DURATION OF THE SEVERAL MITOTIC STAGES IN THE DIVIDING ROOT-TIP CELLS OF THE COMMON ONION

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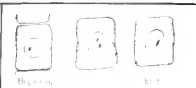
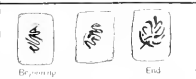
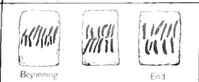
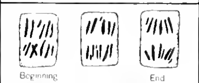
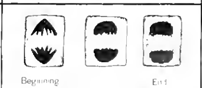
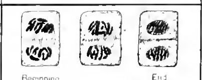
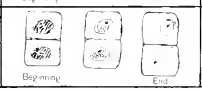
CONTENTS.

Charts, Diagrams, and Tables measuring the relative and absolute durations of the several mitotic stages, and determining the relation between temperature and velocity of each definitely marked stage of the mitotic cycle. (All but the frontispiece in serial order following page 48.)

Summary Chart. The Duration of the Several Mitotic Stages in the Dividing Root-Tip Cells of the Common Onion (Allium cepa)

	Stage Limits	Stage Description	Average Duration At 10° C Percent[a]	At 10° C Minutes	At 20° C Percent[a]	At 20° C Minutes	At 30° C Percent[a]	At 30° C Minutes
Resting Stage		Resting or Metabolic Stage. 1. Nuclear membrane present 2. [illegible] 3. [illegible] present 4. Chromatin in very fine granules	7144 percent	[illegible] minutes —	[illegible] percent	15457 minutes Q_{10} = +1.[illegible]	[illegible] percent	[illegible] minute Q_{10} = +4.946
Stage One		Early Prophase. 1. Nuclear membrane present [illegible]	1448 percent	[illegible] minute —	[illegible] percent	[illegible] minute Q_{10} = −11.40	[illegible] percent	51.4147 minutes Q_{10} = +11525
Stage Two		Early Prophase. 1. Nuclear membrane present 2. Nu[illegible] chromatin aggregations of approximately the same size 3. Focusing up and down does not reveal spireme-like structure	0657 percent	2.1064 minutes —	[illegible] percent	8237 minutes Q_{10} = +2.6822	0172 percent	1.6673 minutes Q_{10} = +4.440
Stage Three		Mid Prophase or Spireme Stage. 1. Nuclear membrane present in all but latest phases 2. Nucleoli possibly recognizable in earlier stage 3. Focusing up and down reveals the typical spireme structure	0268 percent	[illegible] minute —	0122 percent	3.1044 minute Q_{10} = +2.?49	0122 percent	1177 minutes Q_{10} = +2.[illegible]404
Stage Four		Late Prophase. 1. Nuclear membrane gone 2. Spireme broken up into chromosomes 3. Chromosomes "balled-up"	0134 percent	4.704 minutes —	[illegible] percent	[illegible] minutes Q_{10} = +1.385	0124 percent	1.2301 minutes Q_{10} = +2.7593
Stage Five		Metaphase. 1. [illegible] at the equator 2. In the later phases they begin to migrate but do not yet cease to overlap to some degree at the equator	0041 percent	[illegible] minute —	0041 percent	3?41 minutes Q_{10} = +1.4071	0042 percent	[illegible] minutes Q_{10} = +[illegible]
Stage Six		Early Anaphase. 1. Chromosomes cease to overlap at the equator and are moving toward but have not yet reached the poles	[illegible] percent	[illegible] minutes —	[illegible] percent	7651 minutes Q_{10} = −1.1701	[illegible] percent	3.264 minutes Q_{10} = +2.1440
Stage Seven		Mid Anaphase. 1. Chromosomes assembling fan shaped at the poles 2. No sign of cell partition as yet	0021 percent	[illegible] minutes —	0024 percent	0267 minutes Q_{10} = +115[illegible]	0023 percent	2273 minutes Q_{10} = +[illegible]
Stage Eight		Late Anaphase. 1. Chromosomes lose identity in clump at the poles 2. In the earlier phases no sign of cell partition but such may begin to appear at the extreme end of the stage	0057 percent	1.8544 minutes —	0047 percent	11233 minutes Q_{10} = +1.6334	0045 percent	4314 minutes Q_{10} = +2.6038
Stage Nine		Early Telophase or Di-Spireme Stage. 1. Partition present in increasing completeness 2. In the latest phases membranes form around daughter nuclei 3. Nucleoli recognizable in later phases 4. Chromatin masses of stage eight transformed into spireme-like structure	0070 percent	[illegible] minutes —	0065 percent	1.5305 minutes Q_{10} = +1.3329	0072 percent	7054 minutes Q_{10} = +2.1694
Stage Ten		Late Telophase. 1. Division complete, but the twin-ship of the two daughter cells is still patent on account of the close cohesion of their common walls 2. Nuclear membranes and nucleoli present 3. Nucleoli in resting stage	0068 percent	2.6352 minutes —	0095 percent	2.3441 minutes Q_{10} = +1.1240	0077 percent	7579 minutes Q_{10} = +3.0[illegible]
Total Entire Cycle			9978 percent App 10000	[illegible] minutes —	9996 percent App 10000	34097 minutes Q_{10} = +1.139	9996 percent App 10000	9156 minutes Q_{10} = +2.6318

Q_{10} = Velocity increase at a given temperature compared with velocity of same stage at 10° C lower.

NOTE. Kind of onion used: Small white commercial.
Culture: Heavily scored and grown with root-bases submerged in water at constant temperatures 10° C, 20° C, 30° C.
Sampling: From 3 to 5 mm cut from different root-tip ends at 10 minute intervals from 10 a. m. to 1 p. m. inclusive.
Date Sampled: Sept. 9, 1916. Root-tips from 5 to 10 mm long.

[a] In this particular table the Resting Stage is included as a part of the mitotic cycle.

THE DURATION OF THE SEVERAL MITOTIC STAGES IN THE DIVIDING ROOT-TIP CELLS OF THE COMMON ONION.

The ends sought in these studies are, (1) to devise and to prove an accurate method for measuring the relative and aboslute average durations of the several mitotic stages in cell-division; (2) to make use of this method in determining such durations for each of ten arbitrarily marked stages in the mitotic cycle of the dividing cells of the root-tips of the onion (*Allium cepa*), at three different temperatures, namely, 10°, 20°, and 30° C., and thus to learn the effects of such temperature increments upon the duration of the mitotic process as a whole and upon each of its specifically marked stages, with the ultimate view to aiding the analysis of the dynamics of mitosis.

The text-books generally describe the mitotic sequence in considerable detail; but so severe and abnormal an environment for living tissues are the microscopic slide and staining fluids that only recently has special technique developed to the extent of permitting the direct observation of mitotic changes. Especially difficult has been the direct observation of mitosis in any cells other than the first divisions in the transparent fertilized egg in a few organisms. Consequently, most of the data descriptive of mitotic details have been secured from dead samples. This has given a series of pictures of situations at the several instants of killing, which when articulated have restored the whole cycle in correct detail, with these special advantages, that up to instants of killing the tissue may be living in practically normal environment and the high staining may bring out mitotic details as yet unseen in living cells. But this lack of data on the timing and measuring of mitotic processes under definitely controlled environments has prevented the building up of an extensive body of facts on the dynamics of mitosis. The existing knowledge of mitosis is largely descriptive of structure and structural changes.

Ultimately, a process of better staining and viewing live cells may be developed. It may then be possible to trace the normal and unhampered mitotic process in a single living cell and, from direct observation, to time the actual normal duration of each of its successive mitotic stages, and thus from a large series of similar cells easily arrive at the correct average relative and absolute durations of each stage; and further, for the purpose of analysis, to time durations under definitely governed and measured abnormal conditions. But for measuring the velocities of normal activities, it is necessary, in the present stage of development of microscopic viewing of living cells, to find some other method of attack, one in which data are based upon mitotic processes as nearly as possible normal and unhampered up to the instant of

sampling, one in which the mitotic stages may be definitely marked according to arbitrary but fixed standards, and one which will yield numerous samples in order that the average values calculated may have relatively small probable errors.

The method herewith presented is a statistical one based upon stage counts and their classification, within selected microscopic fields taken from closely related and similarly active tissues through a regular succession of observation-instants. The calculations and comparisons shown on the accompanying Method Chart demonstrate the generality and validity, for the purpose designed, of the principle so based, and which is employed in determining the measurements reported in this paper.

PROOF OF PRINCIPLE: HYPOTHETICAL CASE.

The principle here employed is as demonstrable as a geometrical theorem. For the purpose of such proof the case here first presented (see 1. Method Chart) is an hypothetical one in which the mitotic progress is plotted for each of a series of related cells, through an evenly graduated time-scale. Among the cells thus plotted there is fluctuation in (*a*) the mitotic index[1] (M. I.), (*b*) the duration of successive stages within the same cell, and (*c*) the duration of stages of the same order in different cells. This situation, as will be seen later, approximates the actual condition of mitosis in the dividing root-tip cells of the onion. Then transversely across the stage-duration diagram, and parallel to the time-interval lines, are drawn at three time-intervals distant a series of lines marking observation-instants. This graphical presentation of the stage-durations (A. Diagram plotting the situation) lends itself to the actual counting of stages and to measuring their several lengths, thus providing data adequate, by simple arithmetical calculations, to determine the average relative duration (A. R. D.) and the average absolute duration (A. A. D.) of each stage type plotted. Also, it makes possible the construction of Table B, which appears on the lower half of the same chart. The data for this table are secured solely by counting the different mitotic stages (including the resting stage) at the successive periods passed through by the observation-instant lines. From the data thus secured the average relative and absolute durations of the several mitotic stages are calculated.

It is evident that the calculations of the average relative and absolute durations made from actual counting and measure are the correct ones for the particular case presented. The general applicability of the results thus obtained depends entirely upon the representative nature of the sample used; but the reliance which we may place upon

[1] Professor C. S. Minot first used and defined the term mitotic index, "Age, Growth, and Death." Pop. Sci. Mo. 71: 510, 1917. It is the percent-measure of the total number of cells showing mitotic activity in a given sample tissue.

the determinations derived from Table B depends wholly upon the degree of their approach to the results obtained from the actual counting and measuring of the diagrammed stage durations (Diagram A, on the upper half of the chart).

APPLICABILITY OF PLAN.

Since we may, from the study of mitotically homogeneous tissues reared under the same conditions and killed instantaneously at regularly successive intervals, construct a table with all the mathematical properties of Table B at the bottom of the Method Chart, but can not, from directly observed timing of a mitotically active living tissue, plot the details of stage-successions as is done in Diagram A at the top of the chart, it is the immediate task to establish the reliability of measurements calculated from statistical data, as in Table B, and to demonstrate the general applicability of the principles and formulas used.

It is evident that if one kills and mounts, in accordance with modern histological practice, a tissue whose cells are actively dividing, the relative numbers of cells found in the several successive mitotic stages will be dependent upon two factors: (1) the percentage of cells actually dividing at the instant of killing; (2) the mitotic progress each particular cell has made since it began to divide.

If *all* mitotically active cells began to divide at exactly the *same* instant, and *all* had made the *same* progress, then but a single mitotic stage would be seen in the sample. If in, not a single tissue, but in many tissues wherein mitosis had begun at the *same* instant and had made the *same* progress, samples are taken at short time-intervals (shorter than the duration of the shortest mitotic stage), it is evident that, if the total counts per sample be equal, the summations of counts of each of the several types of cells in the whole series of samples will show the greater number of cells to have been killed while passing through the longer stages, and similarly a lesser number during the shorter stages. In such case it is further evident that if in the stage-sequence there is a stage whose length is shorter than the time interval between the observation-instants, it is possible that such a stage may be missed in the sampling, and since under the conditions above referred to all cells of the same sample are in the same mitotic stage, an increase in the number of cells counted in the sample would not supply a chance of including it, nor would such increase in the size of the sample have any bearing upon its representative character.

If, however, *most* of the cells had begun to divide at *about* the same time, and had progressed *about* evenly, an observation early in the process would reveal a *relatively* high number of early stages; similarly, a late observation would reveal a *relatively* large percentage of the late stages. The term *relatively* is here very important, for the cell-

numbers of an observation-instant selected at random, when the mitotic index has not been constant, depends (*a*) upon the number of cells in the sample having begun mitosis previously to the observation-instant, and (*b*) upon the mitotic progress each has made prior to the observation-instant. Thus, if a large number of cells had begun dividing at about the same time, but sufficiently remote and properly timed to bring each of them to a certain very short stage at the time of killing, and also the same number of cells had begun dividing at a different period of time, but properly removed so as to bring their mitotic progress to one of the longer stages at the instant of killing, the numbers of cells actually counted in these two stages in this one sample would be equal and would not, therefore, measure the relative duration of the two stages. If, however, in closely related tissues behaving mitotically in exactly the same manner, a series of samples be taken, both earlier and later than the sample above named, in the later samples the earlier stages become rarer and the later more numerous, and *vice versa* the earlier samples show a rarer number of the later stages and a greater number of the earlier ones.

But if cells of the tissues sampled had begun mitosis at different instants throughout the cycle of the mitotically most advanced cells sampled, at a random instant of sampling there would be found a confusing variety of mitotic stages. This is the situation plotted, and analyzed in the method chart, because (as previously stated) it approximates most closely the actual mitotic condition in the growing root-tips of the onion. As a matter of common knowledge, these differences are known to represent a cross-section and instantaneous view of many cells in varying stages of mitotic progress. Because in the plan followed (*a*) the series of samples is fairly representative of the whole mitotic sequence, and (*b*) the total number of cell-counts per sample, regardless of the mitotic stage, is large and constant,[1] an examination of the method chart shows that even when the mitotic index (M. I.) fluctuates greatly, and the successive stages are of varying durations, these differences coincide and average so that throughout the sampling the summation of the counts of a given definite mitotic stage measures, in proportion to the total number of cells counted for all stages, the average relative duration (A. R. D.) of this particular stage. Thus, not only the duration of the stage but also the mitotic progress which each cell has made up to the instant of sampling must be provided for in any statistical analysis of mitotic progress.

Further, if in this same set of mitotic conditions, sampling and counting, the observation-instants are further removed from each other than the duration of the shortest mitotic stage considered, it is possible that the sampling may omit such stage altogether, but the probability of its being included increases with the number of counts

[1] If not constant, correction can be made by means of the Stage Index (S. I.) (see p. 9).

per sample; and even in this case of very short stage-length, if the sample be large, the stage-length is proportional to the summation of its corrected counts. Unlike one of the hypothetical conditions earlier described, wherein mitotic progress runs exactly parallel in all of the mitotically active cells, in the present case of fluctuating mitotic indices and variously beginning mitoses, the representative character of the sample and the accuracy of the determinations are increased with the number of cell-counts per sample.

STAGE INDEX.

The stage index (S. I.) simply casts into percentage the actual count of each of the several mitotic stages observed in the sample. Thus, for arithmetical purposes, correction is made for the population or size of the sample and for fluctuation in the mitotic index, if the resting stage be not included in the cycle. Mathematically the formula is stated as follows:

$$\text{Stage Index (S. I.)} = \frac{\text{No. of cells in given mitotic stage.}}{\text{Total number of mitotically active cells observed in the same fields.}}$$

If in each sample the cell-count continues until 100 dividing cells are counted, the stage-counts are directly proportional, each to each, to the stage indices. If, however, the count be continued until 100 cells, including the resting cells, are tallied, and the stage index refers to the percentage of the cells actually dividing, *i. e.*, if the resting stage be not included in the cycle, then, as in the first treatment of the actual studies presently to be set forth on mitosis in the root-tip cells of the onion, such simple proportion does not hold good and the stage index must be calculated for each count.

MITOTIC STAGE DURATION AND TIME COMPLEX FOUND IN THE DIVIDING ROOT-TIP CELLS OF THE ONION.

Finally, we come to the actual complex of mitotic conditions found in the growing root-tips of the onion, namely: (*a*) fluctuating mitotic index, implying variation in the numbers of cells beginning and ending mitosis at successive instants; (*b*) stage-lengths varying in successive order in the same cell; (*c*) variations also among stages of the same order in different cells; (*d*) closely parallel mitotic processes in different but similarly appearing root-tips of the same bulb.

FORMULA FOR DETERMINING THE AVERAGE RELATIVE DURATION OF A GIVEN MITOTIC STAGE.

Having determined the effect of each of these complicating factors *a*, *b*, and *c* (factor *d* is treated on p. 13) upon the cell-count of successive mitotic stages, and made corrections for each, we find that if samples of tissues mitotically active as above described be taken at regular and short intervals throughout at least a considerable portion of one cycle,

and if corrections be made by means of the stage index for mitotic index fluctuation and the size of the samples, the summation of the percentage-frequencies (that is, of the stage indices) of a given stage in the several successive samples will measure the average relative duration (A. R. D.) of that particular stage. The one additional complication (factor *c*) not present in the last hypothetical case does not change the rule for this particular type (*i. e.*, average) of measurement. Mathematically stated, the formula for this determination is:

$$\text{Average relative duration (A. R. D.) of a given mitotic stage.} = \frac{\Sigma \text{ S. I. of the } \textit{given} \text{ stage in } \textit{all} \text{ observations.}}{\Sigma \text{ S. I. of } \textit{all} \text{ stages included in the cycle, in the same fields.}}$$

This equals also the average stage index for the particular stage in the series of samples.

Let us now consider the average absolute duration (A. A. D.) of the several mitotic stages. If the mitotic index did not vary, but remained constant throughout the day, and the coefficient of variability for the duration of stages of the same order were low, a single root-tip sample, just as accurately as many samples, would supply data for measuring the average relative durations of the several stages. The accuracy of such measurements would vary with the square root of the number of cells counted within the sample or samples. While such a condition of constant mitotic index would, if it existed, greatly simplify the determination of the average relative durations, it would debar entirely, by the method herein used, the determination of the average absolute durations of the several stages.

It is fortunate, therefore, for the particular investigation in hand, that such fluctuation in the mitotic index really exists in the growing root-tip of the onion. For, in order to make this latter measurement (A. A. D.), it is necessary first to trace through a succession of mitotic stages and time-intervals a definite, recognizable mitotic wave. The conditions conducive to an accurate measurement of the average absolute duration of the several mitotic stages depend upon (*a*) the suddenness and greatness of change in the number of cells beginning the mitotic process during the period of observation; (*b*) the greatness of the number of such waves; (*c*) the greatness of the number of stages traced through each individual wave (if fractional lengths of waves are used and if they are not equally distributed over the whole cycle, they must be applied only in determining the A. A. D. within their respective sections); (*d*) the greatness of distance apart of these waves, especially if some of the stages involve a high percentage of the entire cycle; (*e*) the approximation to constancy in durations of the mitotic stages of the same order.

While variations in none of these five factors would impair in the least the determination of the average relative duration of the several

mitotic stages, the character of each is vital in finding the average absolute duration. And, since the relative duration for all of the several stages is so readily and accurately determinable, it suffices to find only the average absolute duration for a few stages, whereupon determining these latter durations for the whole cycle of stages is a matter of simple calculation. This, also, is indeed fortunate, for, if the waves were closer together than the time-period measuring the duration of the longest mitotic stage, the curves marking their progress would in the longer stages become inextricably tangled. The phenomenon of one wave running into another, thus destroying the recognition of the identity of both in their further progress, may well be called *jamming*. Thus, in the studies made on the onion root-tip it was found advisable to eliminate (for the purpose of tracing definite waves, but not for measuring the average relative durations) the resting stage, which consumes a large percentage of the duration of the entire cycle. In some cases even stage 1 (which, in the onion, when the growing temperature was 30° C., was found to be of even longer duration than the resting stage) may have to be eliminated in order to prevent jamming, but, as was seen above, such elimination does not preclude the determination of the absolute duration of a definite portion of the mitotic cycle, and thence by simple calculation of each definite stage.

PROCESSION INDEX.

Throughout the actual studies on the onion, as in the Method Chart, it was found necessary for the purpose of locating mitotic waves, to calculate for each stage-count not only the stage index (S. I.), but also a procession index (P. I.). The stage index corrects the deviations from the actual wave-course in the stage index table in so far as such are caused by differences in the size of the samples and by variation in the mitotic index. Such correction lends itself directly to the purpose of calculating the average relative durations, but it does not possess properties enabling one, by connecting high values in a succession of such indices (S. I.), to trace a mitotic wave through a succession of time-intervals and stages in which the stage-lengths of different orders vary to any considerable degree. It is necessary, then, for wave-tracing purposes, further to correct the stage-index values by taking into consideration the average length of each stage into which the cycle is divided. This correction is accomplished by means of a *Procession Index* (P. I.). In order to secure this (*i. e.*, the P. I.) for a given count, the stage index (S. I.) is divided by the average relative duration (A. R. D.) of the particular stage. Thus cross-sectioning partially corrects the differences in magnitude of the successive values of stage indices in the path of the mitotic wave, due to the differences in length of the several stages. The correction is complete in latitude and longitude, but is only partial in altitude; it suffices to

trace the wave much as one follows a mountain range, with considerable certainty, but not expecting each successive peak to reach a uniform altitude. In this connection the critical student will examine the procession index tables (Nos. 12, 13, and 14) with the greatest care. He will satisfy himself concerning the definiteness—*i. e.*, the outstanding clarity and unbranching continuity of the waves as indicated by the connecting lines. Also he will seek especially to determine whether the absence of data for observation-instant number 12 in the 20° C. series and for number 2 in the 30° C. series impairs or destroys the possibility of accurate range-tracing.

