Temperature and Growth.

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With eleven Figures in the Text.

INTRODUCTION

THE object of the present paper is to analyse the effects produced upon the growth-process by the temperature factor.

The research was initiated by a casual observation¹, but developed into an attempt to determine the chemical effects of the temperature-factor by studying the pathological portion of the growth-temperature curve; and hence to demonstrate that the time-factor is chemical in its nature, and that its effects can be simulated immediately.

Isolated cells are preferable to bulky plant-bodies for such a purpose if the manipulative difficulties can be overcome, and I have employed as the subject an organism which has been under my observation for the past three years as a pest of the cotton crop—the so-called Sore-shin fungus². It possesses one great advantage as a physiological subject, viz. the absence of any spore-formations, sexual or asexual, so that all the hyphae are both morphologically and physiologically equivalent. The chief novelty in the methods employed is the rapid rate of temperature change. By using so small an organism, and by recording its temperature with fair accuracy, it becomes possible to work through a long range of temperature in a short space of time. Errors due to the time-factor are thus minimized.

The observations given here, and the interpretations based on them, do not profess to do more than attempt to follow the lines along which explanations may be sought of the 'qualitative phenomena' of growth. We are not concerned with the 'quantitative phenomena'. Those internal factors, probably resident in the nucleus, which determine the rate of growth

¹ Khed. Agric. Soc. Year Book, 1905, pp. 184, 190. See also p. 33 *infra*.

² Khed. Agric. Soc. Year Book, 1905. Prel. Note, *ibid.*, 1906. Field Notes, Preventive Measures.

for a cell under standard conditions, belong to a different category from those environmental factors which—the power to grow being granted—determine the relative amounts of that growth under varying external conditions.

It is hardly necessary to point out that the experimental and analytical methods employed are based on Blackman's 'Theory of Limiting Factors'. In 'Optima and Limiting Factors'¹, he points out that 'the way of those who set out to evaluate exactly the effects of changes in a single factor upon a multi-conditioned metabolic process is hard, and especially so when the process is being pushed towards the upper limits of its activity'.

PRELIMINARY WORK

In my first publication² concerning the Sore-shin fungus it was stated that the growth of the fungus was soon inhibited at 34° C. on agar media, and that this inhibition corresponded to the results obtained by raising the temperature of an infected cotton seedling. The wide interval between the 'inhibition temperature' and the death-point led me to examine the matter further, in mass cultures and in hanging drops. The apparatus in common use for the latter purpose was proved to be untrustworthy, and all the results obtained with it have been omitted from this account, being superseded by the later work here given.

Solid Media. The results were at first erratic when the cultures in agar streak tubes were repeated; growth might continue freely over the surface of the jelly, or it might cease after an hour or two, as in the first experiments. The cause of this irregularity was found in the texture of the jelly, the former being the case on soft agar, and the latter on agar which had become dry and tough through storage in the dry air of the laboratory.

Growth on soft agar at 34° C. was not normal; it was marked by a tendency to form aerial hyphae, which grew rather more rapidly than those on the agar, so that the marginal extension of the mycelial disk was carried on by the return of these aerial hyphae to the surface, slightly in advance of those which already formed the edge.

These facts accorded with the expectations based on the hypothesis of poisonous excreta accumulating in the jelly and thus inhibiting the growth of the hyphae, entirely or partly. Renewal of growth at a low temperature would be due to the removal of the excreta, either by decomposition or by diffusion.

Liquid media should not exhibit this cessation of growth until several days had elapsed, owing to the free diffusion of the excreta away from the vicinity of the hyphae.

Liquid Media. Cultures stored at 20° C. develop mycelium with a smooth appearance, the hyphae branching occasionally and being almost

¹ Annals of Botany, April 1905.

² Khed. Agric. Soc. Year Book, 1905.

straight¹. When the surface of the liquid has been covered with mycelium, the hyphae grow up the sides of the flask and may even fill the interior with 'flying hyphae'. Resting cells are formed in abundance.

Cultures stored at 34° C. developed mycelium which had a fluffy appearance owing to the development of numerous short aerial hyphae, which were extensively branched and feathery. The long flying hyphae are not developed, resting cells are but sparsely formed, and growth ceases much sooner than in the cool cultures. This cessation of growth is commonly known to mycologists as 'staleness'.

