Notes on the anaphase mechanism and the energy of chromosome movement.

by

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In the studies on the mechanism of mitosis the estimation of the energy of chromosome movement is of great importance and can help to gain further knowledge about the process cf cell division. As far as the authors of this paper know, there in no sufficient data to calculate the absolute value of this energy, but corsidering the experimental results so far published on the change of chromosome position versus time, it would be possible to compare the values of the energy of two chromosome groups in one cell or in several. The method given below allows to consider the dependence of the energy of the chromosome movement from different parameters such as temperature, age of cells etc.

In order to solve the problem of this energy the authors considered the chromosome movement from a physical point of view. To draw conclusions about the energy it is necessary to consider the force which causes the movements of the chromosomes. This force will be denoted by F_x.

There are two possibilities of the dependence of the force F_x on time:

1. F_x depends only on the position of chromosomes and explicite does not depend on time. The variation of F_x in time is caused by the increase of the chromosome separation. It means that the force F_x at the beginning of the anaphase has at once its maximal value and the movement of the chromosomes begins when the action of this force is abruptly liberated.

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2. F_x explicite depends on time, besides the dependance caused by the change of chromosome separation. It means that at the begining of the anaphase F_x increases gradually and its total dependence on time is the resultant of its direct variation in time, and the variation caused by the increase of the chromosome separation.

It seems evident, that in the anaphase mechanism the first possibility is fulfilled, but it is necessary to stress, that in the latter case, the mathematical considerations given below, would be far more complicated.

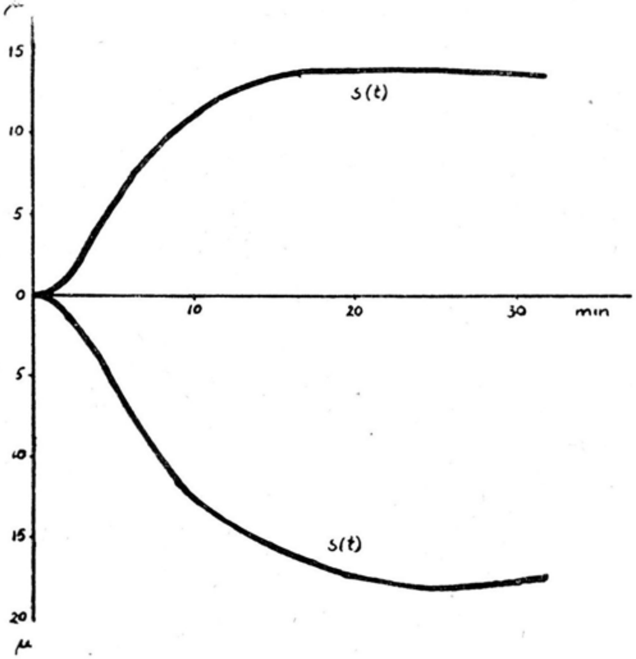

Fig. 1a

Although the hypotheses concerning the mitosis mechanism are numerous (Schrader, 1946), the nature of the force F_x is not known exctly, however Cornmann's (1944) arguments for traction fibers are the most convincing.

Considerations on the force F_x, though without an exact mathematical aproach to the problem, were made by Hughes and Swann (1948), however, in order to draw conclusions concerning the energy, their assumptions must be discussed more fully.

The force F_x is partially balanced by the viscosity resistance F_r, of the spindle material. The resultant of the forces F_x and F_r causes an accelerated motion of the chromosomes with an acceleration a (t)

The condition of balance of the forces acting on chromosomes may be expressed by the equation:

$$F_x = F_r + ma(t)$$

where m — mass of the chromosome.

It is evident from the experimental curves (Barber, 1939, Bajer, 1950) representing the change of the chromosome distance in time s(t) in cells of staminate hairs of *Tradescantia virginica* (Fig. 1a), that in the first stages of anaphase the chromosome movement is accelerated till after 1—2 min the velocity reaches its maximal value, then, the movement becomes retarded and stops. In different specimens the time between the beginning of the movement and the moment in which the velocity reaches its maximal value varies. E.g. in the case of small chromosomes in the chick tissue culture the maximal velocity is observed at the very beginning of the anaphase cf. Fig. 1b taken from Hughes & Swann,

