Partial Mitotic Index and Phase Indices

When a meristematic tissue is in a state of dynamic equilibrium, the mitotic index (% of cells in course of division) is a reflection of the time taken for cell-division in relation to the duration of the interphase period.

Nevertheless, the use of the mitotic index is in most cases a very laborious process from the practical point of view on account of the high counts involved, and the significance of the results is doubtful (MAZIA[1]).

In order to obviate these difficulties, we propose to introduce new indices, such as will help us to distinguish between the various possible interpretations of the mitotic index; namely, the partial mitotic index, and the phase indices. The partial mitotic index will be given by the number of cells in course of division as a percentage of all the meristematic cells. The phase indices express the number of cells observed in each phase per hundred meristematic cells in course of division.

Materials and methods. The onions (*Allium cepa*) were cultivated in the dark at 25°C ± 0.5° in cylindrical glass receptacles of 70 cm³ capacity, using ordinary water, which was renewed every 24 h, aerated by passing bubbles though them with an intensity of 10–15 cm³ of air per min.

The preparations were obtained by TJIO and LEVAN's[2] squash technique, using roots 2–3 cm long.

For the partial mitotic index two readings were taken, up to a total of a thousand meristematic cells, half for each of the roots used in each bulb. The criterion used in distinguishing meristematic cells from those which might have initiated the stage of differentiation was the size of the nucleus, those cells being considered meristematic whose diameter was greater than one third of the longest cell axis. An area was always selected for the count which showed good squashing, and which contained practically only meristematic cells.

The phase indices were obtained by counting 200 meristematic cells in process of division in each of the two roots under study from each bulb, that is, a total of 400 divisions.

Results and discussion. The results obtained by this method are given in Tables I–IV.

As may be deduced from Table I, the mean value of the partial mitotic index is 13%. The observed variability of ± 2% is relatively small and may be considered as being inherent in the method, given the smallness of the actual count. Between these limits of 13 ± 2% we may consider the indices obtained as normal. It is interesting to note that with this method no waves of cell-division were observable under the experimental conditions concerned.

From the data obtained from Tables II, III, and IV we deduce that the physiological prophase index is 47 ± 4%, the metaphase index 13 ± 3%, the anaphase index 9.5 ± 2%, and the telophase index 30 ± 7%, the greatest variability being observed in the telophase index.

Table I. Mitotic indices

Time	Bulb 1	Bulb 2	Bulb 3
0 h	–	14	10.6
2 h	15	11.5	11.2
6 h	–	14.9	15.6
10 h	–	–	13.2
16 h	11.1	–	–
18 h	13.1	–	–
24 h	–	13.3	15.15
48 h	12.1	10.9	14

The method used enables us to obtain the desired indices quickly, and with only a small margin of error. For the reading of all the indices per h per bulb, we have to count 1400 cells, which takes about 40 min.

The partial mitotic index, like the mitotic index, is proportional, but not directly, to the relative duration of the mitotic period within the division cycle. Knowing the mitotic index and the duration of mitosis, we can calculate the duration of the interphase period (HUGHES[3], HOFFMAN[4], and EDWARDS et al.[5]).

The phase indices have the advantage of remaining directly proportional to the duration of each phase, since

Table II. Phase indices – Bulb 1

Phases / Time	Prophase	Metaphase	Anaphase	Telophase
0 h	–	–	–	–
2 h	48,3	11,1	10,4	30
6 h	–	–	–	–
10 h	–	–	–	–
16 h	51,7	13,8	10,4	23,6
18 h	53	12,7	9,1	25,9
24 h	47	18,7	13,3	20,3
48 h	55,5	11,1	8,9	24

Table III. Phase indices – Bulb 2

Phases / Time	Prophase	Metaphase	Anaphase	Telophase
0 h	52,7	18	8,3	24,9
2 h	48,8	15,6	11,3	27,5
6 h	41	11,7	9,2	38,2
10 h	–	–	–	–
16 h	–	–	–	–
18 h	–	–	–	–
24 h	42	12,5	10,5	35
48 h	39	15,7	7,5	37,7

Table IV. Phase indices – Bulb 3

Phases / Time	Prophase	Metaphase	Anaphase	Telophase
0 h	41	12,5	11,5	35
2 h	45,7	10,5	9,2	34,7
6 h	43,7	13,5	9,5	33
10 h	46,5	12,2	8,2	33
16 h	–	–	–	–
18 h	–	–	–	–
24 h	48,2	10,2	7,7	33,7
48 h	47,5	11,7	8	33

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a prophase gives rise to a metaphase, and so on. Thus, the duration of each phase can be calculated by means of the appropriate index once the duration of mitosis is known.