In one experiment where the volume of the medium was about 100 cc. growth continued steadily for four days at 33° C., but on the fifth day the edge of the disk had ceased to grow. When these stale flasks were diluted with sterile water and re-inoculated the fungus grew freely again, although undiluted controls remained as incapable of supporting growth as before.

The time required for the completion of 'staling' is less at high temperatures than at low ones, and it is also affected by the volume of the medium.

Mode of growth. The growth of this fungus is seen to be entirely apical, and never intercalary, when examined under the microscope. A slight contraction may be noted in cooling a culture, but this is probably due to a change in the permeability of the protoplasm.

Hyphae taken from a cool culture show vacuoles, but these disappear on heating, and when a hypha is growing at high temperatures it appears to be homogeneous. The increment in length measured under the microscope thus represents fairly the actual increment in bulk of the protoplasm. Possibly the absence of vacuoles may facilitate the staling process.

The apical zone of growth is very small; the hyphae are not more than 10 microns in diameter, and the longitudinal extension of the zone is not more than 5 microns. Growth can be measured with considerable accuracy by taking some mark, bend, or side branch near the apex as a control point.

The hyphae branch fairly freely and all branches of a hypha—which implies all the hyphae in a culture—show the same temperature relationship, as can be seen when readings are obtained simultaneously from two or more adjacent hyphae.

Chemical Composition of a Stale Culture. The substances which cause staling have not yet been chemically identified, but it has been proved that a culture does not become stale of necessity because its food supply has been exhausted.

For this purpose the Urea Medium was employed²; when all growth had ceased the flasks were re-inoculated for extra assurance of their inability to support growth, and the medium was then analysed quali-

¹ Khed. Agric. Soc. Year Book, 1905.

² Page 563.

tatively for its various known components. Every one of the original components was present, from Urea to Calcium.

This analysis does not dispose of an objection that some substance might be present in minute traces, too small to be identified by ordinary analysis, and that staling was due to its exhaustion. The results of dilution experiments with distilled water dispose of this criticism.

APPARATUS.

KEY TO DIAGRAMS OF APPARATUS.

Copper plate (<i>c.u.</i>) in black. Control-couple in its paraffin bed shaded	
<i>B.</i> Control-bath.	<i>M.</i> Microscope.
<i>C.c.</i> Control-couple.	<i>M.L.</i> „ illumination.
<i>C.</i> Observer's chair.	<i>O.</i> Air space of chamber.
<i>G.</i> Galvanometer.	<i>P.</i> Stirring pump.
<i>G.L.</i> „ lamp.	<i>W.</i> Waste water.
<i>G.S.</i> „ scale.	<i>a.</i> Asbestos sheet.
<i>H.</i> Heating bath.	<i>c.m.</i> Culture medium.
<i>K.</i> Circuit key.	<i>c.u.</i> Copper plate.
<i>L.1.</i> Bunsen of <i>H.</i>	<i>g.</i> Glass carrier-slide.
<i>L.2.</i> Heating lamp of <i>c.u.</i>	<i>w.</i> Water.

The moist chamber. The chamber in which the fungus is grown under the microscope is the most important part of the apparatus employed in this research. Several different forms were devised for the purpose, but only one of these satisfied requirements.

The essential point is that no change whatever shall take place in the concentration of the liquid of the hanging drop. The only way to avoid such change is to immerse the chamber entirely in some liquid; so long as the cover-slip is exposed to the air, even when covered by a jacket, these changes are likely to take place, on account of the uneven heating of various parts of the chamber, and on account of the temperature-lag induced by the relatively massive objective.

The chamber used consists of three parts, as shown in Figs. 1, 2, 3, 4. A crystallizing dish containing water stands on a glass plate which is held in the jaws of the mechanical stage. Inside this water-bath stands a small glass beaker which holds about 30 cc. of culture medium. The moist chamber itself stands inside this beaker: it is made from the top of a test-tube, over which has been cemented a $\frac{3}{4}$ in. circular cover-glass, thus forming a cylinder which is open at the bottom; this cylinder is weighed down in the beaker by a bent and sealed tube full of mercury, to which tube it is fastened by a rubber band; a bent glass capillary tube passes down between the outer wall of the cylinder and the inner wall of the beaker and up inside the former to within a millimetre of the cover-slip; this tube serves to remove the air from the chamber and to introduce the gas required for respiration.