Theoretically, the proper correction of the stage-length, in order to eliminate the difference due to variation in the duration of the several stages, would consist in subtracting from an increased stage index of a given stage, at a given instant of observation, the stage index of the same stage for the next previous observation-instant. Thus corrected, the stage indices would provide a wave of procession indices passing through successive stages and time-intervals and connected by points registering the *same* magnitude. But such mathematical procedure would be possible only in case the normal stage index (that is, of those cells not in the new wave) of each stage in every sample were always proportional to the average relative duration of its own stage. In such a case the procession indices for all stages and time-intervals not in the new wave would be *zero*, while those for the new wave would be marked throughout by points of *equal* magnitude. It is easily determined by the actual counting and classifying of mitotic stages in onion root-tip cells that there exists no such condition as follows: Uniformity in the mitotic index for a considerable number of minutes, then suddenly a much larger and a definite number of cells begin to divide and progress in a thoroughly parallel manner to the end of their several mitotic processes, then at the completion of mitosis, by the suddenly increased number of cells, the mitotic index drops to exactly the same level as existed before the sudden beginning of the new wave. But rather, the facts are, in the material studied, that the mitotic index rises and falls continuously and in small increments, only occasionally presenting a major wave, and even then none too easily recognizable.

All this complicates but does not prevent the location of definite mitotic waves; but we have to be satisfied with a mountain-range effect instead of a dead level in the corrected heights of the points tracing such waves. The formula finally developed for the procession index is not the subtraction-rule above referred to—the actual mitotic complex in the material used precludes that—but is a ratio-rule which, as demonstrated immediately hereinafter, accounts for all of the complicating factors and gives the wave-effect sought. Mathematically stated, the formula for the procession index used is:

$$\text{Procession Index (P. I.)} = \frac{\text{S. I.}}{\text{A. R. D.}}$$

MITOTIC SYNCHRONIZATION IN HOMOLOGOUS TISSUE-SAMPLES.

In connecting procession indices of highest values, one observes that such connecting lines run in the direction expected—that is, they trace, as on the crest of an actual mitotic wave, progressively through successive time-intervals and mitotic stages. This is one of the cardinal proofs of the adequacy of the scheme of attack here followed, because it demonstrates conclusively that the greatest theoretical handicap of the plan (namely, the possibility that the mitotic processes are not running approximately parallel in the homologous tissues sampled) does not exist. For if such parallelism did not exist, no such orderly procession, as is here traced, would be possible.

Additional evidence that, in homologous samples, mitotic processes do run parallel is found in the work of Ward,[1] Kellicott,[2] and Karsten.[3] They show that in mitotically active tissues there is rhythm and, moreover, that the high points of such pulsations occur, more or less specifically, for the same tissues grown under the same conditions, at definite periods of the day. Further evidence consists in the fact that in the growing root-tips of the onion the different tips from the same individual onion, grown under the same condition and having attained the same length and appearance in the same period of time, must have passed through processes of cellular growth and mitosis practically in a parallel fashion. A discussion of an index of mitotic homogeneity is presented later in this paper (p. 30).

CAUTIONS IN METHOD.

There are two other features of the mitotic cycle which should be considered in their bearing upon the relation between stage-count and average relative duration:

(1) The cycle begins in a single cell, while at the end of stage 10 we find, in place of one mitotically active cell, two resting cells. Must rectifications be made looking toward a correction in the determination of the average relative duration of the resting or other stages on this account? The origin of the cells observed makes no difference in the fact that the longer they, on the average, remain in the resting or in any other stage, the more apt they are to be found in that stage at a subsequent random observation-instant. If (*a*) the number of cells in the tissue sampled be small, and (*b*) all must be counted, and (*c*) all mitotic sequences in all cells synchronized exactly, the law of averages would not take care of this doubling factor in its bearing upon average relative duration; but in the tissues studied only a small fraction of the cells were used, and the mitotic indices of these tissues had been fluctuating

[1] Ward, H. M. "On the biology of *Bacillus ramosus* (Fraenkel), a schizomycete of the River Thames." Pro. Roy. Soc. 58: 265–468, 1895.

[2] Kellicott, W. E. "The daily periodicity of cell-division and elongation in the root of *Allium*." Bul. Torr. Club, 31: 529–550, 1904.

[3] Karsten, G. "Über embryonales Wachstum und seine Tagesperiode." Zeit. Bot. 7: 1–34, 1915.

greatly for many previous generations, so that this otherwise sudden doubling effect is entirely lost—scattered over long time-intervals—in the average. The fact that there is a mitotic cycle is, in the kind of study here made, of biological import only. Mathematically, the resting stage and the ten arbitrarily marked subsequent divisions of its mitotic course might just as well have been eleven successive sections from the middle of an indefinitely long process.

(2) There is always a chance that a cell permanently—so far as mitosis is concerned—set aside in the root-structure may be included in the counting. Such inclusion, in the statistical method here followed, would tend to lengthen the average duration of the resting stage, as indeed it should (but would not make it indefinitely long, as would actually timing each cell by the direct observation method); but since this study is primarily one on mitotically active cells, it was sought to eliminate this factor by (*a*) confining the cell-count to cells within two root-tip diameters of the extreme tip, and thus to avoid the region where many non-dividing cells are being left behind; and (*b*) by basing the calculations first upon the ten mitotically active stages and later upon the cycle as a whole.

ADEQUACY OF THE PROCESSION INDEX.

The adequacy of the procession indices and the inadequacy of the actual counts and of the stage indices, to trace mitotic waves which are plotted graphically in Diagram A of the Method Chart, are shown in Table B of the same chart. The solid line through Table B traces an attempt to follow a mitotic wave through successive time-intervals and stages by connecting the high points in the actual count. It can be seen at a glance that by this method, in the situation here plotted, one wave is early confused with the other, and that thereafter the whole is incapable of further analysis.

The line of dashes indicates a similar attempt to trace the same mitotic wave by connecting the highest points of the stage indices. In this case the correction is made for difference in (*a*) size of the sample, and (*b*) variation in the mitotic index. If several successive stages were of approximately the same length, this indeed would suffice to trace the wave (as would in fact the actual cell-count, if also the samples consisted of the same number of counts); but in the stage index a processional correction is not made for variation of length of successive stages of the same cell. One sees, by examining the Method Chart, that tracing by count or by stage index is satisfactory until one comes to stage 4, a very short stage compared with the previous ones. Neither the actual count nor the stage index can, in tracing a wave, cross such a stage—the bridge is shorter (1.2 min. to 4.7 min.) than the width of the chasm (10 min.). Thus, not only the stage-count but also the stage-index method of wave tracing fails.

The dotted line attempts successfully to follow a mitotic wave through successive time-intervals and stages, as one may judge by comparing the actual plotting of the stage successions in the diagram with the wave traced in the table, for here the final correction (in addition to that of the S. I.), namely, that for variation in the average relative duration of the several stages, is partially made. One may glance at this diagram and with the eye readily trace the course of two mitotic waves; first the complete wave (No. 2) in the middle of the plot, and second, earlier than this one, what appears to be the ending of another (No. 1). Then, comparing such actual waves with their mathematical treatment in the table below them, one's confidence in this statistical method of tracing mitotic waves is established, especially since the later stages of the earlier wave overlap, in the same observation-instant, the earlier stages of the later wave. In counting and classifying cell-stages in an isolated sample, this overlapping presents hopeless confusion; in the diagram the counts of successive samples begin to coordinate in orderly manner; but only in the procession indices (P. I.) of the statistical table (B) are the analysis and reorganization of the mitotic pulsations definitely achieved.

FORMULA FOR THE AVERAGE ABSOLUTE DURATION OF A GIVEN MITOTIC STAGE.

The locations of the waves having been established, the duration of definite sections of the cycle is determined in each particular case by counting the number of time units passed through by the particular wave traced, and the average duration of a single stage by dividing the number of time units by the number of stages the wave passes through. In case sections of cycles are included in such determinations, they must on the average equably cover the entire cycle, for in each case a given section of a wave subtends its component stages which may be of varying durations. The average is then made of these several determinations. The average absolute duration for the cycle is calculated by multiplying the number of stages in the cycle by the average absolute duration per stage. The average absolute duration of a particular stage is then determined by multiplying the percentage measuring the average relative duration of the particular stage by the number of time-intervals measuring the average absolute duration of the entire cycle. Mathematically stated, the formulas for the average absolute duration of the entire mitotic cycle and for a particular stage are:

$$\text{Average absolute duration of entire active mitotic cycle (A. A. D. of C.)} = \left\{ \Sigma \frac{\text{Time periods elapsing between two points in a recognizable procession of P. I.}}{\frac{\text{No. of stages covered.}}{\text{No. P. I. waves followed.}}} \right\} \times \left\{ \begin{matrix} \text{No.} \\ \text{of} \\ \text{stages} \\ \text{in} \\ \text{cycle.} \end{matrix} \right\}$$

$$\text{Average absolute duration of a given mitotic stage (A. A. D. of S.)} = \text{A. A. D. of C.} \times \text{A. R. D. of S.}$$

MEASURE OF ACCURACY.

Reverting once more to the Method Chart, we find that by actual count and measure from the diagram, the average relative durations of the five stages run: 0.3213, 0.2609, 0.2167, 0.0398, 0.1611. The same measurement, that is, the average absolute durations of the several stages calculated from the stage indices of Table B, are: 0.2912, 0.2843, 0.2171, 0.0352, 0.1719. Similarly, by actual count and measure from the diagram, the average absolute duration of the stage series measures, in time-units: 13.84, 11.24, 9.70, 1.78, 7.66; a total for the cycle of 44.25; an average of 8.92. While the same measurements calculated through Table B give: 13.32, 13.00, 9.93, 1.61, 7.86; a total of 45.74; an average of 9.14. The close approximation in this test case, of the series of results derived from the table to those calculated from first-hand count and measure in the diagram, establishes the general validity of the principle followed and demonstrates that results secured from such tables alone may be expected to approximate the truth within a relatively small error, provided that the size and representative character of the sample and the closeness and number of observation-instants in an actual case are comparable (in relation to their stage and cycle durations) to the same relations in the hypothetical case. Or, presenting the principle in another manner, granted that the diagram is correct (an exact picture of a representative sample actually taken), Table B derived from it will approximate it in proportion to the greatness of the number of observation-instants. Only by chance would the determinations of the table and the diagram be exactly the same.

The relatively small fluctuation in the duration of average stage length among the waves actually traced (see lower left-hand corner of charts 12, 13, and 14) indicates a consistency in turn indicative of accuracy in measurements and deductions.

We know that if in an actual case we find a definite percentage of cells in a given stage at a given observation-instant, and at the next observation find this percentage changed, there is a net difference, but just where in the interim between observation-instants each particular cell-stage changed we do not know. The closeness of the observation-instants tends to lessen the error due to this fact.

The facts bring us again (see p. 7) to this: From the data secured in observing homologous dead material killed at regularly successive time-intervals, we can not plot an exact diagram of mitotic stage succession in a given cell; nevertheless we can construct the exact anolog to Table B (Method Chart) with all of its mathematical properties, including its characteristic close approach to the actual facts. This is what was done, and thus the data are supplied for the determinations

in both the preliminary and the fuller investigations reported in this paper.

The governing maxim in these studies has been: A maximum of biology and a minimum of mathematics. Continual recourse was had back to actual biological fact. Biometrical formulas mathematically derived are mathematically correct, but if in course of their development a single false biological factor enters, all subsequent derivations are false. Full cognizance of this danger is in mind as the accompanying principles and formulas are set forth. They are nevertheless presented with the confidence that they are sound, both biologically and mathematically. We may safely say that although we can not see the mitotic details in actual process of transformation we may determine the average duration of the successive mitotic stages with fully as great accuracy as would be possible if we were able to follow the normal and unhampered mitotic train directly with our eyes (see charts 1 and 6).

The work of developing the statistical method of interpreting from dead material the facts concerning stage duration in live material and that of conducting a series of preliminary cytological experiments were, of necessity, carried on at the same time; for thus only could these two phases of the investigation mutually suggest and correct. The work was undertaken with the feeling that there must exist a definite mathematically determinable relation between the number of cells found in a given mitotic stage at a given time and the relative duration of that particular stage. The purpose was to find, demonstrate, and formulate such relationships.

To begin the work the only thing to be done was to count and classify the cell-stages in comparable samples of mitotically homogeneous tissues killed in successive order. So far as development of the statistical interpretation was concerned, it was possible only to construct charts and diagrams plotting different hypothetical condition-complexes in reference to mitotic activity, and then inductively from these to work out the mathematical properties of each factor contributory to the complex relationship between the cell-counts as distributed among specific stages and the average and absolute durations of their respective stages. Unless, indeed, one can see and retain in mind the set of complications involved in each different situation, it would seem that such plotting and coordinating of situations in accordance with known biological facts constitute the only safe method of procedure in developing formulas adequate to solving this particular problem. The properties and usefulness, for the end sought, of several of these situation-complexes are summarized in an accompanying table (No. 2) bearing the title "Properties of four condition-complexes in reference to mitotic indices and stage durations." These are way stations reached in seeking the final solution.

PRELIMINARY EXPERIMENTS.

In the first experiments the samples used were the growing root-tips of a reddish commercial onion about 1.5 inches in diameter. They were sprouted in water at an ordinary room temperature which during their period of growth fluctuated around 18° C., thus preventing the possibility of eliminating the temperature factor, but that was not the purpose of the initial study; temperature effects were to be considered in a later investigation. After 5 or 6 days the root-tips had reached a length of 5 to 10 mm. Thirteen samples were taken at 10-minute intervals, from 10 a. m. until 12 noon on the same day early in February 1916. Each sample was dropped immediately into a numbered vial of Fleming's fluid, and each was duly prepared, sectioned longitudinally (6 microns), mounted and stained with Heidenhain's hematoxylin. Then, within two root-tip diameters of the extreme tips, that is, in the mitotically most active region, microscopic fields were selected at random in which the cells were counted and classified as to the stages of their mitotic progress. In each of the 13 successively cut root-tips 1,000 cells, including both those mitotically active and resting, were observed and classified. The same 10 active mitotic stages which were used in the subsequent and fuller study constituted the basis of classification.

The accompanying Summary Chart figures and describes each of these arbitrarily marked sections of the mitotic cycle. Since the mitotic process is a continuous one, there are as many stages in its course as one may care to mark; nevertheless there are striking transformations which appear to occur with relatively great rapidity, and hence their beginnings and ends make suitable mile-posts for studying and comparing absolute and differential progress. When less numerous divisions are required, cytologists generally have named the stages of the mitotic cycle as follows: (1) resting, (2) prophase, (3) metaphase, (4) anaphase, (5) telophase. In these studies ten stages were marked off with arbitrary but definite boundaries in order to provide a more refined analysis of the mitotic cycle than the usual fewer and more indefinite stages just named imply.

AVERAGE RELATIVE DURATIONS OF THE SEVERAL MITOTIC STAGES.

PRELIMINARY EXPERIMENTS.

Applying the principles demonstrated in the method chart, the stage index chart of the preliminary work gives for the average relative durations of the successive stages the following series:

0.4473, 0.2218, 0.0933, 0.0266, 0.0077, 0.0096, 0.0089, 0.0281, 0.0367, 0.1196

These results are based upon 13,000 individual cell-counts, and if the

total population of the several samples were the one controlling factor, these findings would consequently be much more to be relied upon than the total of 708 counts recorded in the Method Chart; but in evaluating the accuracy of these results it must be borne in mind that (*a*) the number of individual cell-counts, the greatness of which tends to increase accuracy, must be considered; (*b*) the greater the number of stages into which the mitotic cycle is divided the greater the chance of error; (*c*) the greater the number of observation-instants the greater the accuracy of the determination; and (*d*) the shortness of intervals between observation-instants conduces to greater accuracy.

PROBABLE ERRORS.

These four factors all tend, in so far as their bearing upon accuracy is concerned, in the directions above indicated, but their incorporation into a single accuracy-measuring mathematical formula has not yet been accomplished. Indeed, none of the several probable-error formulas now used in biometrical study will apply here. In planning the later studies cognizance was taken of the directions in which all of the aforenamed accuracy-factors operate, and the conditions of experimentation, so far as possible and feasible, were modified in accordance with these teachings to make for greater precision in the determinations.

The probable error is a measure of accuracy for certain classes of data, but when (*a*) the data in hand are not from material homogeneous throughout the sampling, or (*b*) the values involved fall below 5 or 6 per cent, or (*c*) if the absolute numbers of individuals in the several classes of the series are low, the probable errors as now calculated are not valid.

The mitotic index is found by applying the following rule:

$$\text{Mitotic index (M. I.)} = \frac{\text{Number of cells dividing.}}{\text{Total number of cells (both resting and dividing) observed in the same fields.}}$$

In these studies on the duration of the several mitotic stages in onion root-tip cells only the mitotic indices lend themselves to the usual probable-error corrections. This is because they alone, of all ratio-results here presented, are measured by high percentages derived from relatively large numbers. But even in case of the mitotic indices each probable error so calculated is comparable with no other like determination of the series, because in each case the material is characteristic of a given time of day, *i. e.*, of a given instant in the mitotic rhythm, and of a given temperature—that is, the population is homogeneous in the given sample only. Nevertheless, the probable-error formula applicable in each particular case is:

$$E_{M.\,I.} = \pm 0.6745 \frac{\sqrt{P_0 \times P_1}}{N}$$

In which P_0 = percentage of cells dividing, P_1 = percentage of cells (dividing and active) in the same field, N = population of sample. The determination of standards with which to compare such probable errors would naturally be a part of any investigation seeking to develop a coefficient of mitotic homogeneity. (See p. 30.)

If a probable error could be calculated for each of the several stage indices of these determinations, it would greatly simplify the calculations of such a measure for all of the subsequently calculated values, because a stage index is an element in each of them. While the stage index is of the same nature as the mitotic index, and normally should be subject to the same probable-error formula, still it is not so easily corrected, for, as a general rule, the values of the stages indices fall below the critical point, namely, 5 or 6 per cent.

The fundamental principles upon which the determination of this study are based are demonstrably sound, but it is not possible, in the present stage of biometrical science, to supply formulas which will measure mathematically the approximation to the actual values of the several calculated determinations. Some other common-sense method of establishing our confidence in their degree of accuracy must be applied; so let us continue by the comparative method to gage the accuracy of the determinations of the hypothetical case, the preliminary study, and the completer experimentations.

It is quite evident that the determinations of the average absolute duration will possess a greater relative error than do those of the average relative duration, because the absolute value of a given stage is based, (1) upon the absolute duration of the whole cycle, which itself is subject to an error, and (2) upon the average relative duration of a given stage, which also possesses an error. An element in reducing error in the average absolute duration is the greatness of the number of waves traced. In the hypothetical studies, in which temperatures were constant, 6 waves were traced through the series grown at 10° C., 6 through that at 20° C., and 7 through that at 30° C.

Taking into consideration only the total populations of the samples, we find that if the populations sampled be homogeneous throughout, accuracy (or the approximation to the truth) is not directly a function of frequency or numbers, but is a function of the square root of such frequency. One must, therefore, if he would halve his approximation to the truth, quadruple the quantity of his observational data. Since in the preliminary study there were 13,000 cell-counts, or 18.35 times the 708 of the Method Chart, it is clear that if the data were taken from a homogeneous population (which is not the present case) the determinations based upon the 13,000 counts would in their approximation to the truth deviate on the average only $\frac{1}{\sqrt{18.35}}$ as far as those based upon 708 counts. In the final studies of this investigation, the first series consisted of 19,000 counts, 26.77 times the number of the Method

Chart, and consequently deductions from such data would be expected, on the average, to vary only $\frac{1}{\sqrt{26.77}}$ as far from the actual values; but other factors enter.

In the hypothetical study 708 cell-counts were distributed among 27 observation-instants and 5 mitotic stages. In the preliminary study, which was made on onion root-tips, 13,000 cell-counts were distributed over 13 observation-instants, and classified among 11 stages (10 active and 1 resting); while the final study consisted of a total of 55,000 cell-counts divided into 3 subordinate studies, the first with 19,000 counts and the second and third with 18,000 each. In the first the counts were distributed over 19 observation-instants and among 11 (10 active and 1 resting) mitotic stages; the second and third were each distributed over 18 observation-instants and among the same 11 stage-types. As was earlier pointed out, until all these factors have been joined in an accuracy-measuring formula, we must be content to balance in judgment the factors which later may be balanced mathematically and with the highest efficiency. In our experimentations we can, therefore, in the interest of accuracy, only increase as much as feasible the quantity of each type of data in the direction proven to make for the reduction of error.

OTHER SOURCES OF POSSIBLE ERROR.