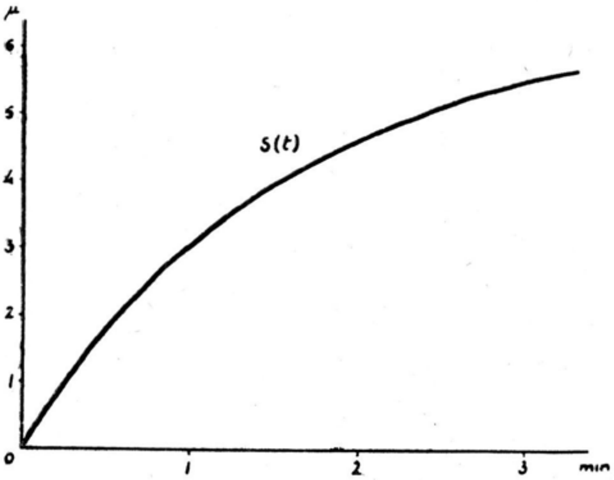

Fig. 1b

(1948 Fig. 16). This is probably caused by the small inertia of the chromosomes due to which the time of the increase of the velocity is extremly short. In reality the picture must be as follows: the chromosome movement is accelerated untill the force F_x is totally balanced by the viscosity resistance F_r, however with the increase of chromosome separation, F_x decreases and is overcome by F_r and so the movement becomes retarded.

On the assumptions that:

1. the shape and the orientation of the chromosomes are not changed during the motion,
2. the viscosity coefficient of the spindle, in which chromosomes move, is constant and independant of their velocity,

the force of viscosity resistance F_r is proportional to the velocity of chromosomes:

$$F_r = A\ v(t)$$

where A is the proportionality coefficient dependent on the viscosity of the spindle and on the shape of the chromosomes.

The first part of assumption 1 is fullfiled, because the shape of the chromosomes does not change untill telophase (contraction, Bělař, 1929). The second part is fullfiled only in the case of small chromosomes. Though the orientation of these chromosomes changes in anaphase as compared to metaphase, however the distance in which this takes place is very short and the relation of this distance to the total distance covered by small chromosomes is equal to 1/10—1/20 or even less. In the case of large chromosomes this relation is much greater (between 1/4 and 1/2). The small chromosomes in the first stages of mitosis anaphase rotate about 90^0. Their arms are very short and usually lie in the plane of the metaphase plate. In meiosis this rotation if at all existing is certainly smaller. In the case of long chromosome the whole chromosome or at least one of its arms changes the position by about 180^0; the reason for this is that the arms do not lie in the plane of metaphase plate, but are usually perpendicular to it. The viscosity resistance of the medium in which the chromosomes move depends on the dimension of the chromosomes and their orientation in anaphase. It is necessary to add that the movement seems to be almost synchronical (all kinetochores move in the same plane perpendicular to the long axes of the spindle), though as we know no exact measurements were done till now.

The objections suggested in the second assumption are more serious. The viscosity of cytoplasm depends on pressure /Pfeiffer, 1937/ while in a normal liquid this dependence does not exist. On the basis of his experiments Pfeiffer maintains that the cytoplasm is not a newtonian liquid and neither *Stockes'* nor *Hagen-Poisseuille's* laws can be applied to it. As a result of its submicroscopical structure (structure elasticity, Frey-Wyssling, 1946) the cytoplasm has elastic properties und for this reason Frey-Wyssling (1947) also shares the opinion that these laws cannot be applied to it. If it were impossible to neglect the dependence of viscosity coefficient on the velocity of the chromosomes, the interpretation of the diagrams given below would have to be modified by taking this dependence into consideration /Makarow, 1948/.

The chromosomes move in the substance of the spindle, but it is not yet known exactly whether the whole chromosome moves in halfspindel „Hlbspindel“ /Schmidt, 1937, 1939) or whether only their kinotochores move in it, while the rest of the chromosome is surrounded by the „Stemmkörper“ (Bělař, 1929). It is also possible that they are surrounded partly by halfspindel and partly bv „Stemmkörper“, exact studies on spindle viscosity have not yet been done. Chambers /1924/ however on the basis of his micrurgical experiments is convinced, that the spindle has greater viscosity than the cytoplasm. Wada /1934/ on the basis of similar studies drew a contrary conclusion. According to both these authors „Stemmkörper“ has a liquid consistance, while Schmidt /1937/ maintains that the „Stemmkörper“ is of a more solid nature. Anyhow the spindle structure is very uniform. It is built of long, longitudinally orientated polypeptide chains. Most probably these chains are extremely thin (according to Hughes and Swann /1948/ about 50 Å) and between them there is a substance of a liquid consistance. This is in agreement with the tactoid hypotesis (Bernal, 1949), and is being accepted by an increasing number of authors (Östergren, 1949, 1950/.

