurements must be arranged in time; as the 0 point in time the beginning of anaphase was chosen, and the time before and after the anaphase beginning was considered as minus and plus respectively. For calculations of mean values measurements, made in different cells, must be considered.

The mean value of viscosity coefficient $\bar{\eta}$ was calculated from the formula:

$$2) \quad \bar{\eta} = \frac{\sum \eta n}{n}$$

The middle of each measurement was taken to be the time of measurement (i. e. if a measurement lasted 6 mins. and ended at 0 time, the time of measurement was considered to be — 3 mins.).

The influence of individual measurements on the mean value depends on their statistical weight (i. e. on the mean value η a measurement where $n = 20$ has much more influence than when $n = 5$). Because of this the mean square error k was calculated, with consideration of the statistical weight, from formula:

$$3) \quad k = \sqrt{\frac{\sum_{i=1}^m v_i^2 n_i}{m(m-1) \sum_{i=1}^m n_i}}$$

where:

m = number of measurements

v_i = deviations from value $\bar{\eta}$

n_i = n in formula 1

Notes on method and its application

In applying P e k a r e k ' s method to the mitotic spindle, following objections may be raised.

The temperature ought to be constant. In the present work the temperature was $18^{\circ} \pm 2,2^{\circ}\text{C}$, but most (at least 70 per cent) measurements were carried in $20^{\circ} \pm 0,5^{\circ}\text{C}$. So the temperature has no great influence on the obtained data.

According to S t r u g g e r (1949) there is however an other more serious objection, i. e. the medium of which the viscosity coefficient is measured ought to be structurless and the particles during oscillation ought not to collide with either element of this structure (cell walls, tonoplast of vacuole, and so on), nor with each other. Though the cytoplasm has a structure, P e k a r e k ' s method can be applied to it but with some restrictions. At any rate, such a conclusion can be drawn from F r e y - W y s s l i n g ' s book (1948 p. 110): „In P e k a r e k ' s viscosity measurements (1930, 1932) which is based on amplitude of oscillation of particles in Brownian movement, the inhomogeneity of cytoplasm finds expression in a much lesser extent, because the oscillatory motion does not cover a large distance through the cytoplasm but can be studied at a fixed point“. It may be also raised that some experiments on the physical properties of cytoplasm (C r i c k and H u g h e s

1950) do not support Frey - Wyssling's theory of cytoplasm structure.

Mitotic spindle differs from cytoplasm by a regular arrangement of particles, the best proof of which is the birefringence of the spindle (Schmidt 1939, Swan 1951 a, b). This regularity of the structure makes the oscillation of the particle more difficult in one direction than in the other, and the viscosity coefficient is greater perpendicularly to the spindle axis than parallelly to it. Because of the structure the true value of the viscosity coefficient is smaller than what is obtained from calculations. To get the real values, it would be necessary to multiply the obtained values by a proportionality coefficient. The calculation of such a coefficient is at present impossible, though it will be possible, when the spindle structure will be known exactly. In my opinion, objection in application Pekarek's method to the mitotic spindle are not very serious and in the case of the pushing body are of little importance, but only if η is measured parallelly to the spindle axis.

The mutual collisions of particles may be dealt with by choosing suitable particles.

These objections will either not or only slightly influence the shape of the curves obtained on Textfig. 2.

OBSERVATIONS

Course of mitosis

The course of mitosis was observed from late metakinesis to resting nuclei (some observations are given by Bajer 1952).

Mitotic spindle is, in most cases, well visible on the background of chondriosomes containing cytoplasm. In numerous cases mitosis is observed in almost bare spindles surrounded by a small quantity of cytoplasm (Plate I, Fig. 3). As mitosis in these spindles is quite normal — such observations do not support Wada's (1950) supposition of spindle poles fixation by „polar plasmic strands“. Also in *Tradescantia* cells mitosis is normal without such plasma connections (cf. Plate I, Fig. 13 Bajer 1950). In *Haemanthus* a normal spindle is quite structurless. Spindle poles are however much better visible in anaphase than in metaphase, in which they often cannot be seen at all. No such membrane as envelopes the mitotic spindle in some plants (*Tradescantia*, Wada 1950, *Hymenophyllum*, Bajer and Molè-Bajer 1952) is detectible in *Haemanthus* endosperm. The arms of long chromosomes

reach very often into the cytoplasm, which seems to prove the non existence of the membrane (this is also indicated by observations on Brownian movement). The nuclear membrane in prophase can be seen very plainly (Plate I, Fig. 1a), and it disappears in the later stages.