Before a new chamber is used it is soaked in warm water for two days in order to remove any soluble bodies from the sealing-wax which cements the cover-slip and from the thin rubber band.

Application of heat. The preliminary experiments referred to above were made in a Pfeiffer Hot-Box. I found, when the thermo-electric method of temperature measurement was adopted, that the temperature of the

FIG. 1. Elevation of control and heating baths.

object on the cover-slip usually lagged behind that indicated by the thermometer in the air of the box by as much as 5°C . when the rate of heating was rapid. Since the temperature of the object could now be determined accurately without employing the cumbersome box, the latter was abandoned in favour of the following plan for applying the heat directly to the microscope stage.

The end of a long plate of thick copper lies under the end of the large glass slide which carries the water-bath. The outer end of this plate is supported on a stand. Injury to the microscope stage by the inner end is prevented by resting it on a sheet of asbestos, through which, as well as through the copper plate, a hole is bored for illumination of the object.

Control Couple in wax plate, with thermometer

Front elevation

FIG. 2 a.

Control Couple.
Plan

FIG. 2 b.

Cover-Glass.

FIG. 2 d.

Culture Chamber and Bath

FIG. 2 c.

- FIG. 2. a. Control-couple plate, with thermometer.
b. Plan of control-couple plate showing connexions.
c. Culture chamber.
d. Plan of cover-slip showing locking-wire.

Below the copper plate a small gas flame stands on the work-bench, and this flame can be moved to any point between the microscope and the outer support; the heat from this flame is conducted along the copper plate to the stage, and there heats the water in the bath, which in its turn heats the medium in the beaker, and the convection currents in this flow

over the cover-slip and slowly raise the temperature of the object. The rate of heating can be arranged for by adjusting the size and position of the flame. This method is very satisfactory in practice in spite of its crude appearance; the temperature of the cover-glass rises uniformly¹, and the rate of heating can be adjusted after a little practice; it is not suitable for constant temperature work, for which a water-bath should be employed

FIG. 3. Semi-sectional elevation of microscope and chamber.

Inspection of the records transcribed at the end of the paper will show its efficiency. Irregular gas pressure in the laboratory mains is the cause of the uneven heating shown in the first curve.

Temperature Measurement. Measurement of temperature is effected by Blackman's thermo-electric couples², using the method of balance.

One couple is varnished and embedded in a thin plate of 60°C. M. Pt. wax, and immersed in a water-bath. The ends of the couple pass into

¹ See curves given below.

² Blackman and Matthaei, Proc. Roy. Soc. B., 1905.

second syphon with a clip serves to carry off the waste water from the bath.

The other couple is also varnished thoroughly to avoid any galvanic effect, and is stretched across the moist chamber below the cover-slip, its ends passing out into the medium and air through the cement with which the cover-slip is fixed. A second plain, thin wire, which lies by the side of this couple, will be referred to later. The ends of the couple are rolled into springs and led through the air to the two unoccupied cups in the head of the paraffin sheet; to permit of this the microscope is placed close to the right-hand side of the bath. This grouping of the cups automatically prevents any error which might otherwise arise in the circuit from subsidiary thermoelectric effects, since the remaining connexions of the switch and galvanometer are paired.

Two of the like terminals of the couples are connected through the cups by a short piece of No. 20 B. W. G. insulated copper wire, while similar silk-covered wires lead to the galvanometer, with a Du Bois Raymond key in circuit.

The galvanometer is an Ayrton-Mather pattern D'Arsonval, made by the Cambridge Scientific Instrument Company, who are also the makers of the copper-constantin couples.