But it must not be concluded that all of the sources of error in a study of this sort are traceable to lack of extreme refinement in statistical methods. For instance, the matter of judging the individual cells and classifying them into their previously determined stages is important, especially since it is indeed difficult to draw a sharp line of demarcation between the end of one stage and the beginning of another. Moreover, in counting and classifying so many (55,000) cells, on the basis of mitotic condition (10 active and 1 resting stage) there is a possible source of error of interest both to biologists and psychologists; the criterion for classification are apt to undergo evolution in the observer's mind. This difficulty was attacked by establishing the criteria set forth in the three figures (see Summary Chart) for each stage marked off. From the examination of these it will be seen that the difference between the last condition of one stage and the first of its successor is very slight and is determined in most cases by a single point of difference, the principle being to characterize these stages not by general conditions descriptive of their means, but to set them off by clean-cut lines. If error crept into the determinations because of this difficulty, it would probably have come in between stages 1 and 2—that is, where the criteria for distinctions are the least well marked. We find in stage 1 but little acceleration in the 20° to 30° C. rise, while in stage 2 in the same temperature rise we find the largest velocity increment in the whole series. This compensating coincidence may lend color to the

theory that a confusion actually occurred here. If stages 1 and 2 actually respond about the same to heat changes, a clean-cut differentiation in classifying them in the early countings and a gradual unconscious evolution of conscious criteria in the later thousands, in which stage 2 was crowded in favor of stage 1, would give the phenomena recorded. At no other point in the determinations is there such a difficult distinction to make, nor is there such another adjacent pair of values that might be accounted for by such an error. However, the much greater duration of stage No. 1 over stage No. 2 precludes the possibility of errors in their distinction, greatly changing the determinations for No. 1, the longer one. When we test this possible error by uniting stages 1 and 2 into a single stage, we find the following:

A. A. D. at 10° C. = 74.36 min.; at 20° C. = 67.49 min.; at 30° C. = 52.67 min. Q_{10} 10° C. to 20° C. = 1.10; Q_{10} 20° C. to 30° C. = 1.28

still giving a stage, sluggish like No. 1, in the 20° to 30° C. temperature-rise response. This indicates strongly that the values calculated for stage 1 are certainly quite correct and those calculated for stage 2 can not be challenged on the grounds of the immediate criticism, and therefore that the striking difference in their calculated temperature reactions is real.

AVERAGE ABSOLUTE DURATIONS OF THE SEVERAL MITOTIC STAGES.

PRELIMINARY EXPERIMENTS.

A further examination of the Stage Index Table (No. 4) of the preliminary study reveals no recognizable mitotic wave passing through a succession of mitotic stages and time-intervals. This confirms the evidence of the Method Chart that connecting the high points of the stage index sequence through mitotic stages and time-intervals will not, in the situation-complex existing in the material used, suffice to determine the average absolute durations of the several stages. The procession indices of the preliminary study were worked out in accordance with the principles analyzed in detail in the Method Chart, and the result shows clearly 3 different progressive waves passing, as would be expected, in an orderly manner through successive mitotic stages and time-intervals. The calculations from these 3 waves give the average duration of the entire mitotic cycle of these 10 active stages to be 172.2 minutes. Dividing this value in proportion to the average relative duration of the several stages, the average absolute duration of the 10 successive stages is as follows (in minutes):

77.02, 38.19, 16.06, 4.58, 1.32, 1.65, 1.53, 4.83, 6.31, 20.59

These results are based upon large portions of 3 waves, while those in the Method Chart were based upon only 2 waves. If, as is seen, the average

absolute durations of the several stages of the Method Chart thus calculated approximate so closely the correct values obtained through actual counting and measure, one is justified in concluding that portions of 3 waves based upon 16 times as many individual cell-counts, although upon twice as many mitotic stage types, and $\frac{13}{27}$ as many observations, would probably as closely approximate the actual facts.

The average relative duration of the resting stage in this preliminary work proves to be 66.12 per cent of the entire cycle, when such cycle is conceived to consist of both the resting stage and the 10 mitotic stages, thus crowding the 10 active stages into 33.88 per cent of the 11-stage cycle. Consequently, the average absolute duration of the resting stage, during the period sampled, is 336.06 minutes, and that of the entire cycle (including the resting stage and the 10 active stages) is 508.26 minutes,[1] which (so far as the number of cells of the region sampled is concerned) means a doubling in about 8 hours, near neither the minimum nor the maximum for such processes.

A word of explanation is perhaps necessary concerning that chart (No. 7) of the preliminary study entitled "Graphs showing orderly succession of procession indices." This chart is simply another method of showing the data tabulated in the Procession Index Table (No. 6) of the same study. The 3 recognizable mitotic waves are traced by the heavy lines connecting successive stages through time-intervals. A heavy line begins at the highest point in the early periods of sampling attained by one of the highest indices of the region. If, by chance, as in wave 1, this happens to be the index for stage 1, at 10^h20^m a. m., the next crest touched must be later than 10^h20^m, and must be that for stage 2, and so on. Thus we connect stages 1, 2, 3, 4, and 5 in one of the straightest lines of the tangle. Wave 2 begins with stage 4, at 10 a. m. This presents a single backward step in that the crest of stage 6 is not quite so far advanced as for stage 5; but, on an average, this line, too, is relatively level. Similarly, wave 3 begins at 10^h10^m a. m. with stage 7, connecting the highest point in the region successively for stages 8, 9, and 10, in not so level a manner as waves 1 and 2, but still relatively so. Indeed, the comparison of the high points of the mitotic wave to the peaks of a definitely traced mountain range holds good in this first actual study. The procession index corrects the stage indexes through the successive periods of a given mitotic wave strongly in the direction of uniformity, but never completely reaches it. They (the procession indices) are the best available means of unraveling the mitotic tangle in the material used, for if, as in the Method Chart, one attempts in this actual study a similar wave tracing in the chart (No. 5) "Graphs showing mitotic and stage indices," he is hopelessly lost. (See pp. 11 and 14.)

[1] If comparison be made with the determinations of the final experiments reported in this paper, account must be taken of the facts that the two experiments differed in temperature, in season of the year, and in variety of onion used (see p. 26).

EXPERIMENTS TO DETERMINE EFFECTS OF TEMPERATURE INCREMENTS UPON THE SEVERAL MITOTIC STAGES.

The results of the preliminary study with the 13 successively taken samples of 1,000 cells each accord with common-sense expectations in reference to the durations of the several stages. Also the ends sought by this investigation lend themselves so completely to a simple cytological and demonstrable mathematical method that it appeared inviting to continue the study with a view to making practical use of the method developed in measuring accurately the effects, in an actively growing tissue, of some selected and controlled environmental factor upon the relative and absolute durations of the several successive mitotic stages and upon the mitotic cycle as a whole.

THE VELOCITY OF CHEMICAL REACTIONS: RESPONSE TO TEMPERATURE DIFFERENCES.

The mitotic process is, no one doubts, a complex of physical and chemical activities. It is known that, in homogeneous chemical systems, within limits generally from 10° to 40° C., the velocity of a chemical reaction is about doubled or trebled for each rise in temperature of 10° C. This is van't Hoff's law, which experimental physiologists have tested out in reference to so many vital phenomena. It was, therefore, decided to select the temperatures 10°, 20°, and 30° C. for the purpose, not only of determining the effect of these different temperature-increments upon mitosis, but also in order to make comparison in reactions to temperature-increments between mitosis and homogeneous chemical reactions. Furthermore, the temperatures selected present two periods of 10° C. each, both still within the growing temperature-range for plants, 30° C. approximating, but still a little lower than the optimum, and 10° C. well above the minimum for growth in the species selected for study. In general the botanists claim that the range for protoplasmic activity in plants varies from zero to about 50° C. As a rule, at a temperature below zero the protoplasm is killed by freezing, and above 50° C. is killed by "heat rigor." Of course, it would have been possible to have tested out van't Hoff's law by making studies with smaller temperature-differences and applying the formula,[1]

$$Q_{10} = \left(\frac{k_1}{k_2}\right)^{\frac{10}{t_1 - t_2}}$$

but in the same quantity of sampling and counting it seemed advisable to increase the cell-count per sample rather than, at the expense of cell-count, to lessen the temperature-intervals. In the absence of a

[1] Snyder, Charles D., "A comparative study of the temperature-coefficients of the velocities of various physiological actions." Am. Jour. Physiol. 22: 311, 1908.

biological necessity of having to resort to the smaller differences, it seemed advisable also to select three temperatures, all between the minimum and optimum for plant growth, and also near the mean temperature most often found in reactions which obey van't Hoff's law. Another reason for basing the first practical measurements (in accordance with the method developed) upon temperature is that the latter is known to exert great influence upon growth, implying bulk increase and mitosis. It is, moreover, one of the external conditions most readily and precisely manipulated.

MATERIAL FOR THE EXPERIMENTS.

Advantage was taken of the facts presented and the experience gained in the preliminary study in planning and executing the completer one. The temperature-range having been decided upon, it is next necessary to select suitable material. The onion, having proven to be so well adapted to the sort of study in hand, was chosen for the completer investigations. Not only has it long been known to show mitotic rhythm, but it presents a homogeneity of samples not so easily obtained in other types of organisms. Their root-tips closely resemble each other and their mitotic processes were shown to synchronize. (See p. 13.) Moreover, one sample may be taken without disturbing the activity of the others, at least during the few hours of sampling. They are not difficult to prepare cytologically. Furthermore, the cells constituting the growing root-tip show comparatively little differentiation. Each possesses a large number of chromosomes, which fact (when the cells are longitudinally sectioned) makes the determination of arbitrarily marked mitotic stages an easy and definite matter. Finally the cells are large and the rate of mitotic activity permits convenient (10-minute) sampling intervals.

Bacteria, such as Ward[1] used in his investigations, divide rapidly, but their smallness and the imperfections of the views obtainable of their transformations render them inferior to many other materials. If one desires to learn how the details of certain other mitotic structures—for example, centrosomes which are not present in plant cells—are influenced during their mitotic transformations by various external agents, other materials would be necessary; but, taking all factors into consideration, the onion presents a very satisfactory source of material for the type of investigation here reported.

Many of the quantitative studies on growth have been based upon the lengthening root-tips of plants. This is suitable material, whether growth proper—*i. e.*, permanent bulk-increase—is considered alone or in relation to mitosis, for the root-tip grows chiefly in one dimension, namely, length. But very rarely do the cells divide other than trans-

[1] See ref. No. 1, p. 13.

versely, and all are about the same size. Thus the cell number, on the average, is roughly proportional to root-tip length in this actively growing tissue.

The onions used in these experiments were uniform in size and external appearance and, while they were purchased in the open vegetable market without their pedigree being known, they were of sufficiently uniform type and sprouted with sufficient uniformity to convince one that their genotypic constitution was quite uniform. An effort was made to divide a single onion into 3 equal vertical sections and to sprout the roots from each section under the 3 different but constant temperatures, thus eliminating a possible genotypic difference. It was found, however, that there were not enough root-tips of uniform size in each section to supply the demands of the study, 57 being required. Five onions were grown in each temperature-constant chamber. The 19 samples required for each temperature-series were cut from these five onions on the basis of uniform length and appearance.

APPARATUS: THERMOSTAT.

Constant temperatures in growing conditions were required and, in the absence of laboratory rooms with equipment especially designed for maintaining constant temperature, a special apparatus had to be built. This consisted of a battery of 3 constant-temperature boxes, each 1 foot by 1 foot by $1\frac{1}{2}$ feet in size, mounted longitudinally about a foot apart upon a board. Each box had a wooden top, bottom, and ends, but the front and back were inclosed with double glass doors. Underneath these chambers ran a wooden tunnel, heated at the extreme right with a small kerosene lamp. Since the CO_2 contents of the 3 chambers must be constant, the fumes from the lamp were not allowed to enter the tunnel, which was separated from the lamp-container by a zinc partition. Along the top of the chambers ran a similar tunnel, connecting from above with a well-insulated ice-box in which the cooling substance (crushed ice and salt) was confined to three-fourths of the space (left-hand) by a wire netting. From each tunnel into each box was an opening covered by a small copper lid slightly controlled by thermostats taken from Hoover incubators. The lids and thermostats were so adjusted that a rise in temperature lowered the lid which covered the warm-air opening, and uncovered further the opening from the cold-air tunnel. When the temperature fell, the reverse action was induced. A centigrade thermometer was inserted through a cork which filled a hole in the top of each chamber; the thermometer was long enough to extend into the water in which the onions grew. In each tunnel on each side of each box were hand-dampers controlling the size of the tunnel. It must be confessed that, even at best, this contrivance was was only partially automatic. In order to keep the temperature of each compartment within the range of 1° C. from the desired standard, it

required to be attended once every 3 or 4 hours during the entire 24; but it worked, and that was the essential thing. Thus the three compartments maintained temperatures at 10°, 20°, and 30° C., respectively, each with a fluctuation throughout the growing period of less than 1° C. above and 1° C. below the standard set. All other environmental factors, including lighting, were apparently very uniform in the three chambers. The machine was set in a cellar admitting light from the north only. In this room the temperature during the period of 3 weeks in which the thermostats were used did not vary more than 2° or 3° C. This aided the maintenance of constant temperatures in the three chambers.

The onions were sprouted in earthen quart crocks and were supported by floating wooden frames so that only the root base of each bulb extended into the water. When onions were first grown, February 1916, for the preliminary work, they sprouted most readily, but in August of the same year, when the constant-temperature apparatus had been built and was in working order ready to receive the onion, the seasonal conditions under which this bulb normally sprouts, or can be induced to sprout, evidently were past. In all 5 varieties of onions were tried out, but after 10 days none sprouted, but this time was well spent in learning to maintain constant temperatures. By the time constant temperatures had been attained in the three chambers, it was found that, after scoring them deeply, the small white onions of quite uniform character, commonly found in the fall vegetable markets, could be induced to sprout roots. (See p. 35.)

SAMPLING AND COUNTING.

As was seen earlier (see p. 22), at a temperature of 18° C. (preliminary study) the whole sequence of these 10 active stages of the mitotic cycle for the onion root-tips, studied during the approach to the natural growing season, occupied approximately 3 hours. This, together with the fact that the highest point in their mitotic activity appeared at 11^h40^m a. m., suggested that the most appropriate time for sampling, if one wished to cover a whole active mitotic wave, would be from about 10 a. m. until 1 p. m. This succession was, therefore, decided upon and 19 observation-instants were chosen, each 10 minutes removed from its predecessor, beginning and ending as above suggested. It is clear that a completer and more refined analysis could be made if the observation-instants were less remotely distant from each other; but it was desired to cover as large a portion of a whole mitotic cycle as possible and to make the cell-counts per individual sample as great as possible; hence the necessity, in the interests of accuracy, to continue the observation-instants in a series 10 minutes removed from each other. Whether this is really economy working for accuracy can be determined only when the relative influences of various factors (previously mentioned)

upon the probable error of the determinations are known. (See pp. 19 and 29.)

One thousand counts per observation having proven satisfactory, the plan of making similar counts was decided upon for the subsequent study.

The task of working out a coefficient (see pp. 13 and 30) of mitotic homogeneity, or synchronization in the mitotic area, was not undertaken, because the preliminary investigation showed in the Procession Index Tables an orderly succession of high points in mitotic waves through successive mitotic stages and time-intervals that would not have appeared had there not been a high degree of parallelism in the mitotic processes in the several samples taken. Judgment, therefore, dictated that it was necessary, in order to make for adequate accuracy, to include in the actual temperature-studies as many cell-counts as possible. Against this one possible handicap of having to use different cells to restore the sequence series, instead of being able to trace the succession of stages in the same cell—that is, in case the index of mitotic homogeneity or synchronization proved to be low—one must balance the fact that many hundreds of stained dead cells can be classed by the statistical method during the time that would be consumed by directly observing and definitely timing, even if it were possible, only a few cells actually moving through their mitotic stages. Remembering that numbers make for accuracy or, to be exact, that accuracy is a function of the square root of the population of the sample, we have only to increase the number of samples counted in order to increase the trueness of our statistical picture. In addition, as was stated earlier (see p. 5), the statistical method has the advantage of taking fresh and naturally developing tissue and killing it almost instantaneously, thus insuring relatively untampered-with normal samples.

On Saturday, September 9, 1916, the samples were taken. The root-tips were 5 to 10 mm. in length and varied but little in this respect in the three different constant-temperature chambers; but it must be remembered that growth and mitosis are different processes. The sampling began at 10 a. m. and, as was planned, continued at 10-minute intervals until 1 p. m., 19 observations in all. There was one person at each temperature-box and at the given signal an onion was lifted out and the root-tip quickly snipped with a pair of scissors and dropped immediately into Fleming's fluid. The temperature in the growing compartments did not vary so much as 0.5° C. during the 3 hours of sampling, although each chamber was opened 19 times; doubtless the volume of water in which the onions were sprouted aided in maintaining the constancy. The root-tips were embedded in paraffin and cut in longitudinal sections 6 microns thick, and were stained with Heidenhain's hematoxylin, due precautions having been taken, as in the preliminary work, carefully to label the vials in which the specimens were prepared, and finally to label the slides upon which the series were mounted.

In order to prevent confusion in counting and classifying the cells, which were viewed under the oil-immersion lens, the field was divided into quarters by means of hairs crossed in the eye-piece of the microscope. Thus in a field containing from 50 to 100 cells it was easy to keep one's bearings. No cells were counted twice, and all cells within a selected field were counted and classified.

Special attention is called to the Procession Index Tables (Nos. 12, 13, and 14). In calculating the average absolute durations of the several stages, only those waves were used which traversed in a definite manner at least three-fourths of the stages of the entire mitotic cycle. Some waves were cut off in their prime by the termination of the sampling at 1 p. m., and because the sampling had a beginning (namely, at 10 a. m.) other waves were found already well advanced. The portions of waves unused in the calculations are indicated by dotted lines.

There are two blanks in these tables, one in the 20° series for the sample at 11^h50^m a. m., and the other in the 30° series for the sample at 10^h10^m a. m. These samples were duly taken and fixed, but were ruined in preparation, so that while the results of the 10° series are based upon the determinations of 19 samples of 1,000 cells each, in the 20° and 30° series each is based upon only 18 samples of 1,000 cells.

In studying the results given in the several tables, attention is called to the fact that, for better comparison between mitotically active and mitotically inactive stages, in some cases the percentages are based upon a cycle consisting of the 10 mitotically active stages only, omitting the resting stage. In other cases the resting stage is considered as a part of the mitotic cycle. Thus, in making comparisons other than those set forth in the same tables, one must make sure that the data apply to the same definition of the mitotic cycle.

FURTHER DEVELOPMENT OF THE STATISTICAL METHOD.

The results of the experimentation reported in this paper invite future statistical investigations as follows:

(*a*) *To work out with more mathematical refinement the measure for accuracy (probable errors) of the formulas here given.*—This involves the determination of the interrelation between the accuracy of the calculations and (1) the size of the individual samples, (2) the number of observation-instants per series, and (3) the closeness of observation-instants; and the working out, as hereinafter suggested, of a coefficient of mitotic homogeneity or synchronization in the successive samples—all of which would permit not only the calculation of probable errors for the several determinations, but also would supply the basis for sound judgment in planning experiments. For example, if only a limited number of observations were feasible, it would enable one to choose, in the interests of accuracy, between closer observation-instants covering

less time and observation-instants farther removed but covering more time.

(*b*) *To find, if possible, a theoretically perfect procession-index.*—The one used in these studies is highly practical and reliable, but, as was pointed out (see p. 11) in the early part of this paper, it lacks certain theoretical refinements.

(*c*) *To work out a coefficient of mitotic homogeneity or synchronization.*—This could be done by sampling a number of similar-appearing root-tips from the same plant at the same instant, counting a large number (say, 1,000) of cells from each, classifying their stages, and calculating the percentage-frequencies of each, as was done in the study herein reported for successive samples. Then one should calculate through the series of samples, for each stage, the average percentage-frequencies. For each calculation, because the material sampled would be homogeneous, the usual probable error of the mean would apply. Then applying the formula $\frac{\%-E\%}{\%} = I.\,H.$, we would have a good index of mitotic homogeneity for each stage. These values could then be coordinated into a single index of mitotic homogeneity for the entire cycle of mitotic stages.

Karsten,[1] in his studies, appears to have taken 4 or 5 samples at about the same time and to have taken data from each of them, but from each sample his cell-counts are low, generally ranging from 50 to 100; which being distributed over the 5 mitotic stages which he used as a basis of classification, would make the calculation of their probable errors valueless. But by further inspection of his tables, one finds a constancy fully in accordance with expectation within the comparative smallness of his samples. This would lead one to expect, in a determination based upon large samples, a low probable error in a coefficient of homogeneity or synchronization. (See pp. 13 and 19.)

FURTHER ANALYSIS OF THE DYNAMICS OF MITOSIS BY THE STAGE-TIMING METHOD.