From early or middle stages of anaphase the action of pushing body („Stemmkörper of B ě l a ř 1929a) usually is pronounced. Changes in character of pushing body action are well known in animals (R i s 1943). In *Haemanthus* this action is most similar to that described by Hughes and Swann (1948) in chick tissue culture, i. e. both shortening of half spindles and pushing body action are simultaneous, and not separated in time as it often happens. In *Haemanthus* the whole cell elongates (in some cases by half of its original length in metaphase — cf. B a j e r 1952 Plate III), it also may constrict (Plate I, Fig. 2), or the pushing body may bend. If a cell has a membrane resting to its elongation (in endosperm there are no cellulose walls) then anaphase chromosome groups flatten, but arms are not bent, which is best noticeable in the case of long chromosomes (of approximately $24\ \mu$). Action of pushing body was noted in all cells, though in different degree. Acting pushing body is very liquified and in no one case the bending of long chromosomes — which H e i t z (1943) found in cells of root tips and explained as the consequence of pushing body action — was observed. During division the cell changes its shape. A similar change of shape, though less pronounced, was found in staminal hairs of *Tradescantia* (B a j e r 1950).

The action of pushing body in *Haemanthus* caeses in telophase, and the cell returns to its original length in metaphase. Usually the cell wall begins to form from the edges and not from the center of the phragmoplast. First stages of middle lamella formation are exceptionally well visible: small spindles (not droplets) parallel to the long axis of phragmoplast originate in it, then they melt together, flatten, and a new middle lamella is formed.

S p i n d l e s t r u c t u r e

Observations on spindle structure were made in cells placed on clean glass squares, cells in demixing (B ě l a ř's (1930) „Entmischung“) and cells after plasmolysis. In the first case cytoplasm begins to creep (cf. B a j e r 1952) and usually as result of this, spindles or nuclei surrounded by a small quantity of coagulated cytoplasm can be observed (Plate II). It is necessary to stress that

these cells during preparation are quite normal, and change just after they are placed on the glass, and that the mechanism of observed changes is not quite clear. In such dead cells the nuclear membrane is well visible in prophase (Plate II, Fig. 4) and is not noticeable in metaphase. Metaphase spindles usually flatten a little, and chromosomes swell and coagulate. In metaphase half spindles are composed of strongly coagulated fibers, between which there usually is little or no structurless substance. In consequence of chromosome coagulation, it is difficult to establish whether fibers end in kinetochores. In metaphase the fibers are parallel to the long axis of the spindle or slightly convergent to it (probably it depends on the stage of metaphase e. g. early or late metaphase) and the spindle poles seem to have a different structure or be fiberless. In anaphase fibers are convergent, and seem to join in one point (spindle pole). In pushing body in late anaphase no fibers are visible (Plate II, Fig. 8).

Cells with reversible demixing are rarely observed (demixing is usually irreversible and cells die). An example of interesting demixing changes can be seen on Plate III, Fig. 10. This cell in metaphase changed quickly its shape, but looked quite normal, and the spindle was homogenous and structurless. Fibers appeared during not more than 2 mins. and the Brownian movement ceased in the spindle simultaneously. Chromosomes, though previously very plainly visible (Plate III, Fig. 10a), changed their refraction coefficient (it decreased), swelled and nearly disappeared. It was impossible to distinguish the particular chromosomes and they seemed to form a shapeless mass. Two types of fibers were easily discernible, i. e.: 1. continuous fibers (Plate III, Fig. 10b) and 2. chromosomal fibers (Plate III, Fig. 10c) — fibers seen between chromosomes and the poles were assumed to be the later ones. Approximate dimensions of these fibers were respectively: 1. breadth $0,3-0,5 \mu$ or somewhat larger, length $35-40 \mu$ 2. breadth $0,3-0,5 \mu$ length $15-20 \mu$. The number of fibers was difficult to establish, as they were slightly convergent to the poles (there seemed to be $30-60$ of them). Though they are invisible at the poles, if the lines formed by them were prolonged, nearly all would intersect at a point which might be considered as the spindle pole. This is only true if the longitudinal section through the spindle centre is considered (cf. Plate III, Fig. c₁). In this cell some hours later anaphase was observed.