The galvanometer scale is graduated continuously (in millimetres) in order to avoid possible errors in the transcription of plus and minus signs; the zero is adjusted (at 30 cm. usually) by moving the scale, if necessary, before each experiment. The deflexion given for a difference in temperature of one degree Centigrade between the couples is about 60 millimetres, as the instrument was set up in this work, so that errors are not likely to be made in the measurement of temperature. In practice the readings are made to the nearest half-centimetre, because the accuracy with which the bath thermometer can be read is considerably less than this; an accuracy of 0.1°C . is all that is aimed at, while even this is less than the errors which are likely to arise in the measurement of slowly growing hyphae.

Culture Media. For general purposes the following formula has been used:—

Peptone	2%	Potassium hydrogen phosphate	0.05%
Liebig's Extract	0.5%	Magnesium sulphate	0.05%
Pure Cane Sugar	0.5%	Tricalcium phosphate	0.01%
Distilled water	97%		

When an exact knowledge of the chemical composition of the original medium was required, the Liebig and peptone were replaced by 1 per cent. of Urea. It may be pointed out for future study that the fungus seemed to find difficulty in growing on this medium at low temperatures (20°C .), but flourished at 32°C .

Solid media were made from these by the addition of 1.5 per cent. agar and 0.5 per cent. gelatine.

EXPERIMENTAL METHODS.

Sterilization. After an experiment the chamber and beaker are washed and dried. Before the next experiment they are soaked in water at a temperature of 70° C. and rinsed with the culture medium. If these precautions are taken, bacteria rarely appear within the three hours which an experiment commonly occupies; the experiment is disregarded for critical purposes if any are seen.

Antiseptics cannot be employed on account of the difficulty of removing all traces of them from the wall and cement of the chamber.

Preparation of the Culture. The plan finally adopted was to inoculate a test-tube which contained 30 cc. of the medium under examination with a very small piece of mycelium from the aerial hyphae of an old, cool-grown, agar tube. The test-tube was left overnight at a temperature of 15° C. to 20° C. (unless some special temperature effect was under study) in order to allow new hyphae to develop. A disk would thus be formed of about 5 millimetres diameter.

A fragment of mycelium would then be taken from the margin of this mycelial disk, and placed in the chamber for observation. The size of this fragment was kept as small as possible, less than a millimetre in diameter and with a probable dry weight of about .0001 grammes.

By thus allowing the fungus to develop in the medium to be tested, we ensure that the new hyphae shall contain the same percentage of the excreted bodies (if any) in their cell-sap as was contained in the medium.

Growth Measurement. The tiny fragment of fungus is clipped in position under the cover-glass by means of the thin wire which lies by the side of the chamber-couple. The chamber is then placed in the beaker and the latter is filled with the culture-medium from the test-tube; the air imprisoned in the chamber is sucked out if necessary, and oxygen or other gas is passed in by the capillary tube; the end of the latter is plugged with a glass rod.

The beaker is then placed in the water-bath, which is filled with water of the desired initial temperature and placed on the stage. The ends of the chamber-couple are led to their mercury cups.

The Zeiss D¹ objective dips into the excess of culture medium which, flowing over the surface of the cover-glass, protects it from the sudden changes of temperature formerly mentioned. The No. 4 Zeiss eye-piece contains a micrometer scale, one division of which is equal to .003 mm. with this combination of lenses. This unit of .003 mm. is the one employed in the curves transcribed at the end of this paper. Under favourable

circumstances it is possible to read to $\cdot 0003$ mm. with this apparatus, but readings to less than $\cdot 001$ mm. are usually to be regarded as doubtful¹.

After some time has elapsed the hyphae resume the growth which had been interrupted by their transference from the test-tube to the chamber; a suitable one is then selected for measurement. The requirements are—rapid growth in a horizontal direction, and the possession of some mark near the apex which may be used to check any general movement.

When the growth at a constant low temperature has been determined, the small flame is put under the copper plate, and the following readings are taken.

Readings. During the progress of an experiment the following readings are taken once every minute:—

- (a) Time.
- (b) Position of control-point of hypha on micrometer scale.
- (c) Position of end of hypha on micrometer scale.
- (d) Temperature of control-bath.
- (e) Deflexion of galvanometer.

In practice the two latter are taken fifteen seconds earlier than the others, and a correction made, if necessary, when the curve is plotted.