By the combined use of the partial mitotic index and the phase indices, we are enabled to calculate the natural or induced modifications in the cell-division cycle with less probability of error.

Résumé. Cet article propose l'emploi de l'index mitotique partiel et des index mitotiques des phases pour l'analyse cytologique quantitative des modifications apparaissant au cours du cycle de division cellulaire, et suggère une méthode rapide pour calculer tous les indices mentionnés.

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A Radioassay for Dopamine-β-Hydroxylase Activity

Dopamine-β-hydroxylase is a mixed function oxidase that catalyzes the terminal step in norepinephrine biosynthesis, namely, the conversion of dopamine to norepinephrine[1]. In addition to dopamine, other phenylethylamines such as tyramine, epinine, amphetamine, α-methyl dopamine, etc.[2–4] can also serve as substrates for the enzyme. The enzymatic activity of dopamine-β-hydroxylase was determined by measuring fluorometrically[5], the amount of norepinephrine formed from dopamine. While this is a relatively simple method for the determination of the enzymatic dopamine-β-hydroxylation, there are no simple methods available for the determination of the β-hydroxylation of other substrates. The specificity of the fluorometric method is also limited since the presence of other fluorescent compounds in the assay mixture may interfere with the determination.

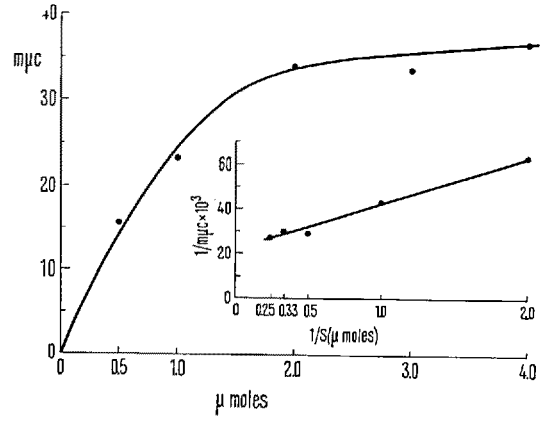
It has been shown that one of the two benzylic tritiums is lost during the enzymatic β-hydroxylation of dopamine-β,β-T[6]. Therefore it is conceivable that the rate of tritium released as water, according to the following reaction sequence:

$$\text{dopamine-}\beta\text{-T} + \text{ascorbate} + O_2 \longrightarrow \text{norepinephrine} + \text{TOH} + \text{dehydroascorbate}$$

is directly proportional to the rate of β-hydroxylation. The recent availability of dopamine-β-T made it possible to measure the release of TOH during the enzymatic β-hydroxylation. The tritiated water which is formed during the enzymatic β-hydroxylation can be separated by ion exchange chromatography from the substrate and product and subjected to radioassay. A similar procedure for the determination of tyrosinase[7] and tyrosine hydroxylase[8] was recently described.

The enzyme was prepared from adrenal medulla[1] and further purified by a previously described procedure[9]. Standard reaction mixtures contained, per tube, dopamine-β-T, 100–200 mμc/μmole (dopamine-β-T, with a specific activity of 137 mc/m*M* was obtained from New England Nuclear Corp., Boston, Mass.). The reaction was started by addition of enzyme, and the tubes were incubated at 37°C for 15 to 45 min. The incubation was stopped by addition of 0.5 ml glacial acetic acid, and the proteins were separated by centrifugation. With highly purified enzyme preparations, the amount of protein was so small that it did not require removal before transfer to the column. To count tritiated water, the reaction mixture was passed through a small Dowex 50 × Na^+ (0.3 × 4 cm) column. The column was washed with water to make an effluent volume of 10 ml. An aliquot (0.5 ml) was dissolved in a liquid scintillation solution which is suitable for counting aqueous samples[10]. A reagent blank, in which all additions were made except for the enzyme, was used to correct the results.

Rates were obtained by assaying TOH at different time intervals. There was a proportional increase of enzymatic activity with time. Reaction rates were also investigated as a function of enzyme concentration. Over the range tested, the reaction was a linear function of enzyme concentration. A typical plot of the reaction velocity vs. substrate concentration, and a double reciprocal plot of the reaction velocity vs. substrate concentration are shown in the Figure. Within a certain range, the velocity of the



Reaction rate (expressed in mμc of TOH formed) vs. substrate concentration (expressed in μmoles) and the double reciprocal plot of the reaction rate vs. substrate concentration.

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