Similar types of fibers (dimension and number) were found after plasmolysis in the dividing cells till telephase. As cells are

surrounded by the embryo sack liquid, and are mounted with it, it is difficult to establish the concentration of the solution which causes plasmolysis. During plasmolysis cells change their shape considerably and fibers are distinctly visible both in half spindles and — what is very strange — in pushing body, though in the later case this probably does not occur in late telophase (Plate III, Figs. 11—13). As the refraction coefficient of chromosome diminishes it is very difficult to distinguish early and late prophase, anaphase and telophase, and often even one stage from the other.

O b s e r v a t i o n s o n B r o w n i a n m o v e m e n t

In metaphase there are small particles only in a part of the mitotic spindles. These oscillating particles are much more numerous in metaphase and anaphase than in metakinesis. In normal cells in prophase the particles were not seen even once. This seem to indicate that the particles pass into the spindle from the cytoplasm. On the other hand the particles are numerous in the pushing body of all observed cells already 10 mins. after the beginning of anaphase.

Observations on the intensity of the Brownian movement, without considering exact measurements, but in full agreement with them, are as follows. In the same stages of different normal cells the intensity of the Brownian movement is not the same, through the difference is usually small. Also in the various stages the intensity of the Brownian movement undergoes changes which are similar in different cells. In all cells the half spindles are fairly stiff as compared to the pushing body, which is always liquefied. The pushing body is liquefied immediately when the particles become visible in it (approximately 10 mins. after anaphase begins); in the earlier stages, and also nearer the kinetochores of anaphase chromosomes, it seems at first to be stiffer and then it liquefies quickly. In half spindles and pushing body the particles oscillate quicker and more easily parallelly to the spindle axis than perpendicularly to it, which is in agreement with Bělař's (1929a), and Becker's (1935) observations on the Brownian movement inside the mitotic spindle. In *Haemanthus* these differences are very distinct in the half spidles and very slight in the pushing body. Particles in half spindles oscillate irregularly: they move quickly, stop, and then move quickly again and each one behaves differently. In metaphase the oscillation of particles at the spindle poles is easier than in the center of the half spindles and also there are marked differences in the two directions (perpendicular and parallel), though the dif-

ferences are lesser here than in half spindles. In all observed normal cells in anaphase, the Brownian movement ceases in half spindles, similarly as at spindle poles.

It is interesting to compare the half spindle and pushing body liquefaction with the degree of pushing body activity. The following conclusion drawn from numerous observations seem to be certain: the more the pushing body is liquefied, the stronger is its activity. The activity of the pushing body is smaller when the half spindle is slightly liquefied, than when it is stiffer. Also chromosome movement depend on the stiffness of the spindle, and in the very liquefied spindles it is very slow; such liquefied spindles are usually found in cells which are not normal and in which often the course of anaphase is disturbed.

The results of measurements of η (in poises) are given on Textfig. 1. It is necessary to stress that the statistical weights of different measurements are not the same (cf. methods) and in most cases very deviating measurements have no great influence on the mean values.

The measurements of viscosity coefficient are in agreement with direct observations. The dispersion of measurements is caused by different factors and was discussed previously (cf. notes on the methods).

On the basis of all measurements Table I is obtained, and from it, curves on Textfig. 2 are plotted. Measurements on spindle poles and half spindles perpendicular to spindle axis are few and for these no curves are plotted. As the value of η seem to be constant in the half spindle till 10 mins. before anaphase, the mean value from all measurements prior to this are calculated. The mean viscosity coefficients of half spindle for the time — 120 mins. till — 11 mins. are: $0,267 \pm 0,008$ and $0,494 \pm 0,019$ parallelly and perpendicularly to the spindle axis respectively. In the same units the viscosity coefficient of water is 0,01, of cytoplasm of *Vicia* germ 0,24 and of *Amoeba* cytoplasm 0.06 (after Frey - Wyssling 1948). In comparison to these values the viscosity of *Haemanthus* spindle is high. The approximate relation of coefficients within the spindle is 1 : 2, i. e. the viscosity across the spindle is almost twice of what it is lengthwise. The beginning of anaphase must be considered carefully. The agglomeration of low values just before and just after anaphase begins, is evident. But in Textfig. 2 (mean values) only two mean values pertaining to the half spindle (parallel to spindle length) are lower. Therefore in view of the dispersion of other points, it