It would be desirable:

(*a*) To conduct experimentations similar to those here reported, but in which every qualitative feature would be more precise and every quantitative factor making for accuracy greatly increased. For instance: Temperature difference of 2° C. from 8° C. to 45° C. (or from the awakening to the maximum temperatures for growth in the particular plant selected), all other environmental factors constant; sampling at 5-minute intervals for 24 hours; 3 or 4 samples per observation-instant; genotypically uniform material; possibly a revision of the successive stages of the mitotic cycle used in this study; at

[1] See ref. No. 3, p. 13.

least 1,000 cell-counts from each sample. This would be a long and arduous task, possibly to be carried out best on a cooperative plan, but it would supply valuable and accurate standards for the further quantitative analysis of mitotic processes.

(*b*) To make studies on the duration of the several mitotic stages at the awakening and end of mitotic activity as affected by temperature changes; also on the effects of light, electricity, moisture, pressure, gravity, foods, and poisons upon stage-durations. Much qualitative work, but none of a quantitative nature, has been done in this direction; for instance, V. Sabline,[1] in subjecting the roots of *Vicia faba* to different temperatures, lack of oxygen, quinin sulphate, sulphuric ether, and other substances and conditions, noted their effects upon mitosis up to the instant of killing. The analysis of vital phenomena by *timing* mitotic stages thus modified is most promising.

(*c*) To follow the clue presented by the effect of temperature on stage 6, in constructing working models simulating this stage of mitotic activity, seeking by a temperature rise to weaken the tension of strands appearing to pull the chromosomes toward the different poles. Indeed, if such strands could be made to appear in a gelatine cell, by a current of electricity, the simulation would be all the more promising as a possible real parallel to mitotic force. (See p. 45).

(*d*) To time in detail the mitotic process, not only in cell-division characteristic of growth in undifferentiated tissue, as in this study, but also in cell-division in tissues undergoing differentiation.

(*e*) To make studies in cell-size, cell-number, mitotic activity, and bulk-increase in the same tissues as affected by temperature-differences. Tissue growth consists in an alternation of cellular bulk-increase and mitosis. The experimentation herein proposed would determine the proportion of the limitation set upon growth by lowering temperatures due to (*a*) slowing-down the mitotic process, and to (*b*) reducing the absorption of food materials and delaying the metabolism necessary to creating the chemical potential which must precede mitosis.

RESULTS AND DISCUSSION.

The accompanying tables and charts give in detail the cell-countings, the mitotic stage-classification, and the determinations derived from them; they give also the formulas used, and finally they set forth graphically and comparatively the results of the experimentation and calculations for each temperature series. Nevertheless, a short discussion is perhaps permissible.

[1] Sabline, V. "L'influence des agents externes sur la division des noyaux dans les racines de *Vicia faba*." Rev. Gen. Bot. 15: 481–497, 1903.

A. RHYTHM IN MITOSIS.

(*a*). GENERAL.

The beginning of the mitotic process in plants is conditioned upon the state of cell-turgor, which in turn implies that under conditions normal to the growing tissue the cell has not only absorbed a definite quantity of water, but also an amount of food materials and oxygen sufficient to set up the necessary physical and chemical potential demanded, in the particular setting of things, to start the mitotic train.

Strictly speaking, growth and mitosis are two distinct processes; growth refers only to permanent increase in bulk; mitosis, on the other hand, refers to cell-division regardless of increase or decrease in the size of the end product. Not only are they distinct processes, but in the same cell at the same time the one practically precludes the other. But while mitosis and increase in bulk are different processes, they must cooperate, if either is long to continue. Cells must divide, because their contact with the external world is through their surfaces and is therefore proportional to the square of their diameters; but their bulk and consequently the amount of metabolic work they are called upon to do vary with the cube of their diameters. A cell active mitotically is resting from its normal metabolic activities; conversely, while a cell is metabolically highly active it can not undergo mitosis. Sachs,[1] in his "Text-book of Botany," says:

"This relation of growth, which is dependent on cell-division, to assimilation, is especially clear in algæ of simple structure (as *Spirogyra, Vaucheria, Hydrodictyon, Ulothrix,* etc.), which assimilate in the daytime under the influence of light, while cell-division proceeds exclusively or at least chiefly at night. . . . .

"We have here a case of division of physiological work which shows us that the cells which have to do with chemical work (assimilation) can not at the same time perform the mechanical labor of cell-division; the two kinds of labor are distributed in the higher plants in space, in very simple plants in time. Provided there is a supply of assimilated reserve-material, cell-division can therefore take place either in the light or in the dark. Whether there are special cases in which light promotes or hinders cell-division is not known with certainty."

Quoting Famintzin,[2] Sachs continues:

"The cell-division of *Spirogyra* has been proved to be dependent on light to the same extent as the formation of starch; but relationship in the former case differs from that in the latter in the following respect: The formation of starch is induced by a very brief exposure to light (about half-hour) and requires that its action be direct; starch is formed only under the influence of light; in its absence the formation at once ceases. Cell-division, on the other hand, is induced only after light has acted for some hours; it then commences in the cells, whether these have been exposed to light for some time or have been removed into the dark."

[1] Sachs, Julius, "Text-book of Botany." (Tr. by A. W. Bennett.) Ch. 3, pp. 659–689.
[2] Famintzin, Mélanges phys. et chim. Petersbourg, 1868, Vol. III.

(*b*). WARD'S WORK.

A very important step in the analysis of vital phenomena was made in 1895, when W. M. Ward,[1] in his classical experiments "On the biology of *Bacillus ramosus* (Fraenkel), a schizomycete of the River Thames," determined that growth (*i. e.*, permanent increase in bulk) while in the long run dependent upon cell-division, does not synchronize but rather alternates with it. He measured quantitatively what other investigators had only caught glimpses of.

(*c*). ADDITIONAL EVIDENCE.

In 1904 W. E. Kellicott[2] published, in a bulletin of the Torrey Club, his paper "The daily periodicity of cell-division and of elongation in the root of *Allium*." In the experimentation upon which this paper was based Kellicott grew onions in wet sawdust until the roots were from 50 to 100 mm. in length. Then, at 2-hour intervals throughout the 24 hours, with the temperature ranging from 14° C. at 1 a. m. to 27° C. at 3 p. m., he took samples of the root-tips and at the same intervals made measurements of the rate of elongation of similar tips. His purpose was to trace the rhythm in cell-division and the rhythm in growth, with a view to determining whether (as Ward nine years previously had found in *Bacillus ramosus*) the maximum of mitotic activity alternates with the maximum of root-tip elongation. His work seems to have confirmed for the root-tip of *Allium* the conclusion of Ward in reference to *Bacillus ramosus*, and thus tended to suggest the generality of the principle.

Besides counting the resting stages in selected areas, he counted also the mitotically active stages, classifying them as early, middle, and late. He reports no further use of this classification other than to add their counts together for determining periods of comparative mitotic activity. His data would hardly suffice for a study of stage duration, for the observation periods were too far apart and the total number of cells counted approximated only 3,000.

Kellicott summarizes his investigations as follows:

"1. In the root of *Allium* there are two maxima and two minima in rate of cell-division during the 24 hours.

"2. The primary maximum occurs shortly before midnight (11 p. m.) and the primary minimum about 7 a. m. The secondary maximum occurs about 1 p. m. and the secondary minimum about 3 p. m.

"3. There is no correspondence between the rate of cell-division and slight variations in temperature.

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"6. Under normal conditions of growth the rate of elongation of the root of *Allium* exhibits a daily rhythm, showing two maxima and two minima during 24 hours.

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[1] See ref. No. 1, p. 12. [2] See ref. No. 2, p. 12.

"7. Elongation is most rapid (primary maximum) about 4 or 5 p. m., the secondary maximum occurring about 7 a. m. The primary minimum is about 11 p. m., and the secondary minimum about noon.

"8. Periods of rapid cell-division coincide with the low rate of elongation and during rapid elongation the rate of cell-division is lowest."

Finally G. Karsten[1] records his investigations of the mitotic rhythm through successive intervals under constant temperature. He traced the fluctuations in mitotic activity through long periods of the day, for the most part through the hours of daylight only. The intervals between his observations were not equal, but varied from 30 minutes to 2 hours. His plants were grown in a thermostat, maintaining a temperature constant at 25° C. From 6 a. m. to 6 p. m. the thermostat was lighted electrically, and from 6 p. m. to 6 a. m. it was permitted to remain dark. His purpose was to eliminate the influence of temperature fluctuations upon the degree of mitotic activity. He determined particularly that the fluctuations in mitotic activity during the course of the day are not due solely to variation in temperature.

In making his cell-counts, Karsten noted five stages, viz., Auflockkerung, prophase, mĕtaphase, anaphase, and telophase, and counted for each species studied a total of from approximately 100 to 400 cells per observation-period. Like Kellicott, he apparently made no further use of his division of stages of mitotic progress other than to sum them for measuring the height of mitotic activity at the given instant of observation. Karsten's view that root-tip cells do not show mitotic periodicity is not well founded, nor is Kellicott's conclusion[2] in reference to temperature and cell-division.

(*d*). SUMMARY OF EVIDENCE OF MITOTIC PERIODICITY.

To sum up the evidence in relation to periodicity, we may say that in growing tissue, so far as the individual cell is concerned, there is a definite alternation between permanent increase in bulk and mitosis. Indeed, if bulk-increase is largely anabolic and cell-division catabolic, as is most probably the case, then opposing activities can not synchronize in the same cell each as a dominant factor of activity. But synchronization of the same activities among many neighboring cells is a different matter. This exists and its degree determines the character of the pulsation observed in rate of growth in actively growing tissues. Even if growing cells did not have to experience this alternation in growth and mitosis, but responded directly and constantly to their environment, we should expect periodicity nevertheless, for the daily cycle of illumination, heat, and moisture, with their concomitant influences, direct and indirect, upon nutrition and metabolism, would make for a rhythm in growth. (See p. 30.)

[1]Karsten, G. "Über embryonales Wachstum und seine Tagesperiode." Zeit. Bot. 7: 1–34, 1915.
[2]See No. 3, p, 32.

Not only would we look for rhythm, as caused by the complex of environmental factors, but the internal organization of the plant permits response at one time or season, but not at another. That is, besides the daily response in mitotic and growth rhythms, due chiefly to external influences, there is a seasonal rhythm due chiefly to internal organization. Thus in February and March the cured onions, which have been stored through the winter, sprout very readily upon being given moisture and light; but in August the same type of onion, as was earlier reported, is hard to awaken to growth. (See p. 27.) Then, too, each individual tissue of each individual animal or plant would be expected, under a definite complex of environmental factors, to present its own specific train of mitotic phenomena, the parallelism in response being governed in such cases by the degree of constancy in the environment-complex and in the genotypic constitution of the tissues compared.

B. HEAT FACTOR IN GROWTH.

(*a*). GENERAL.

Heat is known to exert an important influence upon the velocity (see p. 38) of chemical reactions, and also upon the reaction-rate or strength of practically all of the measurable physical forces known in both the inorganic and the organic worlds. Growth (bulk-increase and mitosis), which is a complex of chemical and physical reactions, can take place only under appropriate temperature-conditions. Other things being equal, the growth response of a specific plant is specific for a given temperature. Many experiments have been conducted upon the rate of growth for the purpose of working out physiological constants for given and various situation-complexes of nature and nurture. So far as temperature-relations are concerned, there have been found cardinal points, specific temperatures, at which growth in a specific plant responds at its minimum, its optimum, and its maximum rates. As a rule, these points are found to vary from slightly above zero to approximately 50° C.

(*b*). PHENOLOGY.

The phenologists have found a certain relationship between the quantity of heat (that is, the number of centigrade-degree days) and the stage reached by a given plant in its development from the dormancy of midwinter. Linsser,[1] in 1867, attempted to formulate this relationship. His conclusions were based upon the theory that a definite quantity of heat is required in order to affect the internal reactions necessary to reach a definite developmental stage; regardless of whether this quantity be distributed over a long or a short season, its end effect was thought to be the same. In general, phenology is an attempt to harmonize the known facts of energy transmutation and conservation in

[1] Linsser, Carl, "Die Periodischen Erscheinungen des Pflanzenlebens in irhem Verholtniss zu den Warmeerscheinunzen." Mem. Acad. Sci., St. Petersb., Ser. VII, Vol. XI, No. 7, 1867.

chemical reactions with the phenomena of growth. It does not, however, take into consideration the differential effect of heat at different temperatures, nor the possibility of physical shock in raising and lowering the temperature, nor the possible wastage and excretion of products before the measured stage is reached.

Ward[1] calls attention to a fact of interest to those who seek to establish physiological constants, namely:

"That the variation in rate of growth which has been going on at an hitherto constant temperature is more pronounced when the rise or fall is 2° C. than when it is only 1° C. will be obvious, and similarly for any other range; but, again, it must be noted that the amount of deflection of the curve for any range of variation depends on the amount of temperature, or the hitherto constant temperature at which the growth has been going on. . . . . The external factors are: (1) Temperature. Variations in the curve are produced by sudden variations in the temperature, and apparently the variations are the more pronounced the quicker the temperature changes and the more extensive their range; but the amount of variation in the curve due to any given rise or fall of temperature in constant time appears to depend on the distance of the temperature (from which the variations is reckoned) from the optimum. In other words, the sensitiveness of the organism to a rise or fall of a degree centigrade varies according to the temperature from which the rise or fall occurs; for if it has been growing at 30° C. constant temperature, for an hour, it shows a more marked deflection in the curve for a sudden rise or fall of 1° C. than for the same sudden rise or fall from 25° C."

He then discusses other factors with which we are here not so concerned.

C. NATURE OF THE COMPLEX IN GROWTH AND MITOSIS.

Physiologists often have attempted to treat the complex of bulk increase and mitotic activity as a unit, fitting in its end-product the simple formula followed by reactions in homogeneous chemical systems. If, by any chance, in a special case, growth (implying an alternation in (*a*) the absorption of food materials, cell turgor, and (*b*) mitotic potential and its consequent mitosis) should be found to follow the same rule in response to one or more external agents as is obeyed by the simpler organic reactions, it would indeed be a matter of chance and not an homologous response due to types of chemical activity being parallel throughout. The one is a relatively simple and direct reaction, and the other a vast complex of inhibitions and activations, with their interplay, giving finally a single measurable resultant of forces. In mitosis we see different structures and can trace their dissolution and reorganization; this shows clearly that mitosis is not a homogeneous chemical reaction. There are many different substances distributed throughout the cell, but their distribution is not so homogeneous as not to require the consideration of the diffusion factor before completing their chemical reactions incident to mitosis. The fact that different structures and substances in the cells, both living and dead, take different stains proves

[1] See ref. No. 1, p. 13.

their different chemical composition and makes possible the microchemical analysis of cell structures, but the same evidence of complexity demands the greatest refinements in measuring unhampered and elementary vital processes. The mathematical formulas for physiological constants are, as a rule, not nearly so dependable as are such velocity-reaction formulas for substances in the world of non-living protoplasm. Doubtless the reason is that in living protoplasm there is a more complex interplay of forces and the consequent manufacture of new products which, in turn, by their presence affect their differential influences upon the whole subsequent course of vital activity. Such can not, without great difficulty, be resolved into its elements and given mathematical interpretation.

D. PHYSICO-CHEMICAL ASPECT.

(*a*). INDIVIDUALITY IN VELOCITY REACTIONS OF THE SEVERAL MITOTIC STAGES TO THE SAME TEMPERATURE CHANGES.

It should be noted that there is a differential response characteristic of each of the several mitotic stages here listed. This is not surprising, for each mitotic stage possesses its own individuality so far as its physico-chemical complex is concerned. This is most strikingly shown in chart No. 18, in the parallelism between the graphs plotting the velocity reactions of the successive stages at 20° C. compared with the velocities at 10° C, and those for 30° C. compared with the velocities at 20° C. as a standard. If the specimens had been grown at temperature-intervals of 2° C., one would expect, from the response shown in table on page 38, through the temperature series a characteristic and orderly increment or decrease in the velocity-response of each arbitrarily marked-off section (mitotic step or stage) of the mitotic cycle, the same as from the cell-organization as a whole, only in slightly less complex manner.

With the microscope it can be seen readily that the mitotic process involves gross molar movements and, as the cycle progresses, differential staining proves the change of minute cellular structures, "the production of structure from metabolism," involving chemical change. In a homogeneous chemical system it is possible to measure the quantity of the homogeneous reaction-product produced in a given amount of time; but in mitotic activity it is the progress of the complex-train with all of its many products that is measured by dividing it into arbitrary but recognizable progress-stages. It is not the mass of its reaction-products that is measured. Thus the end speed of the whole mitotic process is the resultant of many cooperating and conflicting forces; but, regardless of the number of complications, a thing that is measurable and is varied by the change in complicating factors shows orderly change and rhythm. Such measuring is a step in advance because it admits of analysis further than has been made and points the way toward still greater refinements.

Owing to the individuality of the physico-chemical complex characterizing each mitotic stage herein set off, we do not expect orderly fluctuation in the reactions of the successive stages (see chart 18) to the same temperature any more than we expect serial order in the reactions of different organisms selected at random and unseriated; but (see also charts 16 and 17) we do expect to find, in the same organism, that a characteristic and orderly curve plots the reactions to orderly increments in temperature, of the same mitotic stage, of any given combination of mitotic stages, of the entire cell as a unit, or of the more complex organism as a whole.

The effect of temperature increments of 10° C. upon the velocity of each of the several mitotic stages in the dividing root-tip cells of the onion. Q_{10} *values.*

Mitotic stages (see summary chart for definite limits).	Velocity at 20° C. compared with velocity at 10° C.	Velocity at 30° C. compared with velocity at 20° C.
1. Early prophase	0.8818 (*i. e.*, −1.1340)	+1.1525
2. Early prophase	+2.6832	+4.9406
3. Mid-prophase or spireme	+2.9599	+2.6404
4. Late prophase	+1.3859	+2.7593
5. Metaphase	+1.4071	+3.0663
6. Early prophase	0.8546 (*i. e.*, −1.1701)	+2.3440
7. Mid-anaphase	+1.1523	+2.7571
8. Late anaphase	+1.6334	+2.6038
9. Early telephase or di-spireme	+1.3329	+2.1694
10. Late telophase	+1.1240	+3.0931
Resting or metabolic	+1.2215	+4.9463
2 to 10 inclusive	+2.0476	+3.2311
1 to 10 inclusive	+1.1990	+1.3962
Entire cycle, *i. e.*, the 1 resting and the 10 active stages	+1.2139	+2.6218

NOTE.—Each of the above values when preceded by a + or a − sign constitutes the usual Q_{10} calculation.

The above shows, in terms of velocity rather than of duration, the effects of temperature increments of 10° C. upon the increased rapidity of each of the several mitotic stages in the dividing root-tip cells of the onion. (Table 15 and charts 16, 17, and 18 give in detail the comparative effects of temperature upon the duration of the several individual mitotic stages.) In two instances it will be seen that mitotic velocity is slowed down by the 10° C. temperature-increase, while in all other cases it is speeded up. On the whole the increased velocity exceeds the retarding influences, so that a rise in temperature increases the rate of mitotic activity. Stages 1 and 6 are, to a greater degree than any other stages, slowed down by a rise in temperature, while stages 2 and 8 are greatly accelerated by the same change. The former pair (stages 1 and 6) apparently have little in common, while in the latter pair stage 2 is constructing chromosomes and stage 8 is breaking them down.

(*b*). VAN'T HOFF'S LAW.

If van't Hoff's principle is taken to apply only to simple chemically homogeneous reactions, it finds little direct application to the measurements herein reported for the influence of temperature-increments upon mitotic velocity. However, determining the Q_{10} values, *i. e.*, the coefficients for simple or complex physical, chemical, or physiological activities, is a very useful method of analysis. But when we find Q_{10} values of the magnitude of van't Hoff's expectation, namely, of from +2.0 to +3.0, we must not consider therefore that we have of necessity located a simple homogeneous chemical reaction. We may or we may not have found such. As many as possible of the contributing factors must be taken into consideration and each duly weighted. Every chemical and physical activity has its characteristic velocity-response to a 10° C. rise in temperature. Generally these values vary from −2.0 to +5.0. Because in these experiments with mitosis the value of Q_{10} is never greater than +4.95 and never less than −1.18, the evidence points strongly toward the nature of mitotic forces being chiefly chemical and physico-chemical, but without further analysis this evidence tells little more as to what combination of a great repertoire of activities may be involved in the mitotic stage-complex whose activities are measured as a unit.

The fact that influences are both specific and measurable is the encouraging thing. The measuring of two complexes differing only in one factor supplies a measure of this differential. If finally a vital reaction is analyzed and one of its elements closely accords in behavior with some simple reaction, well and good, for such indicates approach to the elementary, and elemental formulas relating to such a complex can be synthesized; but calling a patently and unanalyzed complex elementary because it responds like such in one or more respects hardly makes for progress. Doubtless the component processes of mitosis are of a chemical and physico-chemical nature and their individual responses to temperature-changes are of the expected nature and degree. But the interplay of activities may cause the complex as a unit to synchronize with certain selected elements or the conflict of forces may greatly retard or accelerate the common progress. For instance, the production of enzyme A may be proceeding at a chemically expected rate in response to its surrounding temperature. But when enzyme A comes in contact with enzyme B, which is being similarly produced, their interaction may introduce another factor, accelerating or retarding general or specific progress. Also, anticatalysis (or the influencing of the velocity of production of a chemical product by the unremoved product itself) is a factor. It is a mass of such individual activities that we measure in most physiological activities, and especially is this true in mitosis.