When the rise in temperature of the chamber has carried the galvanometer to zero, the hot-water syphon is opened and the bath temperature raised about 1° C. To ensure thorough mixing of the newly-added hot water with that already in the bath, it is stirred up by blowing air through with a small hand-pump, the tube from this pump being weighted with lead to prevent it from jumping about in the bath and injuring the couple. The pump is worked with the left hand while the other is writing the note of the last reading.

One galvanometer reading is omitted after thus adding water, in order to make sure that the bath-couple, thermometer, and water are all at the same temperature. The amount added is adjusted by rule of thumb so that the galvanometer shall return to zero after two or three minutes.

The fungus may move slightly in the field; the adjustment to correct this is made by the mechanical stage. It is fortunate that the later stages of the experiment, when the fungus is near to the stopping-temperature, are rarely troubled by this movement, which seems to be due to the expansion of the copper plate.

The process might appear to be rather feverish from this outline, but in practice there is always time to spare. After some experience one experiment can be completed in about two or three hours, the last hour

¹ See curve 'Growth per Minute' in Curve 1.

being spent in taking consecutive readings, with practical certainty of a result. This does not, of course, include the time spent in the preparation of the culture.

Improvements. Though the apparatus has served its purpose up to the present, it suffers from a great many faults, chiefly, however, faults of convenience. By the help of an expert glass-blower, and by the use of steam-resisting cement, it should be possible to modify the chamber so that a number of cultures might be set up under aseptic conditions and examined successively, avoiding the loss of time which now follows transference from tube to chamber.

The heating apparatus also requires to be improved, as it is at present satisfactory for only rising or falling temperatures.

EXPERIMENTAL RESULTS.

KEY TO CONSTRUCTION OF CURVES.

Typical sample experiments have been taken in each class.

Ordinates represent the actual length of the hypha, and not the growth per minute. The latter can be seen by inspection of column 5 of the experimental data attached to each curve. Growth per minute is given in addition on Curve 1 to indicate error of measurement.

When the control-point on the hypha has been changed during the experiment, the control-point has also been changed in plotting the curve, so as to give a continuous line representing the position of the tip of the hypha.

The curves had to be plotted fairly steeply in order to show clearly the falling off of the growth-rate. The temperature curve had to be on the same sheet, so that a glance along the ordinate would show the temperature at the time of growth-cessation. Consequently, in Curves 1 and 5, the growth-curve crosses the temperature curve, making an apparent confusion, which can be rectified by consulting the side indices.

The graphic ratio of the units of time, temperature, and length is the same in all the curve except No. 4, where the graphic temperature unit is doubled.

Curve 2 is half the scale of the rest, but with the same graphic ratio.

Curves 5*a* and 5*b* are plotted somewhat curiously in order to bring the graphic representation of growth-cessation in each to coincident points. This shows up the difference in growth-rate, time of appearance of the time-factor, and alteration in the stopping-point temperature more clearly than would have been the case with any other plotting.

Small crosses on the temperature curves represent times when the galvanometer was at, or very near to, zero, i. e. when bath and culture were at the same temperature, and the error of temperature determination at a minimum.

The Standard Cultures.

The temperature relationships exhibited by freshly grown hyphae when supplied with fresh culture media—the said hyphae not having been previously raised above 20° C.—has been made the subject of some thirty preliminary experiments. The results are quite consistent.

The growth-curves plotted from the results of these experiments show a steady acceleration of growth with rise of temperature, which corresponds fairly well to the expectation based on Van 't Hoff's law until 30°C. is reached. Above 30°C. this acceleration decreases, and the curve begins to flatten as the 'time factor' comes into noticeable operation. At a fairly definite temperature growth ceases and the curve becomes horizontal. The temperature at which this cessation of growth takes place is commonly called the 'maximum', but in view of the results obtained in this research it seems advisable to drop the word for the present; I shall refer to this point in the growth-temperature curve by the provisional title of 'stopping-point'.