Textfig.1. Values of η (statistical weights not considered) plotted against time (0 — beginning of anaphase; minus — before, plus — after anaphase begins). Triangles — half spindle, circles — pushing body; black — parallel to spindle length, white perpendicular to it. Crosses — spindle pole, large, thick — parallel to spindle axis, small, thin — perpendicular to spindle axis. Note the agglomeration of triangles at the beginning of anaphase.

TABLE I

Time in mins.: minus before, plus — after anaphase begins; 0 — beginning of anaphase. Triangle — half spindle, circle — pushing body. Viscosity coefficients (in poises) measurements parallel or perpendicular to spindle length.

Explanation of n and m given in formulas 1 and 3 respectively.

time	Δ ○ +	 ⊥	$\bar{\eta}_l$ or η_l	n	m	time	Δ ○ +	 ⊥	$\bar{\eta}_l$ or η_l	n	m
—120—81	Δ		0.383	26	3	+ 15 + 19	Δ		0.333 ± 0.035	24	3
	Δ	⊥	0.725	8	1		Δ	⊥	0.539 ± 0.105	13	4
— 80 —71	Δ		0.267	14	2		+		0.113	10	1
	+		0.186	10	1		○		0.163 ± 0.013	84	8
— 70 —61	Δ		0.288	20	2	+ 20 + 24	Δ		0.421 ± 0.043	29	7
	Δ	⊥	0.436	10	1		Δ	⊥	0.434	3	1
— 60 —51	Δ		0.296 ± 0.021	35	4		+	⊥	0.282	12	1
— 50 —41	Δ		0.242 ± 0.022	52	5		○		0.134 ± 0.017	74	6
	Δ	⊥	0.723 ± 0.019	23	4		○	⊥	0.125 ± 0.010	109	8
— 40 —31	Δ		0.270 ± 0.012	47	4	+ 25 + 29	+		0.636	6	2
	Δ	⊥	0.387 ± 0.016	36	3		○		0.127 ± 0.027	146	14
	+		0.239	18	2		○	⊥	0.145 ± 0.009	45	4
— 30 —21	Δ		0.252 ± 0.010	72	9	+ 30 + 34	Δ		0.425 ± 0.017	28	3
	Δ	⊥	0.400	6	1		Δ	⊥	0.992	3	1
	+		0.450	16	2		+		0.482	6	1
	+	⊥	0.580	7	1		○		0.117 ± 0.004	171	14
— 20 —11	Δ		0.307 ± 0.032	80	10		○	⊥	0.128 ± 0.017	79	7
	Δ	⊥	0.262	10	1	+ 35 + 39	Δ		0.835	9	1
	+	⊥	0.246	11	1		+		0.506	3	1
— 10 — 6	Δ		0.333 ± 0.136	18	2		○		0.123 ± 0.005	155	15
	Δ	⊥	0.530 ± 0.020	26	5	+ 40 + 44	+	⊥	0.367	12	1
	+	⊥	0.262	11	1		○		0.124 ± 0.008	261	22
— 5 — 1	Δ		0.242 ± 0.018	92	10		○	⊥	0.150 ± 0.008	135	11
	Δ	⊥	0.209	10	1	+ 45 + 49	○		0.157 ± 0.003	150	14
	+		0.234	15	2		○	⊥	0.124 ± 0.031	89	7
0 + 4	Δ		0.175 ± 0.016	54	5	+ 50 + 54	○		0.175 ± 0.003	180	18
	Δ	⊥	0.315 ± 0.012	31	3		○	⊥	0.194 ± 0.014	43	3
+ 5 + 9	Δ		0.358 ± 0.029	51	8	+ 55 + 59	○		0.149 ± 0.007	135	14
	Δ	⊥	0.343 ± 0.033	24	4		○	⊥	0.157 ± 0.015	73	5
	+	⊥	0.246	21	3	+ 60 + 69	○		0.165 ± 0.004	228	22
+ 10 + 14	Δ		0.315 ± 0.017	37	6		○	⊥	0.241 ± 0.015	56	7
	Δ	⊥	0.313	13	2	+ 70 + 79	○		0.183 ± 0.023	97	7
	+		0.323	10	2		○	⊥	0.254 ± 0.022	95	10
	+	⊥	0.153	6	1	+ 80 + 89	○		0.137 ± 0.011	136	11
	○		0.127 ± 0.008	99	9	+ 90 + 99	○		0.219 ± 0.017	61	6
							○	⊥	0.405 ± 0.115	23	3
						+ 100 + 120	○		0.261 ± 0.014	60	6
							○	⊥	0.405 ± 0.013	23	3