While the experiments and discussions of this paper are confined to the method of mitotic analysis based upon velocity-responses characteristic of definite temperatures, which method doubtless will continue to yield profitable returns, the study of specific mitotic stage-duration as affected by other physical forces, such as light, electricity, pressure, and gravity, and by chemical agents, and finally by given complexes of these forces and agents, must be resorted to for a better determination of the details of mitotic dynamics. The method of measuring the durations of mitotic stages presented in this paper is applicable equally well to each of these situations.

Gradually the physiological complex of the cell is being analyzed, each factor measured, and coefficients and indices of reaction of definite living organisms to controlled environmental conditions are being worked out so far as velocity-reactions to temperature are concerned. The fact that mitosis in its complexity does not behave throughout like a uniform and simple chemical reaction is to be expected. In mitosis there exists a microcosm of chemical and physical forces, each with its characteristic response to temperature-increments. Indeed the differential reactions of the several stages of the mitotic process-train present the only possible but nevertheless a most promising key to further analysis of the forces involved in cell division by the method of measuring velocity-response to temperature-changes. Especially valuable will this key be if used under a wide range of controlled conditions and applied to mitotic stages of very definite but small differences. Finally, of course, velocity-analysis in its various relations will (like temperature-analysis) reach its limits of usefulness, but its possibilities in determining the nature of the dynamics of mitotis are thus far only sampled.

(c). ISOLATION OF FACTORS.

Elimination by comparative experimental evidence.—When a physiologist confines his investigations to a definite, localized, relatively homogeneous reaction, he may expect results more closely approximating those of the chemist dealing with homogeneous systems. But even then the varying factors may act upon processes controlling the one sought to measure alone. Riddle[1] experimented with four species of cold-blooded vertebrates, with a view to determining the velocity of digestion in relation to temperature. He recognized the difficulty in measuring the effects of temperature upon the digestive process alone. In regard to complicating factors he says:

"The data indicate that the effects of temperature on the digestive processes must be considered under two heads: First, the accelerating action of increased temperature on the chemical processes involved; and second, the retarding action of very high or very low temperatures due (*a*) to the production by the animal of smaller amounts of digestive enzymes under these

[1] Riddle, Oscar. "Rate of digestion in cold-blooded vertebrates." Amer. Jour. Physiol. **24**: 447 *et seq.*, 1909.

conditions or (*b*) to the actual destruction of enzymes by these extreme temperatures."

After executing his experiments in a manner as nearly as possible eliminating these perturbing influences, he finds:

"Within certain not very wide ranges of temperature the rule of van't Hoff applies to the digestive processes in living cold-blooded vertebrates, the average of eight valid coefficients being 2.62."

And, in further interpretation of his results in which the velocity increase for a 10° C. temperature-increment varied from 0.93 to 7.81, he says:

"Those numbers which are greater than 3.00 indicate that the lower temperature of the two temperatures compared exercises a destructive or inhibitive action on the digestive secretions; whereas numbers smaller than 2.00 indicate that the higher temperature of the two temperatures compared likewise inhibits or destroys ferment action."

It is clear that he regards uncomplicated peptic digestion as a simple and purely chemical process which would, therefore, for moderate temperatures, show the characteristic Q_{10} value of from +2.0 to +3.0. For these reasons, of the 13 determinations made 5 were rejected as not valid. His 8 valid coefficients, above mentioned, were determined for temperatures approximating the optimum for peptic digestion in each of the several species experimented with. Thus the cardinal temperature-points for the particular activity characteristic of the particular species and individuals used in the experiment and must be taken into account in interpreting temperature-indices based upon physiological systems.

A single index for two factors.—Livingston[1] attacked the problem of physiological constants. As he points out in his investigation, he "takes account of the principle of temperature minima, optima, and maxima." Thus, "basing the indices upon a physiological rather than an exponential system," he finds "the van't Hoff-Arrhenius principle, upon which is based the exponential series, appears to hold for the elongation of young maize shoots only for a temperature range from about 20° to about 30° C. (Lehenbauer), and the physiological system is approximately true for all temperatures from 12° to 43°C., at least for the conditions of Lehenbauer's experiments." Subsequently the same author (Livingston) worked out "A single index to represent both moisture and temperature conditions as related to plants."[2]

There is always great difficulty in attributing to an elementary and uncomplicated physiological process the Q_{10} values found in any given measurement, so great in the living organism is the interrelation of activities. The analysis must, however, strive to isolate the factors and thus seek data based upon relatively simple processes. Formulas duly weighing each factor can then be synthesized.

[1] Livingston, Burton E. "Physiological temperature-indices for the study of plant growth in relation to climatic conditions." Physiol. Res. 1: No. 8: 399, 1916.

[2] Physiol. Res. 1: No. 9: 421–440, 1916.

(*d*). DIFFERENCE BETWEEN PHYSIOLOGICAL AND PURELY CHEMICAL TEMPERATURE-VELOCITY REACTIONS.

Physiological processes.—Harvey,[1] in his investigations of the rate of conduction of the nerve impulse in the medusa *Cassiopea*, calls attention to the fact that within medium temperatures—that is, from 18° to 38° C.—the velocity-increment per definite temperature-rise for physiological processes declines as the temperature increases, whereas in purely chemical reactions the velocity-increment increases as the temperature rises. He gives the accompanying table showing the former principle for the experiment above named.

Temp. (C.).	Q_{10}.
18° to 28°	2.40
19 29	2.24
20 30	2.08
21 31	1.93
22 32	1.82
23 33	1.73
24 34	1.58
25 35	1.41
26 36	1.25
27 37	1.10
28 38	0.96

In interpreting this behavior Harvey says:

"If the rate of nerve conduction depends on the velocity of some chemical reaction in the nerve, the above-mentioned difference in its temperature curve remains to be explained. It is possible, indeed probable, that yet another factor than reaction velocity determines conduction rate, and the resultant curve of the two factors is the one actually observed. . . . . Different enzymes exhibit maxima at different temperatures. Most of these are rather high, much higher than the maximum for nerve-conduction, which lies at about 33° C. The same ferment obtained from different sources may exhibit different maxima . . . . we may say that the propagation of the nerve impulse is not only dependent on the velocity of a chemical reaction, *but that the reaction is further accelerated by the presence of an enzyme.* Thus the characteristic difference in the form of curve from that of a simple reaction."

Growth or permanent bulk increase.—Lehenbaur,[2] presents the table shown herewith. The purpose of his experiments was to test the applicability of van't Hoff's principle to the rate of growth in the stem-shoots of maize seedlings. He points out that his results approximate van't Hoff's law in the medium temperatures only, that is, from 20° to 30° C., where the concomitant temperature-coefficients range from +1.88 to +2.40. The table is indeed a most interesting one, for growth alone is considered, and this he studied in its more restricted sense, namely, permanent increase in bulk disregarding mitotic activity. There is no constant velocity-increment with each temperature-rise of 10° C., but it will be

Temp. range.	Range of growth-rate.	Coefficient.
	mm.	
°C.	1.01	
12 to 22	9 to 59	6.56
13 23	10 64	6.40
15 25	20 75	3.75
18 28	28 98	3.50
20 30	45 108	2.40
21 31	53 109	2.06
22 32	59 111	1.88
25 35	75 86	1.15
32 42	111 11	0.09
33 43	101 6	0.06

[1] Harvey, E. Newton. "Effects of different temperatures on the medusa Cassiopea, with special reference to the rate of conduction of the nerve inpulse," Carnegie Inst. Wash. Pub. No. 132, pp. 27–39, 1910.

[2] Lehenbauer, Philip A. "Growth of maize seedlings in relation to temperature." Physiol. Res. i: No. 5: 281, 1914.

seen that the lower the temperature the higher the coefficients. It is evident that here increasing temperatures exert a progressively declining accelerative effect upon growth.

Mitosis.—Not only is there, in a relatively simple physiological complex, a decrease in Q_{10} values as the temperature increases, but if growth, which is most complex physiologically, is measured in terms of permanent bulk-increase, we find the same phenomenon.

In comparing the values found in the mitosis velocity-measurements at different levels on the temperature scale with the two types of velocity-increments which Harvey points out, the striking thing is that in mitosis all of the stages measured in the present investigation show a greater velocity-increment for a rise of 10° C. from 10° to 20° C. than from 20° to 30° C. Thus, unlike the rate of nerve conduction in *Cassiopea*, and the increase of length in the root-tips of the seedling maize along with physiological activity generally, mitosis behaves in its velocity-increments to temperature-increments like the simpler chemical reactions. This does not mean that mitosis is a "simple chemical reaction." Far from it it is a vast complex of physical and chemical activities. By chance the resultant of the actions and interactions of these processes present, when measured as a whole, an aspect resembling in this one feature a simple chemical reaction.

Many biological curves are shaped like an elongated and slanting capital letter S—thus  ; for instance, the curve of auto-catalysis, when time (abscissæ) and quantity of product (ordinates) are plotted. If the temperature at which the onion root-tips of the present study were sampled had extended beyond the cardinal temperature points for mitosis in the specimens used, we would have found ultimately a breaking-point and a decrease in velocity increment in the higher temperatures, such as Harvey found in the velocity of nerve conduction in *Cassiopea* at 28° C. to 38° C., and Lehenbauer in the growing root-tips of maize at 32° C. to 42° C. The curves for velocity of physiological reactions in response to temperature-changes are the shape of the upper end of the elongated  , while the curves for mitosis and also for the simpler chemical reactions take the direction of the lower half. The range of temperature in the mitosis experiment (10° C. to 30° C.) is somewhat lower on the temperature scale than those used by Harvey (18° C. to 38° C.) and by Lehenbauer (12° C. to 43° C). In the region of the medium temperatures this particular contrast between the velocity-gradients of mitosis and of physiological processes generally and the closer resemblance of the mitosis-gradient to that of the simpler chemical reactions is undeniable. We must look for its meaning not in position on the temperature-scale, but in a physiological (physico-chemical) complex in which the many specific elementary reactions to temperature-changes give a resultant in which the many aberrations from the velocity-gradient characteristic of a simple chemical process are mutually canceled.

(e). THE REACTIONS OF DEFINITE MITOTIC STAGES.

General survey.—The temperatures 10°, 20°, and 30° C. at which the plants experimented with were grown are medium in the sense in which the term is used in relation to physiological experiments generally. At these temperatures, with mitosis as with other physiological processes, we find Q_{10} values of the expected magnitude. Here also, as is usual with both simple chemical and complex physiological processes, accompanying an arithmetical change in temperature, we find a geometrical change in reaction velocity.

In some stages, such as No. 2, it appears that the activity is chiefly chemical, or at least diffusional involving most minute bodies, for a high-power microscope reveals few structural changes. If the products of reaction were immediately removed, if auto-catalysis and other activating or retarding factors were absent, such a stage might, in its behavior, be expected more nearly to approach van't Hoff's rule than would a stage whose changes appear to be mostly physical, such as, for instance, stage 6, which seems chiefly a physical shift. This surmise in reference to stage 2 holds good in the temperature-difference 10° to 20° C., but falls down utterly in the 20° to 30° C. rise. While other stages—Nos. 4 to 10—which seem to be characterized chiefly by gross structural changes, in the 10° to 20° C. change generally respond with a Q_{10} value less than van't Hoff's expectation, but in the 20° to 30° C. change are well within the range of such prediction. These differences indicate an interplay of forces specific for each stage. Doubtless the non-removal of products, which become thereby factors influencing subsequent activities, constitutes a very great if not the principal cause of difference between the response of a mitotic stage and a homogeneous chemical reaction to temperature-changes.

A cell through a given mitotic stage is apt to be more homogeneous, *i. e.*, simpler, in its physico-chemical complex than the same cell traced throughout its whole mitotic cycle; also the activities of a given mitotic stage may be chiefly chemical or chiefly molar. We should, therefore, expect to find individual stages presenting velocity-gradients more elementary (*i. e.*, less composite) than the same gradient characteristic of mitosis as a whole. Examination of the data shows that for the mitotic cycle as a whole (*i. e.*, the 10 active stages), an increase of 10° (from 10° to 20° C.) causes a reduction in duration from unity to 0.8342 (velocity increase of +1.1990), while an increase of 10° C. (from 20° to 30° C.), taking 20° as the standard, causes a reduction in duration for the 10 active stages from unity to 0.7158 (velocity increase of +1.3926). Thus the cumulative effect of increasing temperature upon the velocity of mitosis is, in the present experiments, greater in the higher than in the lower temperatures, in this respect resembling the simpler chemical reactions. (See pp. 39 and 43.)

Further, if we take each of the 10 active stages singly, we find that the same rule applicable to the 10 stages as a whole holds good, with the single exception of stage 3, the spireme stage, in which an increase of temperature from 10° to 20° C. causes an increase in velocity of 2.9599 times, while from 20° to 30° C. velocity is increased only 2.6404. This decrease is slight, but it operates in the direction of general physiological rather than simple chemical expectation. (See pp. 38 and 42.) Nevertheless the values are so close that, considering stage 3 only, the fitting to van't Hoff's rule is most striking. Thus, judged by the van't Hoff rule alone, from its reactions to heat, stage 3 seems to be a comparatively simple chemical reaction; but, as seen through the microscope, it is characterized by molar changes also. So it is probable that the close approximation of its Q_{10} value to +3.0 at both the upper and lower temperature ranges is due to its being the resultant of a number of conflicting higher and lower values, else all processes involved were alike in having the same Q_{10} characteristics, which latter is possible, but not probable.

The movement of chromosomes.—Stages 4 to 7, as designated in this study, involve the movement of chromosome-bodies within the cell. Although the chromosomes may be attached by strands, it may be profitable to make comparison with the action of heat upon the rate of movement of other bodies in protoplasm. In Davenport's "Experimental Morphology" a diagram[1] shows the relation between temperature and the rate of movement of the chlorophyll-grains floating in the protoplasm of the cells of three species of green plants. These curves show a rapid rise in rate of movement from slightly above 0° C. to from 33° to 39° C., and then a rapid falling off. Before their breaking points they are essentially the shape of the curves plotted for velocity-reactions of most of the mitotic stages to temperature-changes. The curve is specific for each particular species. So, with the specific mitotic stages, there is a specificity of reactions due, doubtless, as among the different species and processes above referred to, to a distinctive complex of physiological (*i. e.*, physico-chemical) properties.

The peculiar reaction of mitotic stage No. 6.—From the present experimentation one of the most interesting results is in reference to mitotic stage No. 6, in which the chromosomes are moving from the equatorial plate toward the poles. One would naturally suspect that a rise in temperature would increase the speed of these moving bodies, as a rise in temperature increased the rate of movement of the chlorophyll-granules above referred to, but such is not the case. Whereas it is true that a rise in temperature increased the speed of the whole mitotic process, it actually decreased the speed of this particular stage. The unexpected response of this stage to temperature-

[1] Davenport, Charles B. Experimental Morphology, p. 226, 1899. Data from Velten, W. Die Einwirkung der Temperatur auf die Protoplasma-bewegung. Flora 59: 177–217, 1876.

increments might indeed be considered as a mistaken interpretation due to bad statistical methods, or to experimental errors, if we did not have corroborative evidence. If the temperature-response of stage 6 in cells growing at 20° C. is compared with those growing at 10° C. we find a slowing-down, both relatively and absolutely, caused by an increased temperature, and when we take the duration at 10° C. or that at 20° C. as a basis, we find also that at 30° C. there is a similar response, namely, a slowing-down relatively to the velocity increments of the preceding and following stages. This is seen graphically in Chart No. 18 and is too consistent to have been due to error. The decrease in the velocity of stage 6 caused by a rise in temperature is outstanding and real. This brings within range of profitable experimentation work seeking to determine the nature of the forces moving the chromosomes from the equator toward the poles.

From whatever angle viewed, the problem of the nature of mitotic forces enters the field of physical chemistry, and consequently a more refined analysis of its dynamics is being sought with greatest profit in the realm of this science. Analysis by differential temperature-reactions is only one means of attacking the problem, but its possibilities are promising. In a supplementary study[1] there were brought together, for the purpose of aiding in the analysis of the mitotic potential, (*a*) the facts concerning the velocity-reactions to temperature-differences of the several mitotic stages of the growing root-tips of the onion as determined in the present investigation, and (*b*) data from the experiments recorded in scientific literature on the temperature-coefficients of a number of elementary and complex physical, chemical, and physiological processes.

SUMMARY.

(1) This study sets forth and demonstrates the mathematical and biological soundness of a statistical and cytological method of measuring both the relative and absolute durations of the several arbitrarily delimited progress-stages in cell-division.

(2) The net results of this investigation are given in concise form in the accompanying table (No. 3) "Principles and formulas for determining the relative and absolute durations of the several mitotic stages," and in the "Summary Chart," which constitutes the frontispiece and which gives in detail the measurements and ratios found by applying the demonstrated principles to three actual cases, namely, to measuring and comparing the duration of the ten active and one resting mitotic stages in the dividing root-tip cells of the common onion (*Allium cepa*) at 10°, 20°, and 30° C.

[1]Laughlin, Harry H. The Dynamics of Cell-Division. Pro. Soc. Exp. Med. and Biol., XV, 8, No. 179 (1357), pp. 117–122. May 1918.

(3) From the Q_{10} values derived from these comparisons it is found that each mitotic stage presents characteristic velocity-reactions to temperature-increments. These reaction-values approximate van't Hoff's expectations, thus indicating that most probably the repertoire of activities constituting each such mitotic stage is composed of the actions and interactions of those much more elementary physical and chemical forces which measured in more isolated relations have been shown to react in this same velocity-fashion.

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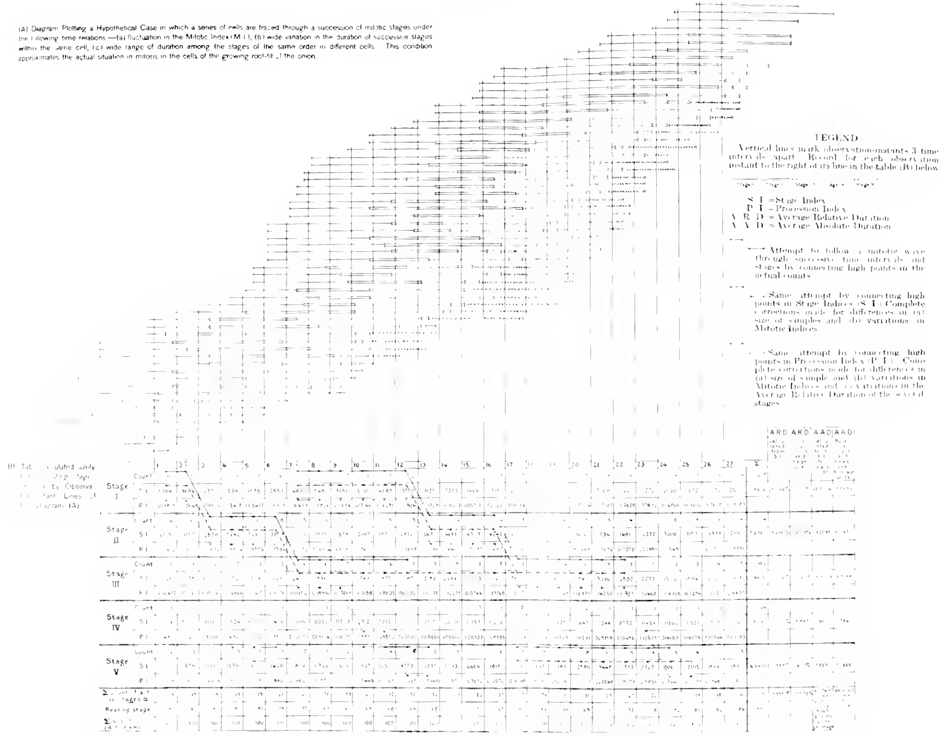
1.—*Method Chart.*

(A) Diagram Plotting a Hypothetical Case in which a series of cells are traced through a succession of mitotic stages under the following time relations:—(a) fluctuation in the Mitotic Index (M. I.), (b) wide variation in the duration of successive stages within the same cell, (c) wide range of duration among the stages of the same order in different cells. This condition approximates the actual situation in mitosis in the cells of the growing root-tip of the onion.



*Also, since in this particular series the total count per each observation instant divided by 10 = Mitotic Index, M. I.