From the above data it results that the *Stokes'* law cannot be applied to cytoplasm without restrictions. Makarow (1948) suggests however that this restrictions are slight. Nevertheless in the case of chromosome movement the range of the velocity changes is very small and the substance in which the chromosomes move has most probably other properties than the cytoplasm. In consequence the application of *Stokes'* law to chromosome movement it more justifiable

If the *Stokes'* law is to be applied the Raynold's number R must be $\ll 1$.

$$Re = \frac{dv\rho}{\eta}$$

where d is the dimension of the chromosome, ca 1μ.

v — the velocity of chromosome,
ρ — the density of substance in which chromosomes move, ca. $1 g/cm^3$ (water)
η — the viscosity coefficient, taken as 10 cP

With these values in the case of the chromosome movement the Raynold's number is equal

$$Re = 7.10^{-9}$$

The values given above are rather arbitrary, but even if they were considerably changed, Re would be much less than 1.

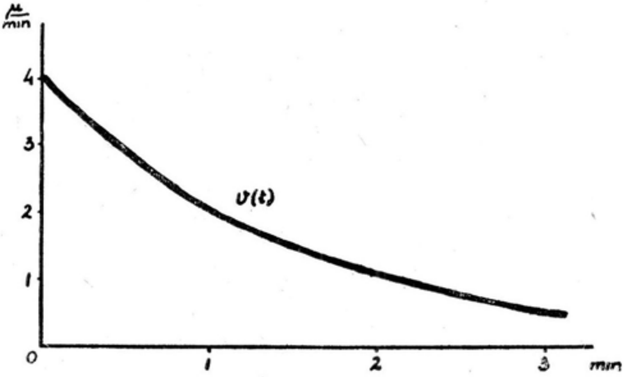

Fig. 2

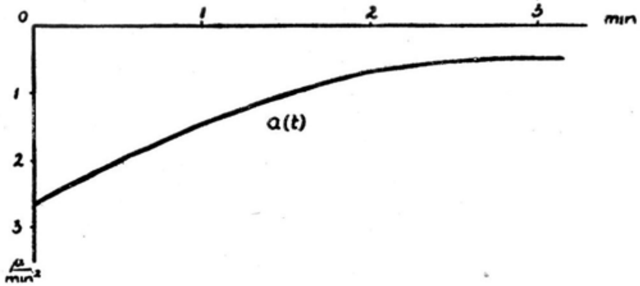

Fig. 3

The coefficient A in the formula

$$F_r = A.v(t)$$

can be represented in the form

$$A = \eta k$$

where η — the viscosity coefficient k — the factor dependent on the shape of the chromosome (in the case of a spherical one $k = 6\pi r$)

Then the equation /1/ will take the form:

$$F = \eta k v(t) + m a(t) \tag{2}$$

The diagrams of the functions v(t), a(t) can be easly obtained by graphical differentiation of s(t). Such diagrams for anaphase in the cells of chick tissue culture (obtained from the experimetal curve of Hughes and Swann, 1948)) are represented in Figs 2 and 3.

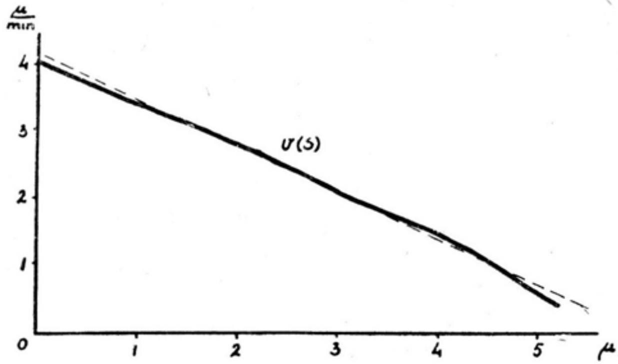

Fig. 4

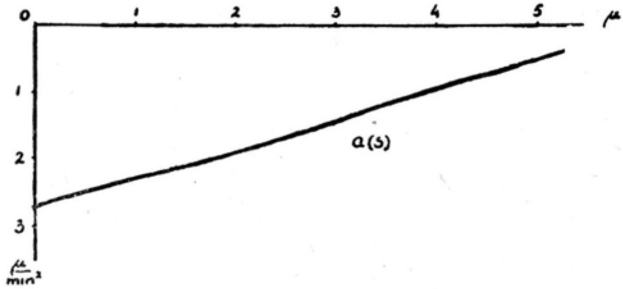

Fig. 5

To draw conclusions about the nature of the force F_x we must know the dependence of velocity and accelaration on the distance. From the diagrams of s(t), v(t) and a(t) it is easy to obtain the diagrams of v(s) and a(s). These diagrams are represented in Figs. 4 and 5.