Textfig. 2. Curves of changes in viscosity coefficients plotted against time. (0 — beginning of anaphase, minus before, plus after anaphase begins). Triangles — half spindle, circles — pushing body; black — parallel to spindle length, white perpendicular to it. Curve for half spindle perpendicular to spindle length is not plotted. In half spindle thin line at the beginning of anaphase indicates the probable beginning in decrease of viscosity coefficient (cf. text).

These curves are plotted from data given in Table I.

seems that the lowering of viscosity at the beginning of anaphase, though very probable, cannot be considered as proved. In anaphase the viscosity coefficient of the half spindle rises quickly. The increase of viscosity coefficient is simultaneous with pushing body action, of which the viscosity coefficient is approximately half that in the half spindle (viscosity coefficient of pushing body, parallel to long axis is approximately 0,12) In comparison to the half spindles the pushing body is highly liquified. The differences of η in pushing body (parallelly and perpendicularly to long axis) are slight in anaphase, and they increase in telophase during middle lamella formation. In telophase viscosity coefficient of pushing body increases because of its transformation into the phragmoplast. The increasing of viscosity coefficient in phragmoplast at the beginning of telophase was found by W a d a (1935).

DISCUSSION

The results obtained here are similar to the data on cytoplasm viscosity during mitosis obtained by other methods (C a r l s o n 1946, 1952, H e i l b r u n n and W i l s o n 1948 and numerous earlier papers — most cited by C a r l s o n 1946).

C a r l s o n ' s (1946) observations on changes of cytoplasm viscosity in different stages of mitosis in neuroblast cells of grasshopper are somewhat different, than those obtained in the course of this work. He reported the decrease of viscosity coefficient in the pushing body, but according to him, the half spindles are liquid. However the result of his later studies fully support the present observations on *Haemaphysalis*. C a r l s o n (1952), on the basis of his micrurgical experiments on neuroblast cells of grasshopper, reports: 1. the rigid consistence of mitotic spindle in metaphase, 2. the lowering of the viscosity coefficient in pushing body and 3. the attachment of spindle fibers to spindle poles in anaphase. He thinks that the decrease of viscosity in anaphase half spindles takes place between kinetochores and the pole. It is difficult to state whether this is the case in *Haemaphysalis*, as observations refer only to the central part of the half spindle, between the kinetochores and the pole. In endosperm of *Haemaphysalis* the viscosity coefficient of the spindle increases in anaphase, but it is very probable that it decreases just before anaphase begins. It is very interesting that the curve for pushing body viscosity (Textfig. 2) if prolonged, would begin in the probable of maximum lowering of half spindle viscosity. If such lowering would really take place, it will be possible to draw conclusions concerning the mechanism of anaphase beginning (i. e. this would indicate that anaphase begins just after changes of submicroscopical structure of metaphase spindle). The increase of viscosity in the half spindle in anaphase and its decrease in the pushing body is caused by structural changes in the mitotic spindle. Coagulated spindle fibers during creeping of cytoplasm, the appearance of fibers during demixing and especially during plasmolysis confirm the well known fact that the spindle is composed of two types of fibers: 1. continuous fibers, 2. chromosomal fibers. Fibers have different appearance, than those seen on fixed material (cf. photos of Ö s t e r g r e n 1951) and seem to be of the same breath throughout their length. There arises the question in what degree these fibers resemble those existant in normal cells. Though this cannot be answered on the basis of present investigations, it appears to me that these are real fibers (the two last latter

cases i. e. demixing and plasmolysis) of normal cells from which water has been partially removed i. e. they are slightly dehydrated fibers. Fibers may be somewhat convergent in the metaphase half spindle, but the poles are not distinctly visible. In anaphase they seem to join at the pole. According to W a d a (1950) the spindle is composed of fibers and an interfibrillar substance. It is not known whether this substance is anisotropic or isotropic (S w a n n 1951a,b). However, it seems evident that the Brownian movement takes place in it. This work does not solve this question (due to the presence of fibers differences of intensity in the Brownian movement both parallel and perpendicular to the spindle length, may be no indication of a differentiation in the substance), but two properties of this substance are noted: 1. the ability to become liquefied, which enables it to flow from the spindle, 2. reversible denaturate, which stops the Brownian movement in it.