The form of the upper part of the curve as it falls off to the horizontal is very variable; even at the same rate of heating it is not necessarily the same, and it is flatter when the cultures are heated more slowly than the usual rapid rate employed. In a few cases the curve almost coincided with the theoretical expectation, the falling off of the growth-rate being scarcely noticeable until a minute before complete cessation. It will appear subsequently that these variations are probably due to differences in the freedom of osmosis from cell to medium, of the poisonous excreta.

The 'optimum' thus appears as the temperature at which the time factor and the temperature acceleration are in equilibrium, and it is not in any way a definite point. The word will not be used again in this connexion.

More interest centres round the stopping-point. When all allowances have been made for errors of observation, it is certain that with the present apparatus the point can be determined to within 0.3°C. of accuracy, or even less. The differences actually recorded are much more than this observational error; the stopping-points lie between 37°C. and 38.3°C. , with one exception which will be described shortly. These variations will be seen at a later stage of the paper to be in all probability due to varying freedom of osmosis, provided that the rate of heating is approximately the same in each experiment.

The exceptional case, which in itself supports this idea, was the first reading of a double observation. The hypha almost stopped at 37.5°C. , bent slightly at the tip, and continued to grow slowly until 40.5°C. was reached. The new portion bore the appearance of having burst out from the end of the hypha through a weak, though unbroken area in the cell-wall; this mark enabled the slight growth to be determined with absolute certainty. The second determination on this hypha after cooling down gave a reading of 37.4°C. The fact has some importance, for it shows that the cessation of growth is not due to any inability of the protoplasm to perform constructive metabolism beyond 38°C.

The principal point to notice, in view of subsequent results, is that in no case was the stopping-point depressed below 37.0°C.

STANDARD CULTURE. CURVE I.

Standard culture: see Curve I.

24/4/1907. 100 % fresh medium. Stored overnight at 20° C.

Set up 10 a.m. Pure oxygen. Galvanometer zero at 30 mm.

Lamp put in to begin heating at 11.57 a.m.

Readings of micrometer eye-piece scale in length-units of .003 mm.
(Columns 2, 3, 4, 5).

1 <i>Time.</i>	2 <i>Control Point.</i>	3 <i>End of Hypha.</i>	4 <i>Length under Observation.</i>	5 <i>Growth per Minute (by difference).</i>	6 <i>Temperature of Bath.</i>	7 <i>Reading of Galvanometer.</i>	8 <i>Notes.</i>
12.0 p.m.	3	26.9	23.9	—	—	—	
1	—	28	25	1.1	23.3° C.	29½	
2	2.7	29	26.3	1.3	—	—	
3	2.3	29.8	27.5	1.3	24.6	34	
4	2.7	31.2	28.5	1.0	—	31	
5	2.5	32.3	29.8	1.3	—	28	
6	2.2	33.3	31.1	1.3	—	—	
7	2	34.3	32.3	1.2	—	—	
8	2	35.6	33.4	1.1	26.5	33½	
9	—	36.8	34.6	1.2	—	31	
10	2.3	38.2	35.9	1.3	—	28	
11	—	39.4	37.1	1.2	—	—	
12	—	40.5	38.2	1.1	27.5	30½	
13	—	41.8	39.5	1.3	—	—	
14	2.2	43.2	41	1.5	28.5	32	
15	—	44.7	42.5	1.5	—	29	
16	—	45.8	43.6	1.1	—	—	
17	11.0	31	20	—	29.4	32	New control.
18	—	32.3	21.3	1.3	—	29½	
19	—	33.8	22.8	1.5	—	—	
20	—	35.1	24.1	1.3	31.1	31½	
21	—	36.8	25.8	1.7	—	29½	
22	—	38.2	27.2	1.4	—	—	
23	—	39.8	28.8	1.6	32.6	34½	
24	—	41.4	30.4	1.6	32.5	30½	
25	—	43	32	1.6	—	27	
26	—	44.8	33.8	1.8	—	—	
27	—	46.4	35.4	1.6	34.5	38	
28	—	48	37	1.6	34.4	36	
29	9.0	18.0	9.0	—	34.3	34	New control. Opened lamp.
30	—	19.3	10.3	1.3	34.2	31½	
31	—	20.3	11.3	1.0	34.2	29½	
32	—	21.4	12.4	1.1	—	—	
33	—	22.3	13.3	.9	—	—	
34	—	23.3	14.3	1.0	36.0	36	
35	—	24.4	15.4	1.1	—	33	
36	—	25.3	16.3	.9	35.9	30	
37	—	26.3	17.3	1.0	—	—	
38	—	27	18	.7	37.0	31½	
39	—	27.2	18.2	.2	36.9	29	
40	—	27.4	18.4	.2	—	—	
41	—	—	—	.0	38.1	31½

CURVE I. Standard culture.