If it were possible to find the coefficients in equation /1/ the force F_x as the function of the distance could be obtained. From the shape of this curve the conclusions on the nature of F_x can easily be drawn, which was done for the first time by Hughes and Swann /1948/.

Among the different types of forces existing in nature there are three, which can be considered here:

1. Elasticity forces, which according to Hooke's law can be represented by the equation

$$F = B - C.s$$

2. Electrostatic forces, which are according to Coulomb's law inversly proportional to the distance

$$F = D/s^2$$

3. Forces acting between electrical or magnetical dipoles inversly proportional to the cube of the distance

$$F = E/s^3$$

Diagrams of these forces are represented in Fig. 6 a, b, c. Units in these diagrams are arbitrary.

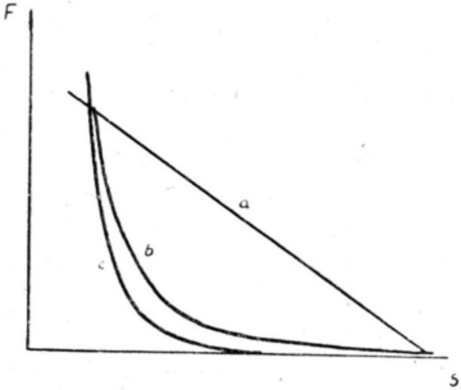

Fig. 6

It is of a great consequence whether the term ma(s) causes a change in the shape of the force F_x. Hughes and Swann /1948/ neglect this term without an exact mathematical argumentation but to find whether this is justifiable the orders of magnitude of the term F_r and of the correctional term ma(s) must be calculated. For this purpose the following values of the coefficients in the equation /1/ are assumed:

$\eta = 10$ cP
$k = 10\mu$ (for the spherical chromosomes of $0.5\ \mu$ radii)
$m = 4/3\ \pi r^3 \rho = 0{\cdot}25.10^{-12}$ g.

It follows from further considerations that even a great change in orders of magnitude of these values has no influence on the conclusions drawn.

The above values give:

$$F_r = \eta k v_{max}(s) = 10\ \text{cP}.\ 10\ \mu.\ 4\ \mu/\text{min} = 7.\ 10^{-10}\ \text{dyn}$$
$$ma_{max}(s) = 0{\cdot}25.10^{-12}\ \text{g}.\ 3\ \mu/\text{min}^2 = 2{\cdot}10^{-20}\ \text{dyn}$$

It is obvious, that the correction introduced by the term ma(s) is extremely small in comparison with the viscosity resistance F_r and, that it can be neglected. Owing to this fact, the diagram of the force F_x has the same shape as the diagram v(s). It follows that the conclusion drawn by Hughes and Swann from the shape of the curve v(s), that the force F_x is an elastical one, is correct.

From the diagram of v(s) it is possible to find the relative differences of the energy of the translation motion of both chromosome groups in anaphase. The energy of the chromosome motion is given by the equation:

$$E = \int_0^{s_{max}} F_x(s).ds = A \int_0^{s_{max}} v(s).ds$$

Then the shaded areas in Fig. 7 are proportional to the energies of the movement of each chromosome group. These areas can be easly found by means of planimetry.

In all papers on chromosome movement (except Bajer, 1950), separate curves for both groups of anaphase chromosomes are not given. In order to base the present considerations on a concret example, the curves represented in Fig. 7 (Function s(t) for this curve is given in Fig. 1a) are calculated from the experimental curves for *Tradescantia,* though this material is very unsuitable as the chromosomes are long and their seperation rather short.

The method used by the authors of this paper for obtaining the relative energy differences can be applied to the solving of the following problems:

1. Whether or not the energies of both chromosome groups in anaphase are equal to each other in one or more cells.
2. Whether the energy of chromosome motion is the same in the case of the different chromosome separations.

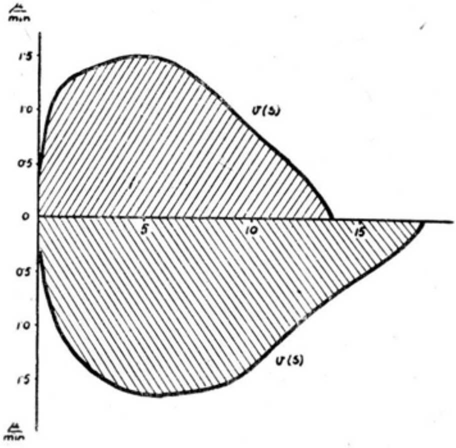

Fig. 7

3. Whether the energy of the chromosome movement depends on temperature. It is possible that on this base some conclusions concerning the viscosity could be drawn.
4. Whether in cells of different age the energy of the chromosome movement is the same.

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