The increases of viscosity coefficient in anaphase half spindle may be caused by two factors: 1. displacement of fibers from a more or less parallel (metaphase) to a convergent (anaphase) arrangement, i. e. in anaphase half spindle fibers are much closer to each other than in metaphase, and during anaphase they approach still more, 2. the decrease or change in structure of the substance between the fibers. Though, the action of both these factors is quite plausible, it is difficult to decide for certain, whether they act together or only one of them is responsible. At any rate only one of these factors (the existence of the first one is proved) is sufficient to stop the oscillatory movement of particles within the spindle during anaphase.

The viscosity of the metaphase half spindle is about twice that of the pushing body. In the pushing body the difference in viscosity coefficients measured lengthwise and crosswise to long axis is small, but in my opinion it surpasses the possible error in measurements. This difference may be caused by a small number of fibers or interzonal connections which may be present in the pushing body. It would be interesting to know, whether the substance which would remain, if the fibers from metaphase spindle were removed, would have a similar viscosity coefficient as the pushing body. Probably this would not be the case, and most authors think that the pushing body originates after submicroscopical changes in the spindle. The above confirm studies on animal cells in polarised, light, according to which the pushing body is not birefringent (S c h m i d t 1939, S w a n n 1951). In *Haemanthus* during the first stages of anaphase

particles, when seen in the pushing body, oscillate very slowly, and they behave similarly near the kinetochores. On the other hand in later stages in the center of pushing body particles move with greater freedom. This also seem to indicate that this interfibrillar substance changes in anaphase.

The viscosity coefficients of pushing body changes. These changes must be considered during: 1. anaphase, when the pushing body acts while it is most liquefied (untill 40—50 mins. after anaphase begins), 2. the changes of pushing body in telophase i. e. during phragmoplast formation. „Stemmkörper“ (pushing body) and traction fibers hypotheses were questioned by Carlson (1952), who on the basis of micrurgical experiments states: „Since the interzonal region during middle and late anaphase is fluid except for fine interzonal connections between the distal ends of sister chromosomes, the only pushing force one can visualize is that resulting from the lengthening of these fibers by either active elongation, or by accretion of new material at their ends“. Such interzonal connections I have also observed both in vivo and after acetocarmine in *Haemanthus* pollen tubes (Bajer 1952 unpubl.). In my opinion the facts known at present are unsufficient for a full elucidation of „Stemmkörper“ hypothesis. However, the presence of interzonal connections and also low viscosity of pushing body are not necessarily an evidence against this hypothesis. If interzonal connections had an active influence, the orientation of individual chromosomes in anaphase would be different, than what is observed. The fluidity of pushing body may support „Stemmkörper“ hypothesis and need not be an evidence against it. It is well known, that on the boundaries of two mediums with different surface tensions a force directed toward the medium with the higher surface tension acts. Half spindles and pushing body have different viscosity coefficients and most probbaly their surface tensions also differ, if that is so, then the pushing body would act not on individual chromosomes but on both half spindles.

During phragmoplast formation some changes in pushing body are unexpected and difficult to understand. An increase viscosity coefficient of phragmoplast in telophase may be caused by denucleinization which in *Haemanthus* is very rapid (chromosomes begin to disappear often 40 mins. after anaphase beginning). According to Jacobson and Webb (1952), ribonucleoproteins in telophase pass from chromosomes to the space between chromosome groups. Viscosity coefficient of RNA is very high and it can cause