2.—*Properties of four condition-complexes in reference to mitotic indices and stage durations.*

Type	Condition-complex	Relative stage frequency (i. e. S. I.) in a selected observation	Σ S. I. for a given stage through successive observations	P. I.	Use of P. I. in determining the A. A. D.	Possibility of determining the A. A. D. by S. I. or P. I.
I	Stage durations equal. M. I. constant.	∝A. R. D.	∝A. R. D.	Constant for all stages and observations	None	Impossible
II[a].	Stage durations equal. M. I. varying.	Not ∝A. R. D.	∝A. R. D.	Not constant, but bearing a constant relation to its concomitant S. I. of the same stage through successive observations	Superfluous; S. I. and P. I. coincide. Orderly procession of S. I. in relation to successive mitotic stages and successive observation-intervals is adequate to determining the absolute duration of a definite portion of the entire mitotic cycle.	Possible by either S. I. or P. I.
III.	Stage durations unequal. M. I. constant.	∝A. R. D.	∝A. R. D.	Constant for all stages and observations	None	Impossible
IV[a].	Stage durations unequal. M. I. varying.	Not ∝A. R. D.	∝A. R. D.	Not constant, but bearing a constant relation to its concomitant S. I. of the same stage through successive observations	Essential. In complex cases the P. I. restores the recognizable and orderly procession of the S. I. in relation to successive mitotic stages and successive observation-intervals, thus making it possible to measure the absolute duration of a definite portion of the entire mitotic cycle.	Possible by P. I. only.

a The key situation into which the situation *b*, that actually found in mitosis in onion root-tip cells, tends to be corrected by means of the P. I. The condition-complex of Type IV is the one analyzed in the method chart (Chart No. 1) because of this direct applicability to the case in hand.

For meaning of formulas see table No. 3, "Principles and Formulas."

NOTES:

1. Only when M. I. is constant (but regardless of variation in successive stage-durations) is S. I. of a given stage in a given observation ∝A. R. D. in a single sample.

2. Σ S. I. for the same stages is always ∝A. R. D., regardless of variation in successive stage duration or constancy in M. I.

3. *Jamming* is the confusion of the orderly processions of S. I. which results when one fluctuation in M. I. follows another so closely that a considerable percentage of cells beginning mitosis in the first fluctuation have finished so small a portion of the cycle that the same stage in both the first and second waves is recorded in the same time interval. The shortening of observation-intervals tends to diminish, but can not totally correct, this difficulty.

4. The amount of fluctuations in M. I. is not essential (however, the greater and more sudden the fluctuation the easier the determination) to determining absolute duration by the P. I. method, but the time intervening between pulsations (i. e., changes in M. I.) is very important—relatively long intervals simplifying, relatively short intervals complicating, the determination.

3.—*Principles and formulas for determining the relative and the absolute durations of the several mitotic stages.*

PRINCIPLES.

1. The duration of a mitotic stage is directly proportional to the summation of its percent-frequencies (*i. e.*, stage indices [S. I.]) observed at successive intervals, in accordance with the principles of sampling, during the mitotic process.

2. The absolute duration of a succession of mitotic stages is measured by the time interval between two points in a recognizable procession through time intervals and mitotic stages of the procession indices [P. I.], marking, respectively, the first and last stages in the selected succession.

FORMULAS.

1. Mitotic index.

$$\text{M. I.} = \frac{\text{No. cells dividing.}}{\text{Total number of cells (both resting and dividing) observed in the same fields.}} \pm .6745\sqrt{\frac{\text{p. ct. } P_0 \times P_1}{n}}$$

2. Stage index.

$$\text{S. I.} = \frac{\text{No. cells in a given mitotic stage.}}{\text{Total number of mitotically active cells (\textit{i. e.}, excluding the resting cells) observed in the same fields.}}$$

3. Average relative duration of the active cycle.

A. R. D. of C. = 1 when "resting" is not included as a stage; = 1 − A. R.D. of R. when "resting" is included as a stage.

4. Average relative duration of a given mitotic stage.

$$\text{A. R. D. of S.} = \frac{\Sigma \text{ S. I. of the } \textit{given} \text{ stage in all observations.}}{\Sigma \text{ S. I. of } \textit{all} \text{ stages included in the cycle, in all observations.}} \text{ or } \frac{\Sigma \text{ S. I. of the } \textit{given} \text{ stage in all observations.}}{\text{No. of observation-instants.}}$$

= also the average stage index of the given stage.

5. Procession index.

$$\text{P. I.} = \frac{\text{S. I.}}{\text{A. R. D. of S.}}$$

6. Average absolute duration of the entire active mitotic cycle.

$$\text{A. A. D. of C.} = \left(\Sigma \frac{\text{Time periods elapsing between two points in a recognizable procession of P. I.}}{\text{No. of stages covered.}} \Big/ \text{No. P. I. waves followed.} \right) \times \text{No. stages in cycle.}$$

7. Average absolute duration of a given mitotic stage.

$$\text{A. A. D. of S.} = \text{A. A. D. of C.} \times \text{A. R. D. of S.}$$

NOTE: An observation consists of 1,000 cells from the same root-tip selected by counting all cells within a sufficient number of microscopic fields selected at random within two root-diameters of the extreme tip.

4.—*Stage index table.* (*Preliminary study.*)

Stage		10h 00m a.m.	10h 10m a.m.	10h 20m a.m.	10h 30m a.m.	10h 40m a.m.	10h 50m a.m.	11h 00m a.m.	11h 10m a.m.	11h 20m a.m.	11h 30m a.m.	11h 40m a.m.	11h 50m a.m.	12h 00m noon	Σ Count Σ S.I.	Average Relative Duration * (A.D.R.)
Resting	Count	682	668	696	744	795	846	684	789	530	507	477	505	653	8,596	*
	S.I.															
1	Count	193	189	200	133	114	82	111	82	115	141	158	232	107	1,857	
	S.I.	.6069	.5692	.6578	.5195	.5560	.5324	.3512	.3886	.2446	.2860	.3021	.4686	.3083	5.7912	.4473
2	Count	53	59	27	54	38	35	31	42	146	121	148	130	85	1,029	
	S.I.	.1666	.1596	.0888	.2109	.1853	.2272	.2879	.1990	.3106	.2454	.2829	.2626	.2449	2.8717	.2218
3	Count	22	15	113	17	16	18	37	22	69	36	53	53	49	420	
	S.I.	.0691	.0451	.0427	.0664	.0780	.1168	.1163	.1042	.1468	.0730	.1013	.1070	.1412	1.2079	.0933
4	Count	18	6	6	6	3	3	4	5	21	21	14	6	11	123	
	S.I.	.0566	.0180	.0197	.0234	.0146	.0194	.0125	.0236	.0446	.0423	.0267	.0121	.0317	.3454	.0266
5	Count	2	3	3	4	1	0	0	1	6	5	3	5	5	38	
	S.I.	.0062	.0090	.0098	.0156	.0047	.0000	.0000	.0047	.0127	.0101	.0057	.0101	.0144	.1000	.0077
6	Count	1	2	6	5	2	0	5	1	6	9	3	2	2	44	
	S.I.	.0031	.0060	.0197	.0195	.0096	.0000	.0157	.0047	.0127	.0182	.0057	.0040	.0057	.1246	.0096
7	Count	2	6	7	5	1	0	5	2	3	11	4	1	4	51	
	S.I.	.0062	.0180	.0023	.0195	.0048	.0000	.0157	.0094	.0063	.0223	.0076	.0020	.0115	.1163	.0089
8	Count	3	14	15	11	7	2	1	3	27	22	7	5	10	127	
	S.I.	.0094	.0421	.0493	.0429	.0341	.0129	.0031	.0142	.0574	.0446	.0134	.0121	.0288	.3643	.0281
9	Count	6	12	11	12	5	3	9	5	31	42	20	5	14	175	
	S.I.	.0188	.0361	.0361	.0468	.0243	.0194	.0284	.0236	.0659	.0851	.0382	.0121	.0403	.4751	.0367
10	Count	18	26	16	9	18	11	53	48	46	85	113	56	60	559	
	S.I.	.0566	.0783	.0526	.0351	.0878	.0714	.1677	.2274	.0978	.1724	.2160	.1128	.1729	1.5488	.1196
Σ Count 1-10		318	332	304	256	205	154	316	211	470	493	523	495	347	4,424 12.3453	
Σ Count R+1-10		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	13,000	
Mitotic Index (M.I.)		.318	.332	.304	256	205	.154	.316	.211	.470	.493	523	.495	.347		

Stage Index (S. I.) = $\frac{\text{No. cells in a given mitotic stage}}{\text{No. of all mitotically active cells (i. e., excepting the resting cells) observed in the same fields.}}$

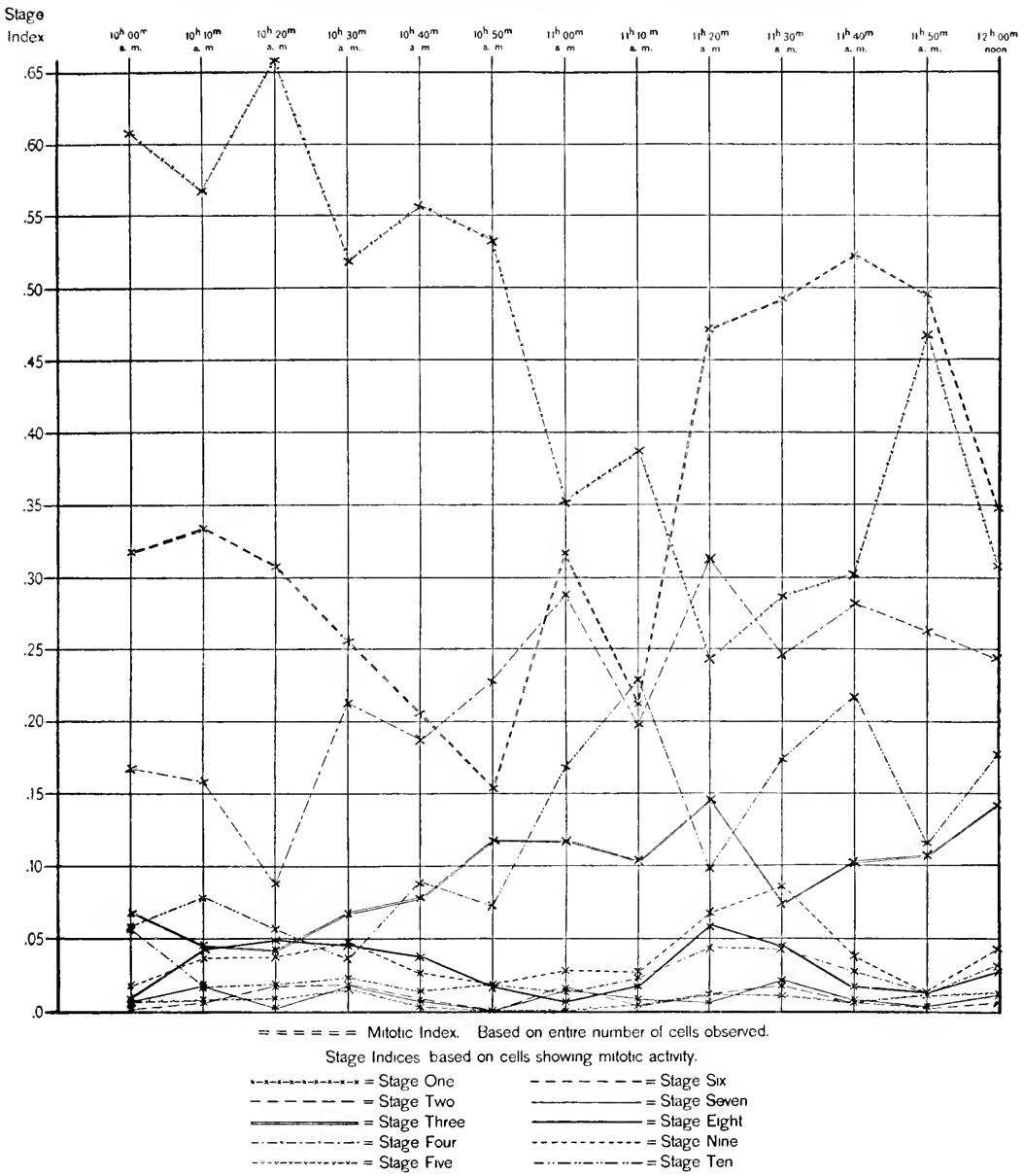
* If the resting stage had been included in the cycle considered, and the duration of the 10 active stages and the resting stage rated at unity, A. R. D. of the resting stage = .6612 and A. R. D. of all 10 active stages = .3388.

———— = Connects highest S. I. of each stage.
– – – – – = Connects lowest S. I. of each stage.

There is no systematic procession of a recognizable S. I. through the successive mitotic stages during the definite time periods.

If there were fair constancy of duration of the several mitotic stages, and if there were a rhythmic fluctuation in the Mitotic Index, then the Stage Index procession would be quite pronounced; provided the duration of the several stages were equal. Hence the necessity of converting the several Stage Indices into indices which would have appeared had all stages been of equal duration. The Procession Index (P. I.) does this.

5.—*Graphs showing mitotic and stage indices. (Preliminary study.)*



6.—*Procession index table.* (*Preliminary study.*)

Stage	A. R. D.		1	2	3	4	5	6	7	8	9	10	11	12	13
			$10^h\ 00^m$ a.m.	$10^h\ 10^m$ a.m.	$10^h\ 20^m$ a.m.	$10^h\ 30^m$ a.m.	$10^h\ 40^m$ a.m.	$10^h\ 50^m$ a.m.	$11^h\ 00^m$ a.m.	$11^h\ 10^m$ a.m.	$11^h\ 20^m$ a.m.	$11^h\ 30^m$ a.m.	$11^h\ 40^m$ a.m.	$11^h\ 50^m$ a.m.	$12^h\ 00^m$ noon
1	4473	S. I.	.6069	.5692	.6578	5195	5560	5324	.3512	3886	2446	2860	.3021	4686	3083
		P. I.	1.3628	1.2524	1.9706	1.1614	1.2430	1.1902	7851	8685	5468	6393	6753	1 0476	6892
2	2218	S. I.	1666	1596	0888	2109	1053	.2272	2879	1990	3106	2454	2829	.2626	.2449
		P. I.	7511	7195	.4003	.9508	.8354	1.0243	1.3016	.8972	1.4013	1.1064	1.2754	1.1839	1.1041
3	0933	S. I.	.0691	0451	.0427	0664	0780	1168	1163	1042	1468	.0730	1013	1070	1412
		P. I.	7406	.4833	4576	.7116	.8360	1 2518	1.2465	1.1117	1.5734	7824	1.0851	1.1468	1.5133
4	.0266	S. I.	0566	.0180	0197	.0234	.0146	.0194	.0125	0236	0448	0425	0267	.0121	0317
		P. I.	2.278	.6766	7406	.8796	5488	.7293	.4699	.8872	1.6766	1.6992	1.0037	4548	1.1917
5	0077	S. I.	.0062	.0090	.0098	0156	.0047	.0000	.0000	.0047	.0127	0101	.0057	.0101	.0144
		P. I.	.8051	1.1688	1.2727	2.0259	.6103	.0000	.0000	.6103	1.6493	.6346	7402	1.3116	1.8701
6	.0096	S. I.	.0031	.0060	.0197	.0195	.0036	.0000	.0157	.0047	.0127	.0182	.0057	.0041	.0057
		P. I.	.3229	.6250	2.0520	2.0312	1.0000	.0000	1.6354	4895	1.3229	1.8958	.5937	.4166	.5937
7	.0089	S. I.	.0062	.0180	.0023	0195	.0048	.0000	.0157	.0094	.0063	.0223	0076	.0020	.0115
		P. I.	.6966	2.0224	.2584	2.1910	.5393	.0000	1.7640	1.0561	7078	2.5056	.8534	2247	1.2921
8	.0281	S. I.	.0094	.0421	.0493	.0429	.0341	.0129	.0031	.0142	0574	.0446	.0134	.0121	.0288
		P. I.	.3345	1.4982	1.7544	1.5266	1.2131	.4590	.1103	.5053	2.0427	1.5871	4768	.4306	1.0249
9	.0367	S. I.	.0188	.0361	.0361	.0468	.0243	.0194	.0284	.0236	.0659	.0851	.0382	.0121	.0403
		P. I.	.5122	.9836	.9836	1.2752	.6621	.5286	.7738	.6430	1.7956	2.3188	1.0408	.3297	1.0980
10	.1196	S. I.	.0566	.0783	.0526	.0351	.0878	.0714	.1677	.2274	.0978	1724	.2160	.1128	.1729
		P. I.	.4732	.6546	.4937	.2934	.7341	.5969	1 4021	1.9093	.8177	1.4414	1.8060	.9431	1.4456

Calculating the Absolute Duration of the Mitotic Cycle.

———— = The movement of a recognizable Procession Index through 5 stages of equal duration in 100 minutes
– – – – = The movement of a recognizable Procession Index through 6 stages of equal duration in 90 minutes
·—·—·— = The movement of a recognizable Procession Index through 3 stages of equal duration in 50 minutes

Procession Index	Time	Average Time per Stage
5 stages	100 min.	20 min.
6 "	90 "	15 "
3 "	50 "	16.66 "

Giving equal weight to each procession 3)51.66 min.

Average Duration of 1 step, i. e., ⅕ the cycle = 17.22 "

∴ The Average Duration of the entire active mitotic cycle = 172.2 "

The Average Duration of the resting stage = 336.06 "

The Average Duration of the entire cycle including the resting stage = 508.26 "

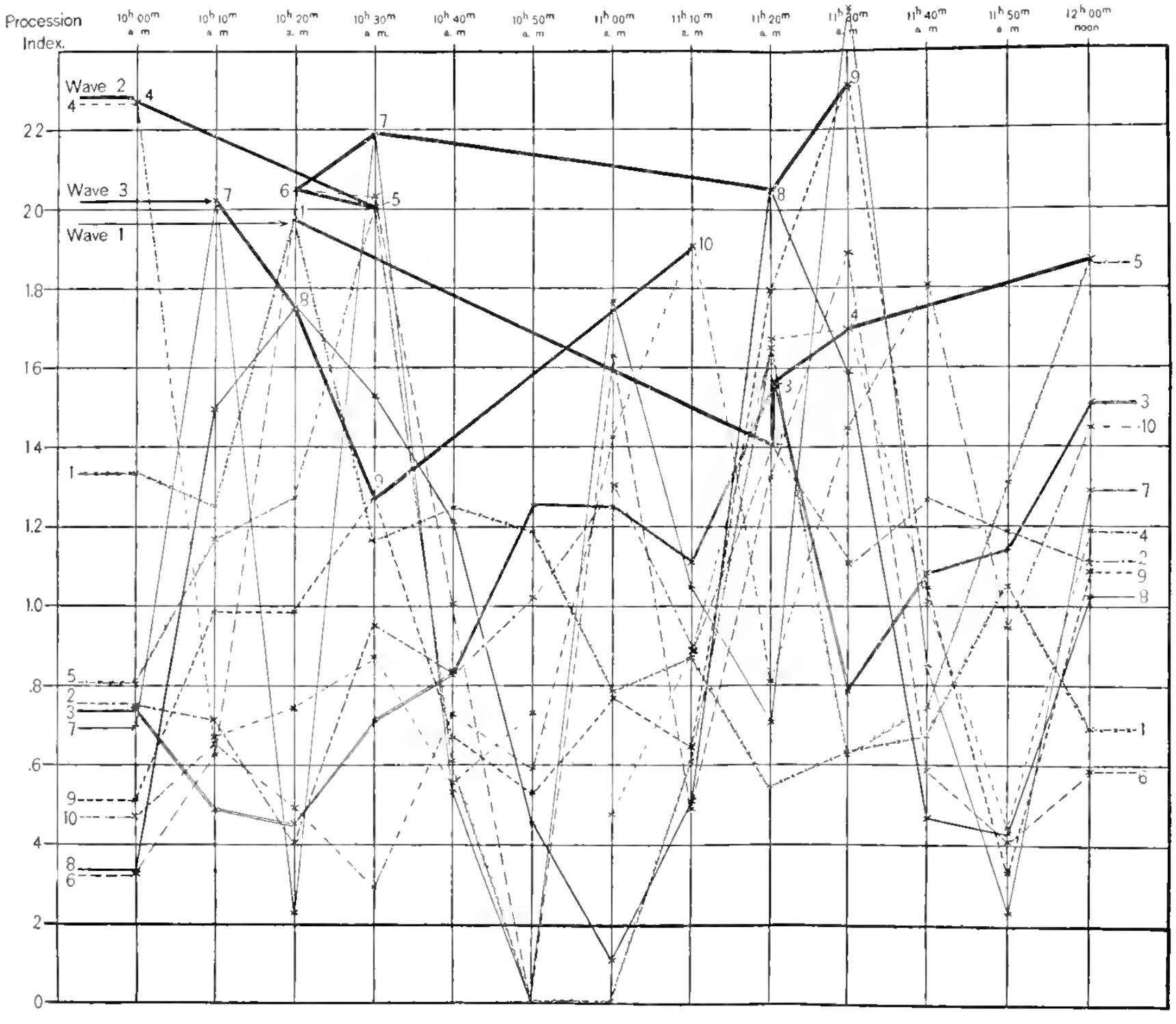
Average Absolute Duration of the Entire Active Mitotic Cycle (A. A. D. of C.) = { Time elapsing between 2 points in a recognizable procession of a definite P. I. / No. of stages covered / No. P. I. followed } × No. of stages in cycle

Average Absolute Duration of a given Mitotic Stage (A. A. D. of S.) = A. A. D. of C. × A. R. D. of S.

∴ In this onion root-tip experiment the Average Absolute Duration of the successive mitotic stages is as follows:

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8	Stage 9	Stage 10
77.02 min.	38.19 min.	16.06 min.	4.58 min.	1.32 min.	1.65 min.	1.53 min.	4.83 min.	6.31 min.	20.59 min.

7.—*Graphs showing orderly succession of procession indices. (Preliminary study.)*



——— = Lines connecting highest points of procession indices in successive stage and time order.

-·-·-·-·- = Procession Indices of Stage One
– – – – = " " " " Two
═══ = " " " " Three
– – – – = " " " " Four
-·-·-·-·- = " " " " Five
– – – – – = Procession Indices of Stage Six
——— = " " " " Seven
——— = " " " " Eight
- - - - - - = " " " " Nine
— – — – = " " " " Ten

S.—*Mitosis in onion root-tip cells at 10° C. Stage index table, and calculation of average relative duration of the several mitotic stages.*

Mitotic stage		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Σ Count Σ S. I.	Average stage index. = also average relative duration (A R D.)	Σ S. I.	A. R. D. when R "resting" is considered a stage.
		10h00m a. m.	10h10m a m	10h20m a m	10h30m a. m.	10h40m a m	10h50m a m.	11h00m a. m.	11h10m a. m.	11h20m a. m.	11h30m a. m.	11h40m a. m.	11h50m a m	12h00m n.	12h10m p. m.	12h20m p. m.	12h30m p. m.	12h40m p. m.	12h50m p. m.	1h00m p. m.				
Resting	Count	785	722	773	760	742	780	660	641	745	735	781	776	705	606	792	547	639	739	647	13,575			7144
1	Count	136	166	117	136	129	131	171	228	78	160	173	119	187	202	82	183	122	186	141	2,847	5354	10 1699	.1498
	S. I.	6325	5971	5154	5066	5000	5051	5029	6350	3088	6037	7899	5312	6338	5126	3042	4039	3379	7126	3994	10 1699			
2	Count	38	36	43	67	66	56	107	67	69	50	37	45	54	84	55	124	105	47	100	1,250	2265	4 3034	.0657
	S. I.	1767	1294	1894	2791	2558	2545	3147	1866	2705	1886	1689	2008	1830	2131	2644	2737	2008	1890	2832	4 3034			
3	Count	17	44	25	18	24	22	28	25	32	30	7	25	25	38	25	39	47	12	28	511	0943	1 7918	.0268
	S. I.	0790	1582	1101	0750	0930	1000	0823	0996	1254	1132	0319	1116	0817	0964	1201	0860	1301	0459	0793	1 7918			
4	Count	11	31	25	11	22	6	10	9	14	10	1	11	3	18	18	20	15	1	19	235	0482	9169	.0134
	S. I.	0511	1115	1101	0458	0852	0272	0294	0250	0549	0377	0045	0491	0101	0156	0865	0441	0415	0038	0538	9169			
5	Count	0	0	4	2	5	2	9	7	7	3	0	3	1	8	11	7	7	0	3	79	0142	2715	.0041
	S. I.	0000	0000	0176	0083	0193	0090	0264	0194	0274	0113	0000	0133	0033	0205	0528	0154	0193	0000	0084	.2715			
6	Count	1	0	0	3	3	1	1	5	3	2	0	3	1	1	1	3	4	0	2	39	0067	1273	.0020
	S. I.	0045	0000	0000	0125	0116	0045	0029	0139	0117	0075	0000	0133	0033	0025	0048	0176	0110	0000	0056	.1273			
7	Count	1	0	4	2	5	0	1	5	5	3	0	2	1	4	2	4	1	0	0	40	0074	.1409	0021
	S. I.	0046	0000	0176	0083	0193	0000	0029	0139	0196	0113	0000	0089	0033	0101	0096	0088	0027	0000	0000	.1409			
8	Count	4	0	8	1	2	2	5	4	15	5	0	5	6	12	2	20	10	1	8	110	.0188	.3588	0057
	S. I.	0186	0000	0352	0041	0077	0090	0147	0111	0588	0188	0000	0223	0203	0304	0096	0111	0277	0038	0226	.3588			
9	Count	2	0	0	0	1	0	6	3	14	1	0	5	8	9	5	33	20	2	22	133	0209	.3975	0070
	S. I.	0093	0000	0000	0000	0038	0000	0176	0139	0549	0037	0000	0223	0271	0228	0240	0728	0554	0076	0623	.3975			
10	Count	5	1	1	0	1	0	2	4	18	1	1	6	9	18	7	15	30	12	30	161	0270	.5139	0068
	S. I.	0232	0035	0044	0000	0038	0000	0058	0111	0705	0037	0045	0267	0305	0456	0336	0331	0831	0459	0849	.5139			
Σ Count 1 to 10.		215	278	227	240	258	220	340	359	255	265	219	224	295	394	208	453	361	261	353	5,425	Σ A. R. D. .9994	ΣΣ S. I. 18 9919	Σ 10 active 2834
Σ Count R+1 to 10.		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	ΣΣ Count 19,000			ΣR+10 active 9978
Mitotic Index (M. I.)		215	278	227	240	258	220	340	359	255	265	219	224	295	394	208	453	361	261	353	Σ M. I. 5.425	Average M. I. .2855		

9.—Mitosis in onion root-tip cells at 20° C. Stage index table, and calculation of average relative duration of the several mitotic stages.

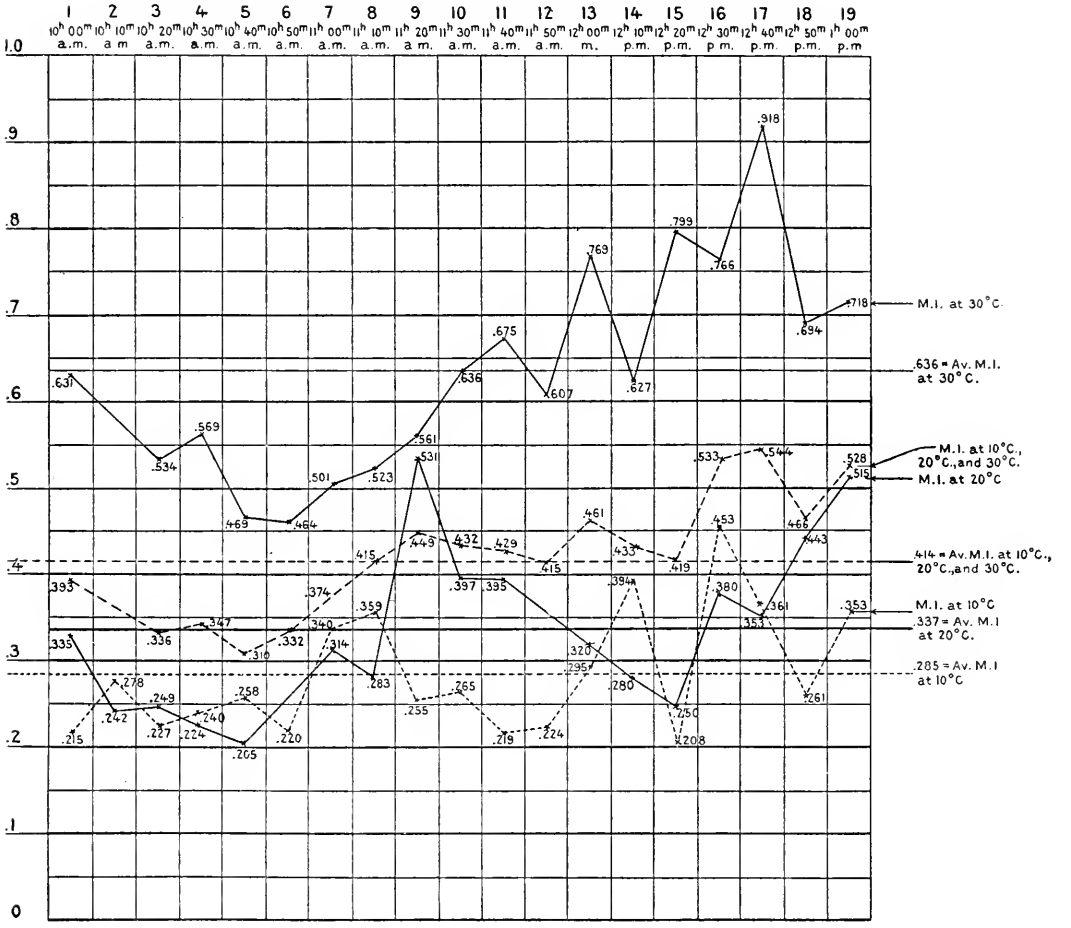
Mitotic stage		1 10h00m a m	2 10h10m a m	3 10h20m a m	4 10h30m a m	5 10h40m a m	6 10h50m a m	7 11h00m a m	8 11h10m a m	9 11h20m a. m	10 11h30m a m	11 11h40m a m.	12 11h50m a m	13 12h00m n	14 12h10m p. m	15 12h20m p m	16 12h30m p m.	17 12h40m p. m.	18 12h50m p m	19 1h00m p m	Σ Count Σ S I	Average stage index = also average relative duration (A R D)	ΣΣ S. I	A. R. D when R "resting" is considered a stage.
Resting	Count	665	758	751	776	795	686	717	635	469	603	605		680	720	750	629	647	557	485	11,919			6621
1	Count S I	253 7552	192 7933	186 7469	146 6517	138 6731	104 6178	222 7844	281 7698	433 8154	267 6725	207 7518		215 6718	203 7250	145 5800	200 7031	271 7077	356 8036	389 7553	4,178 13 0984	7280	13 0984	2487
2	Count S I	38 0835	21 0867	31 1244	36 1607	24 1170	13 1569	25 0883	30 0821	37 0696	46 1158	21 0531		36 1125	25 0892	44 1760	28 0736	26 0736	35 0799	51 0990	587 1 8210	1012	1 8210	0326
3	Count S I	6 0179	10 0413	7 0281	7 0312	9 0430	17 0541	17 0600	9 0246	19 0357	13 0327	16 0405		10 0312	12 0428	23 0920	14 0368	13 0368	9 0203	9 0174	220 0873	0382	.6873	0122
4	Count S I	4 0119	7 0289	8 0321	15 0669	10 0487	15 0477	5 0176	11 0301	10 0188	17 0428	32 0810		23 0718	14 0500	15 0600	9 0236	8 0226	22 0496	24 0466	249 7507	0417	.7507	0138
5	Count S I	0 0000	1 0041	2 0080	1 0044	1 0048	6 0194	1 0035	8 0219	6 0142	4 0100	8 0202		9 0281	2 0071	9 0360	4 0105	6 0169	4 0090	2 0038	74 2186	.0121	2186	0041
6	Count S I	3 0089	0 0000	1 0040	3 0135	2 0097	5 0159	1 0035	4 0109	4 0075	4 0100	3 0075		7 0218	2 0071	5 0200	3 0078	4 0113	0 0000	6 0116	57 1708	0094	1708	0031
7	Count S I	2 0059	1 0041	2 0080	3 0133	3 0146	7 0222	0 0000	4 0104	4 0075	5 0125	2 0050		2 0062	2 0071	2 0080	1 0026	2 0056	0 0000	3 0058	45 1393	.0077	.1393	0025
8	Count S I	4 0119	1 0041	4 0160	6 0267	3 0146	9 0286	4 0141	6 0164	8 0150	12 0302	4 0101		2 0062	1 0035	2 0080	7 0184	1 0028	2 0045	9 0174	85 2485	.0138	.2485	0047
9	Count S I	12 0358	2 0082	5 0200	4 0178	5 0243	7 0222	3 0106	5 0136	3 0056	10 0251	6 0151		7 0218	6 0214	3 0120	12 0315	7 0198	2 0045	15 0291	114 3384	0188	.3384	.0063
10	Count. S I.	23 0686	7 0289	3 0120	3 0133	10 0487	11 0330	5 0176	7 0191	7 0131	19 0478	6 0151		9 0281	13 0464	2 0080	12 0315	15 0424	13 0293	7 0135	172 5184	0288	5184	0095
Σ Count 1 to 10		335	242	249	224	205	314	283	365	531	397	395		320	280	250	380	353	443	515	6,081	Σ A. R. D 9097	ΣΣ S. I. 17 9914	Σ 10 active 3375
Σ Count R+1 to 10		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000		1,000	1,000	1,000	1,000	1,000	1,000	1,000	ΣΣ Count 18,000			ΣR+10 active .9996
Mitotic Index (M I.)		335	242	249	224	205	314	283	365	531	397	395		320	280	250	380	353	443	515	Σ M I 6.081	Average M I. .3378		

Specimens ruined in preparation

10.—*Mitosis in onion root-tip cells at 30° C. Stage index table, and calculation of average relative duration of the several mitotic stages.*

Mitotic stage.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Σ count Σ S. I	Average stage index = also average relative duration (A.R.D.)	ΣΣ S. I.	A. R. D. when R "resting" is considered a stage.
Resting	Count	369		406	431	531	536	490	477	439	364	325	393	231	373	201	234	82	306	282	6,539			3632
1	Count	547		454	464	370	389	429	467	476	563	575	482	715	583	747	716	885	653	673	10,193	8819	15 8687	5662
	S. I.	8958		8501	8242	7889	8389	8562	8929	8481	8852	8518	7940	9297	9298	9349	9347	9640	9469	9373	15 8687			
2	Count	29		15	28	22	27	13	8	9	21	18	29	13	13	13	12	8	23	9	310	0286	.5163	0172
	S. I.	0459		0280	0492	0469	0581	0259	0152	0160	0331	0266	0477	0169	0207	0162	0156	0087	0331	0125	.5163			
3	Count	7		12	9	8	15	27	12	10	9	16	21	18	10	12	11	9	6	9	221	0202	.3648	.0122
	S. I.	0110		0221	0158	0170	0323	0538	0229	0178	0141	0237	0345	0234	0159	0150	0143	0098	0086	0125	3648			
4	Count	22		24	18	25	8	18	4	14	10	21	10	6	10	5	7	7	2	13	224	0211	3809	0124
	S. I.	0348		0419	0316	0533	0172	0359	0076	0249	0157	0311	0164	0078	0159	0062	0091	0076	0028	0181	.3809			
5	Count	4		3	6	4	5	2	5	5	3	8	8	3	1	0	0	1	0	0	58	0055	0999	0032
	S. I.	0063		0056	0105	0085	0107	0039	0095	0089	0047	0118	0131	0039	0015	0000	0000	0010	0000	0000	0999			
6	Count	3		1	9	3	1	4	3	3	3	8	9	1	2	3	5	3	1	1	63	0056	1014	.0035
	S. I.	0047		0018	0158	0063	0021	0079	0057	0053	0047	0118	0148	0013	0031	.0037	0095	0052	0014	0013	1014			
7	Count	1		1	6	3	3	1	4	5	1	5	5	0	1	0	3	0	1	2	42	0039	.0715	0023
	S. I.	0015		0018	0105	0063	0064	0019	0076	0089	0015	0074	0082	0000	0015	0000	0039	0000	0014	0027	0715			
8	Count	3	Slide ruined in mounting.	6	1	5	5	2	7	5	5	7	11	4	2	4	4	0	3	4	NI	0074	1348	.0045
	S. I.	0047		0112	0070	0106	.0107	0039	0133	0089	0078	0103	0181	0052	0031	0050	0052	0000	0043	0055	1348			
9	Count	12		8	14	14	4	4	6	15	4	10	16	3	0	7	4	3	1	5	130	0121	2195	.0072
	S. I.	0190		0149	0246	0298	0086	0079	0114	0267	0062	0148	0263	0039	0000	0087	0052	0032	0014	0069	2195			
10	Count	3		10	6	15	7	1	7	19	17	7	16	6	5	8	4	2	4	2	139	.0130	2345	.0077
	S. I.	0047		0187	0105	0319	0150	0019	0133	0338	.0267	0103	0263	0078	0079	0100	0052	0021	0057	0027	2345			
Σ Count 1 to 10		631		534	569	469	464	501	523	561	636	675	607	769	627	799	766	918	694	718	11,461	Σ A. R. D. 9993	ΣΣ S. I. 17 9923	Σ 10 active .6364
Σ Count R+1 to 10		1,000		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	ΣΣ count 18,000			ΣR+10 active 9996
Mitotic Index (M. I.)		631		534	569	469	464	501	523	561	636	675	.607	769	627	799	766	918	694	718	Σ M. I. 11 461	Average M. I. 6367		

11.—*Graphs showing mitotic indices at 10° C., 20° C. and 30° C.*



12.—*Mitosis in onion root-tip cells at 10°C. Procession index table, and calculation of average absolute duration of the several mitotic stages.*

Stage	Average relative duration (A.R.D.).		1 10h00m a. m.	2 10h10m a. m.	3 10h20m a. m.	4 10h30m a. m.	5 10h40m a. m.	6 10h50m a. m.	7 11h00m a. m.	8 11h10m a. m.	9 11h20m a. m.	10 11h30m a. m.	11 11h40m a. m.	12 11h50m a. m.	13 12h00m noon.	14 12h10m p. m.	15 12h20m p. m.	16 12h30m p. m.	17 12h40m p. m.	18 12h50m p. m.	19 1h00m p. m.	Σ S. I. Σ P. I.	Average absolute duration (A. R. D.) in minutes
1	.5354	S. I.	.6325	5971	.5154	.5666	.5000	.5954	.5029	6350	.3058	.6037	.7899	5312	.6338	.5126	.3942	4039	.3379	.7126	3994	10 1699	52 2550
		P. I.	1.1813	1 1152	.9626	1 0582	.9338	1.1120	.9392	1.1860	.5711	1.1275	1 4753	.9921	1.1837	.9574	.7362	7543	.6311	1 3309	.7459	18 9938	
2	.2265	S. I.	.1767	.1294	.1894	2791	2558	.2545	3147	.1866	2705	.1886	.1689	2008	.1830	.2184	2644	.2737	.2908	1800	2832	4.3034	22 1064
		P. I.	.7801	.5713	.8362	1 2322	1 1293	1.1236	1.3894	.8238	1.1942	.8326	.7456	8865	.8079	.9108	1 1673	1 2083	1.2830	.7947	1 2503	19.0016	
3	0943	S. I.	0790	.1582	.1101	.0750	0930	.1000	0823	.0696	.1254	1132	.0319	.1116	.0547	.0964	.1201	0860	1301	.0459	0793	1.7918	9 2036
		P. I.	8377	1.6776	1.1675	.7953	.9862	1 0601	8727	.7380	1 3297	1.2084	.3382	1 1834	8981	1.0222	1.2735	.9119	1 3796	.4867	.8409	19 0000	
4	0482	S. I.	.0511	.1115	.1101	.0455	.0852	.0272	0294	0250	.0549	.0377	.0045	.0491	.0101	.0456	.0865	.0441	.0415	.0038	.0538	.9169	4 7043
		P. I.	1 0601	2 3132	2 2842	.9502	1.7676	.5643	.6099	.5186	1.1390	.7821	0933	1 0186	.2095	.9460	1.7946	.9149	.8609	.0788	1 1161	19.0219	
5	0142	S. I.	.0000	.0000	0176	.0083	.0193	.0090	.0264	.0194	.0274	.0113	.0000	.0133	.0033	.0203	.0525	.0154	.0193	.0000	.0084	.2715	1.3859
		P. I.	.0000	.0000	1.2594	.5845	1.3591	.6338	1.8591	1.3661	1.9295	.7957	.0000	.9366	.2323	1.4295	3.7183	1 0845	1 3591	.0000	.5915	19.1190	
6	0067	S. I.	.0016	.0000	.0000	0125	.0116	.0045	.0029	0139	.0117	.0075	.0000	0133	.0033	.0025	.0048	0176	.0110	.0000	.0056	.1273	6539
		P. I.	.6865	.0000	.0000	1 8656	1.7313	.6716	.4328	2.0746	1.7462	1.1194	0000	1 9850	.4925	.3731	7164	2.6268	1.6417	.0000	.8358	18.9993	
7	.0074	S. I.	.0046	.0000	.0176	.0083	0193	.0000	.0029	.0139	0196	0113	.0000	0089	.0093	0101	.0096	.0088	.0027	0000	.0000	1409	7222
		P. I.	.6216	.0000	2 3783	1.1216	2.6081	.0000	.3918	1.8783	2.6466	1.5270	.0000	1.2027	.4459	1 3648	1 2972	1.1891	.3648	0000	.0000	19.0392	
8	.0188	S. I.	.0186	.0000	.0352	.0041	.0077	.0090	0147	.0111	.0588	.0188	.0000	0223	.0203	.0304	0096	0441	0277	.0038	.0226	.3588	1.8348
		P. I.	.9893	0000	1.8723	2180	.4095	.4787	7819	.5904	3.1276	1 0000	0000	1 1861	1.0797	1 6170	.5106	2 3457	1.4724	.2021	1 2021	19 0544	
9	.0209	S. I.	.0093	.0000	.0000	.0000	.0038	0000	.0176	.0139	0549	.0037	.0000	.0223	0271	.0228	.0240	.0728	.0554	.0076	0623	.3975	2.0398
		P. I.	.4449	.0000	.0000	.0000	.1818	0000	.8421	.6650	2 6267	.1770	.0000	1.0669	1 2966	1 0960	1 1483	3.4832	2.6507	3636	2 0808	19.0185	
10	0270	S. I.	.0232	.0035	.0044	.0000	.0038	.0000	.0058	.0111	0705	.0037	0045	.0267	.0305	0456	0336	.0331	0831	.0459	0849	5139	2.6352
		P. I.	8592	.1296	.1639	.0000	.1407	0000	2148	.4111	2 6111	.1370	.1666	.9888	1 1296	1 6888	1 2444	1.2259	3.0777	1 7000	3 1444	19 0326	
																						ΣΣ P. I. 190 3637 Average 19 0363	97.5411 min. A A.D. of cycle, *i. e.*, 10 active stages exclusive of R.