the increase of viscosity in phragmoplast. It is very surprising that the two curves for pushing body in Textfig. 2. separate and from this the only conclusion which can be drawn is, that a structure parallel to the long axis of pushing body is being formed. In fact, on fixed material the structure is visible in the phragmoplast. This structure is not birefrigent in polarised light and on the basis of that kind of experiments the phragmoplast is structurless. The fibers visible during the earlier stages in pushing body most probably do not exist in the phragmoplast. There is a connection between changes occurring in the phragmoplast and cell wall formation. During middle lamella formation pectine substances are precipitated in the shape of small droplets (Y a s u i 1939), which are seen in electron microscope (R o z s a and W y c k o f f 1952). In *Haemanthus*, instead of droplets, small spindles are visible. It is possible that in order to transport pectine substances a structure arises, which makes the oscillation perpendicular to phragmoplast axis more difficult than the one parallel to it. The nature of the changes in the phragmoplast and the pushing body may be elucidated by further exact measurements such as changes in volume, studies of chemical properties etc.

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

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SUMMARY

As a material *Haemanthus* endosperm was used.

Observations on spindle structure of living cell confirm the well known fact that the spindle is composed of two kinds of fibers (chromosomal and continuous fiber) and of interfibrillar substance. The existence of continuous fibers or fibers of the same character was confirmed in pushing body (B ě l a ř ' s Stemmkörper). Two properties of interfibrillar substance in metaphase half spindle are confirmed:

1. It can be liquefied, then it flows out of the spindle almost completely after which bare coagulated fibers are seen.
2. It can be denaturated reversibly (in reversible demixing) or irreversibly (coagulation) and then it hardens and all Brownian movement in it stops completely.

Viscosity coefficient in spindle and pushing body is greater in the direction perpendicular than parallel to spindle axis (in half spindles almost double and in pushing body by $1/5$ — $1/4$). Viscosity coefficient of pushing body is nearly half that of the half spindle in metaphase. The values (in poises) of these coefficients are given.

Viscosity coefficient of the spindle in metaphase does not change and increases quickly in anaphase. The increase of the viscosity coefficient values is simultaneous to the pushing body action (in endosperm of *Haemanthus* the pushing body action is similar to what was found in chick tissue culture). In late metaphase and at the beginning of anaphase viscosity coefficient of mitotic spindle probably decreases considerably. However this process cannot be considered as proved. The pushing body acts when it is most liquefied. The viscosity coefficient of the pushing body increases in telophase during phragmoplast formation which may indicate that a new structure is formed in it at this stage.

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EXPLANATION OF PLATES

Phase contrast microscope; magnification approximately 800 ×

Plate I

- Fig. 1. a—d. Mitosis in the same cell; a — note nuclear membrane on lower right part of the nucleus; b—c — two stages of anaphase; d — late telophase with almost resting nuclei. b — 44 min. after a, c — 1 h 19 min. after a, d — 4 h after a.
- Fig. 2. Action of pushing body — the cell constricts.
- Fig. 3. Late telephase, phragmoplast and nuclei almost without cytoplasm. Note long chondriosomes.

Plate II

Cells placed on bare glass squares (cf. text). Dead cells.

- Fig. 4. Prophase; note nuclear membrane.
Fig. 5. Spindle injured during preparation — coagulated spindle.
Figs. 6—7. Normal cells after preparation, flattened spindle, cytoplasm creeps.
Fig. 8. Late anaphase, half spindle flattened. Note that chromosomes arranged on circumference of circle which seems to indicate that spindle fibers are fixed at the spindle poles. In pushing body no fibers.
Fig. 9. Late telophase.

Plate III

- Fig. 10 a—e. Cell with reversible demixing as described in text. a — metaphase before demixing; b—c — cell in demixing, b — continuous, c — chromosomal fibers, c₁ — c in which most visible fibers are drawn in, — note their crossing almost in one point. Thickness of fibers markedly uniform. This is partly obliterated on photograph due to light refraction on cytoplasm structure. Chromosome form shapeless mass, their refraction coefficient is much reduced. d — e — anaphase in the same cell; note change of shape. b — 16 min. after a, c — 31 min. after a, d — 1 h 42 min. after a, e 2 h 34 min. after a.
Figs. 11—13. Plasmolysed cells. Metaphase or early anaphase; note fibers. 12 — ana-telophase, note fibers in pushing body. 13 — late telophase no fibers in pushing body. All cells changed shape owing to strong plasmolysis; refraction coefficient of chromosomes much reduced.

Plate I.

Plate II.

Plate III.