Calculation of Average Absolute Duration (A.A.D.)

Wave No.	No. of stages passed through	Minutes	Av. stage duration = No. of min. ÷ No. of stages
1	7	60	8.571
2	9	80	8.888
3	9	100	11.111
4	9	90	10.000
5	9	90	10.000
6	9	90	10.000
			Total 58 570

58.570 min. ÷ 6 = 9 76 min. = Average absolute duration of stages.
10 × 9.76 min. = 97 6 min. = Average absolute duration of mitotic cycle.

Average duration of the entire mitotic cycle when the resting period is considered a stage.

R (*i.e.* resting stage) = 71 14 per cent of entire duration = 194 92 min.
10 active stages = 28 55 per cent of entire duration = 97 60 min.

10 active stages + R = 100 00 per cent of entire duration = 292 52 min.

13.—Mitosis in onion root-tip cells 20° C. Procession index table, and calculation of average absolute duration of the several mitotic stages.

Stage	A R. D.		1 10h00m a. m	2 10h10m a. m	3 10h20m a. m.	4 10h30m a. m.	5 10h40m a. m.	6 10h50m a. m	7 11h00m a. m.	8 11h10m a. m	9 11h20m a. m.	10 11h30m a. m	11 11h40m a. m	12 11h50m a. m.	13 12h00m n.	14 12h10m p. m	15 12h20m p. m	16 12h30m p. m.	17 12h40m p. m.	18 12h50m p. m.	19 1h00m p. m.	Σ S. I. Σ P. I.	Average absolute duration (A. A. D.) in minutes.
1	7280	S. I.	7552	7933	7469	6517	6731	6178	7844	7098	8154	6725	7518		6718	.7250	5806	7631	7677	8036	7553	13 0984	.59 2592
		P. I.	1 0373	1 0896	1 0259	8951	9245	8486	1 0774	1 0374	1 1200	9237	1 0326		9228	9958	7967	1 0482	1 0545	1 1038	1 0375	17 9914	
2	1012	S. I.	0835	0867	1244	1907	1170	1369	0983	0821	0696	1158	0531		1125	0892	1760	0736	0735	.0790	0990	1 8210	8 2376
		P. I	8250	8867	1 2282	1 5870	1 1561	1 3327	.8725	8112	0877	1 1442	5247		1 1116	8814	1 7581	7272	7272	.7806	9792	17 9932	
3	0382	S. I.	0179	0413	0281	0312	0139	0541	0600	0246	0357	0327	0305		.0312	0328	0920	0368	0368	0203	0174	6873	3 1094
		P. I	4685	1 0816	7356	8167	1 1492	1 4202	1 3760	6439	9345	8560	1 0202		8167	1 1281	2 4083	9633	9633	5314	4554	17 9913	
4	0417	S. I	0119	0289	0321	0669	0487	0477	0176	0301	0188	0428	0840		0718	0500	0600	0236	0226	0496	0466	7507	3 3943
		P. I	2853	6930	7057	1 6043	1 1678	1 1438	4220	7218	4508	1 0263	1 9424		1 7218	1 1990	1 4388	5659	5419	1 1894	1 1175	18 0013	
5	0121	S. I	0000	0041	0080	0044	0048	0191	0035	0219	0112	0100	0202		0281	0071	0360	0166	0169	0090	.0038	2186	9849
		P. I.	0000	3388	6611	3636	3966	1 5783	2892	1 0099	9256	8264	1 6694		2 3220	7867	2 9752	8077	1 3966	7438	3140	18 0059	
6	0094	S I	0059	0000	0040	0133	0097	0150	0035	0000	0075	0100	0075		0218	0071	0200	0078	0143	0060	0116	1708	7651
		P I	9468	0000	4255	1 4148	1 0319	1 6911	3723	1 1593	7978	1 0883	7978		2 3191	7553	2 1276	8297	1 2021	0000	1 2340	18 1694	
7	0077	S I	0059	0074	0080	0133	0146	0222	0000	0000	0075	0125	0050		0062	0071	0080	0026	0056	0000	0058	1393	6267
		P I	7662	5324	1 0389	1 7272	1 8801	2 8831	0000	1 4155	9740	1 6233	6493		8051	9220	1 0389	3376	7272	0000	7532	18 0000	
8	0135	S. I	0119	0041	0160	0267	0146	0285	0141	0164	0150	0302	0101		0062	0035	0060	0184	0028	0043	0174	2185	1 1233
		P. I	8623	2971	1 1594	1 9347	1 0579	2 0724	1 0217	1 1884	1 0859	2 1884	7318		4492	2536	5797	1 3383	2028	3260	1 2608	18 0064	
9	0188	S. I.	0358	0082	0200	0178	0243	0222	0105	0136	0056	0251	0151		0218	0214	0120	0315	0198	0045	0291	3384	1 5303
		P. I.	1 9040	4361	1 0638	9468	1 2935	1 1808	5638	7234	2978	1 3351	8031		1 1595	1 1382	6382	1 6755	1 0531	2393	1 5478	17 9988	
10	0288	S. I.	0686	0289	0120	0133	0487	0350	0176	0191	0131	0478	0151		0281	0464	0080	0315	0424	0293	0135	5184	2 3443
		P I	2 8819	1 0034	4166	4618	1 6809	1 2152	6111	6631	4548	1 6597	5243		9757	1 6111	2777	1 0937	1 4732	1 0173	4687	17 9992	
									1		2		3				4			5			

ΣΣ P. I.	S1 3751 min.
180 3071	A. A. D. of cycle,
Average	i. e., 10 active
18 0307	stages exclusive of R.

Calculation of Average Absolute Duration (A. A. D.).

Wave No.	No. of stages passed through	Minutes	Av. stage duration = No. of min. ÷ No. of stages
1	9	50	5 555
2	9	60	6 666
3	9	70	7 777
4	9	90	10 000
5	9	80	8 888
6	8	80	10 000
		Total	48 886

48 886 mm ÷ 6 = 8 14 min. = Average absolute duration of stages.
10 × 8 14 min. = 81 4 min. = Average absolute duration of active mitotic cycle

Average duration of the entire mitotic cycle when the resting period is considered a stage

R (i. e., resting stage) = 66 21 per cent of entire duration = 159 57 min.
10 active stages = 33 78 per cent of entire duration = 81 40 min

10 active stages + R = 100 00 per cent of entire duration = 240 97 min.

14.—*Mitosis in onion root-tip cells 30° C. Procession index table, and calculation of average absolute duration of the several mitotic stages.*

Stage	(A.R.D.)		1 10h00m a. m.	2 10h10m a. m.	3 10h20m a. m.	4 10h30m a. m.	5 10h40m a. m.	6 10h50m a. m.	7 11h00m a. m.	8 11h10m a. m.	9 11h20m a. m.	10 11h30m a. m.	11 11h40m a. m.	12 11h50m a. m.	13 12h00m n.	14 12h10m p. m.	15 12h20m p. m.	16 12h30m p. m.	17 12h40m p. m.	18 12h50m p. m.	19 1h00m p. m.	Σ S. I. Σ P. I.	Average absolute duration (A. A. D.) in minutes.
1	8819	S. I.	8668		8501	8242	7889	8389	8562	8929	8484	8852	8518	7940	.9297	9298	9349	9347	9640	9409	9373	15 8687	51 4147
		P. I	9828		9639	9345	8945	9512	9708	1 0124	9620	1 0037	9658	9003	1 0542	1 0542	1 0000	1 0598	1 0930	1 0669	1 0628	17 9928	
2	0286	S. I	0459		0280	0492	0169	0581	0259	.0152	0199	0331	0286	0477	0169	0207	0162	0156	0087	0331	0125	5163	1 6073
		P. I	1 6048		9790	1 7282	1 6398	2 0314	9055	.5314	.5594	1 1573	9300	1 6678	5909	.7207	5664	.5454	3041	1 1573	4370	18 0514	
3	0202	S. I.	0110		0224	0158	0170	0323	0538	0229	0178	0141	0237	0345	0234	0159	0150	0143	0098	0086	0125	3648	1 1776
		P. I.	5445		1 1089	7821	8415	1 5990	2 6683	1 1356	8811	6980	1 1727	1 7079	1 1584	7871	7425	7079	4851	4257	6188	18 0586	
4	0211	S. I	0348		0449	0316	0533	0172	0359	0026	0249	0159	0311	0164	0078	0159	0062	0091	0076	0028	0181	3809	1 2301
		P. I	1 6492		2 1279	1 4976	2 5260	8151	1 7014	3601	1 1860	7440	1 4739	7772	3696	7535	2938	4312	3601	1327	8578	18 0511	
5	0055	S. I	0063		0056	0105	0085	0107	0039	0095	0089	0047	0118	0131	0039	0015	0000	.0000	0010	0000	0000	0999	3212
		P. I	1 1454		1 0181	1 0990	1 5454	1 9454	.7090	1 7252	1 6181	8545	2 1454	2 3818	7090	2737	0000	0000	1818	0000	0000	18 1628	
6	0056	S. I	0047		0018	0158	0063	0021	0079	0057	0053	0047	0118	0118	0133	0031	0037	0055	0032	0014	0013	1014	3264
		P. I	8392		3214	2 8214	1 1250	3750	1 4107	1 0178	9464	8392	2 1071	2 6728	2321	5833	.6607	1 1607	5714	2500	2321	18 1065	
7	0039	S. I	0015		0018	0105	0063	0064	0019	0076	0089	0015	0074	0082	0000	0015	0000	0039	0000	0014	.0027	0715	2273
		P. I.	3846		4615	2 6923	1 6153	1 6410	3871	1 9167	2 2820	3846	1 8974	2 1025	0000	3846	0000	1000	.0000	3589	6923	18 3328	
8	0074	S. I	0047		0112	0070	0160	0107	0039	0133	0089	0078	0103	0181	0052	0031	0050	.0052	0000	0043	0055	1348	4314
		P. I	6351		1 5185	9459	1 4324	1 4459	5270	1 7972	1 2027	1 0540	1 3918	2 4459	7027	4189	.6756	.7027	.0000	5810	7432	18 2155	
9	0121	S. I	0190		04491	0246	0298	0086	0079	0114	0267	0062	0148	0263	0039	.0000	0087	0052	0032	0014	0069	2195	7054
		P. I.	1 5702		1 2314	2 0330	2 4628	.7107	.6528	.9421	2 2056	.5123	1 2231	2 1735	.3223	.0000	7190	4297	2644	1157	5702	18 1398	
10	0130	S. I	0047		0187	0105	0319	0150	0019	0133	0338	.0267	0103	0263	0078	0079	0100	0052	0021	0057	0027	2345	.7579
		P. I.	3615		1 4384	8076	2 4538	1 1538	1461	1 0230	2 6000	2 0538	7923	2 0230	.6000	.6076	.7692	4000	1615	4384	2076	18 0376	
								1				2	3	4	5						6	ΣΣ P. I. 181 1489 Average 18.1148	58.2593 min. A. A. D. of cycle, i. e., 10 active stages exclusive of R.

Calculation of Average Absolute Duration (A.A.D.).

Wave No.	No. of stages paved through	Minutes	Av. stage duration = No. of mm ÷ No. of stages.
1	9	40	4 444
2	9	60	6 666
3	9	40	4 444
4	9	40	4 444
5	9	40	4 444
6	9	80	8 888
7	8	60	7 500
		Total	40 830

40 830 mm ÷ 7 = 5 83 mm = Average absolute duration of stages
10 × 5 83 mm = 58 3 mm = Average absolute duration of mitotic cycle.

Average duration of the entire mitotic cycle when the resting period is considered a stage.

R (*i. e.*, resting stage) = 36.32 per cent of entire duration = 33.26 min.
10 active stages = 63.67 per cent of entire duration = 58.30 min.

10 active stages × R = 100.00 per cent of entire duration = 91.56 min.

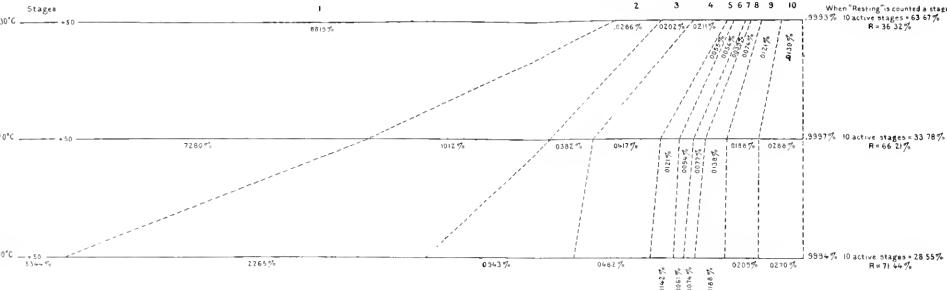
15.—*Mitosis in onion root-tip cells. Summary and comparison by stages and temperatures.*

Mitotic stage	10° C.		20° C.				30° C.					
	A R D	A A. D.	A. R D		A A. D.		A. R. D.			A A. D.		
	Per cent measure.	Minutes measure	Per cent measure	Compared with same at 10° C	Minutes measure.	Compared with same at 10° C.	Per cent measure	Compared with same at 10° C.	Compared with same at 20° C.	Minutes measure	Compared with same at 10° C.	Compared with same at 20° C.
1*	5354	52 2550	7280	1 3597	59 2592	1 1340	8819	1 6471	1 2114	51 4147	9839	8676
2*	2265	22 1064	1012	4467	8 2376	3726	0286	1262	2826	1 6673	0754	2024
3*	0943	9 2036	0382	4050	3 1094	3378	0202	2142	5286	1 1776	1279	3787
4*	0482	4 7043	0417	8651	3 3943	7215	0211	.4377	5059	1 2301	2614	3624
5*	0142	1 3859	0121	8521	9849	7106	0055	3873	4545	3212	2317	3261
6*	0067	6539	0094	1 4029	7651	1 1700	0056	8358	5957	3264	4991	4266
7*	0074	7222	0077	1 0405	6267	8679	0039	5270	5064	2273	3147	3626
8*	0188	1 8348	0138	7340	1 1233	6122	0074	3936	5362	4314	2351	3840
9*	0209	2 0398	0188	8995	1 5303	7502	0121	5789	6436	7054	3158	4609
10*	0270	2 6352	0288	1 0666	2 3443	8896	0130	4814	4513	.7579	2876	3232
Cycle 10 active stages *	1 0000	97 60 min	1 0000	1 0000	81 40 min	8342	1 0000	1 0000	1 0000	58 30 min	5971	7158
Resting stage **	7147	194 92 min	6621	9264	159 57 min.	8186	3632	5081	5485	32 26 min	1655	2021
Entire cycle 10 active stages and R **	1 0000	292 52 min	1 0000	1 0000	240 97 min	8237	1 0000	1 0000	1 0000	91 56 min	3130	3799
Stages 1 to 10 inclusive **	2855	97 60 min	3378	1 1831	81 40 min	8340	6367	2 2301	1 8848	58 30 min.	5973	7162
Stage 1 *	5354	52 2550	7280	1 3597	59 2592	1 1340	8819	1 6471	1 12114	51 4147	9839	8676
Stages 2 to 10, inclusive,*	4640	45 2861	2717	5855	22 1159	4883	1174	2530	4321	6 8446	1511	3094
Average M I	At 10° C. = 285		At 20° C = .337 Compared with av M I at 10° C. = 1 1824				At 30° C. = 636 Compared with av. M. I. at 20° C = 1 8872 Compared with av. M I at 10° C. = 2 2315					

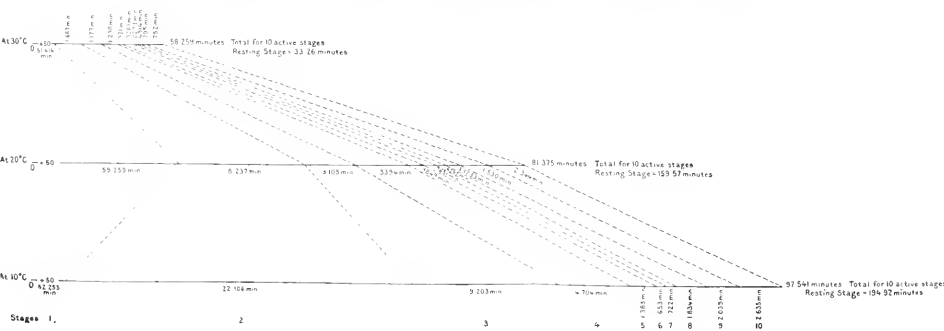
* Cycle = 10 active stages only
A R D = Average relative duration.

** Cycle = 10 active stages and the resting stage.
A A D = Average absolute duration.

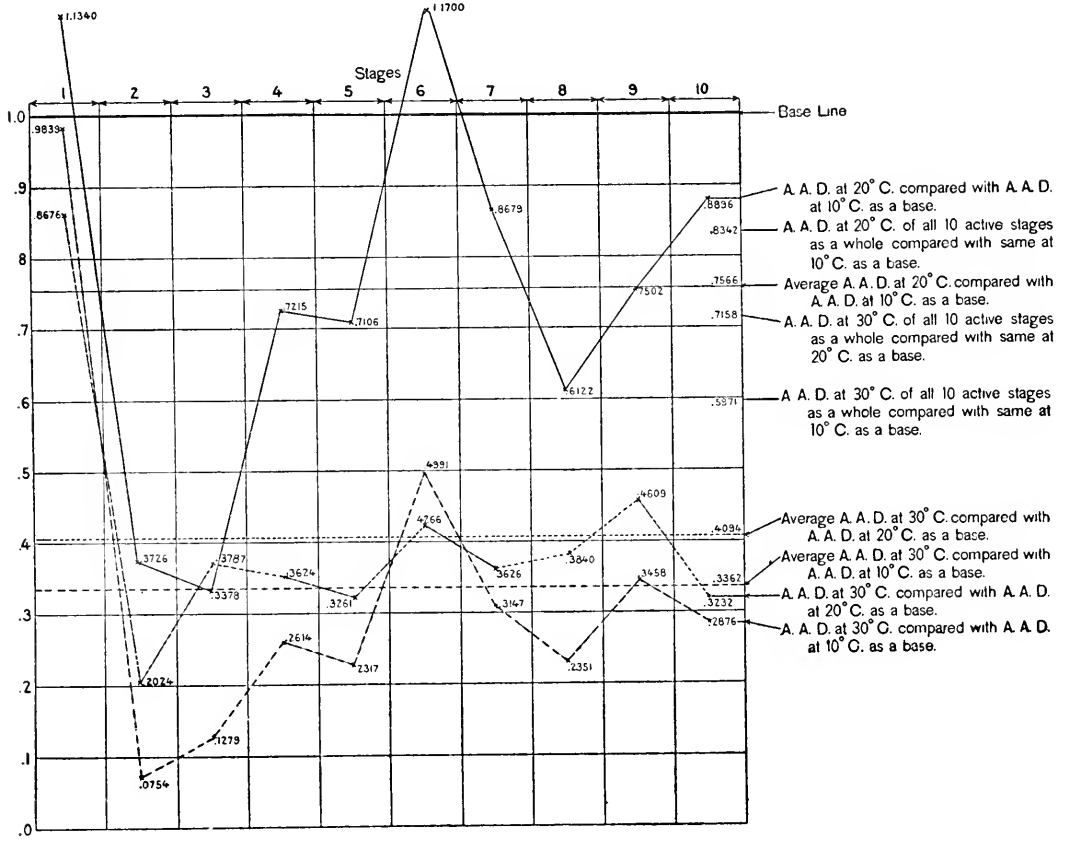
16.—*Comparison at 10° C., 20° C. and 30° C. of the average relative duration of the several mitotic stages.*



17.—*Comparison at 10° C., 20° C. and 30° C. of the average obsolute duration of the several mitotic stages.*



18.—*Graphs showing comparative measures at 10° C., 20° C., and 30° C. of the average absolute durations of the ten active mitotic stages.*



Data from Table 15.