TRIPLE READING. CURVE 2.

Fresh Salomonson medium, 100 %, pure oxygen.

Tube inoculated evening of 9/3/07.

Set up 3 p.m., 10/3/07. Galvanometer zero, 30 cm.

Readings of micrometer eye-piece scale in length-units of .003 mm. (Columns 2 3, 4, 5).

1 <i>Time.</i>	2 <i>Control Point.</i>	3 <i>End of Hypha.</i>	4 <i>Length under Observation.</i>	5 <i>Growth per Minute (by difference).</i>	6 <i>Temperature of Bath.</i>	7 <i>Reading of Galvanometer.</i>	8 <i>Notes.</i>
5.19 p.m.	—	32.0	30.0	1.1	29.4° C.	32	
20	—	—	—	—	—	—	
21	3	35	32.0	—	29.0	29	
22	2.0	34.8	.8	0.8	28.8	26	
23	0.0	33.6	33.6	0.8	—	—	
24	—	34.6	34.6	1.0	30.5	30.5	
25	—	—	—	—	.3	25	
26	2.0	38.8	36.8	1.1	—	—	
27	0.0	38	38	1.2	33.1	37.5	
28	—	—	—	—	32.7	31.5	
Changed to a branch of the same hypha.							
5.29	9.0	31.5	22.5	—	32.4	25.5	
30	10.0	33.0	23.0	0.5	—	—	
31	9.5	33.5	24.0	.5	35.7	38	
32	10	34.4	24.4	.4	.4	32.5	
33	—	35.0	25	.6	.1	26.5	
34	—	35.2	25.2	.2	37.1	30 ?	
35	9.0	34.2	25.2	.0	36.9	29	
36	—	34.2	—	—	.5	23	
37	—	—	—	—	38.5	27 ?	
38	—	—	—	—	.1	24	Partially re- [moved lamp]
39	—	—	—	—	37.7	20.5	
40	—	—	—	—	—	—	
41	—	—	—	—	39.8	30	
42	—	—	—	—	.3	31	
43	—	—	—	—	38.8	—	
44	—	—	—	—	.5	—	
45	—	—	—	—	.1	30	
46	—	—	—	—	37.8	29.5	
47	—	—	—	—	.6	29	
48	—	—	—	—	.3	28.5	
49	—	—	—	—	37.0	28	
50	—	—	—	—	36.7	27	
51	—	—	—	—	—	—	
52	—	—	—	—	—	—	
53	—	—	—	—	—	—	
54	—	—	—	—	—	—	
55	10.0	35.3	—	—	37.5	27	
56	—	—	—	—	.1	27.5	Removed lamp.
57	—	—	—	—	36.8	27	
58	—	—	—	—	.5	26.5	
59	—	—	—	—	.2	26	
6. 0	—	—	—	—	35.9	25	
1	10.0	35.4	—	—	—	—	
2	—	—	—	—	36.4	28.5	
3	—	35.6	—	—	.1	39 ?	
4	—	—	—	—	35.8	30	
5	—	—	—	—	.5	30.5	

1 Time.	2 Control Point.	3 End of Hypha.	4 Length under Observa- tion.	5 Growth per Minute (by difference).	6 Temperature of Bath.	7 Reading of Galvano- meter.	8 Notes.
6.6 p.m.	10.0	35.6	—	—	35.3	32	
7	—	—	—	—	.0	33	
8	—	—	—	—	—	—	
9	—	35.8	25.8	0.2	34.6	30	
10	—	—	—	—	34.2	31	
11	—	—	—	—	—	—	
12	—	—	—	—	33.9	37 ?	
13	10.0	36	26.0	0.2	.5	32.5	
14	—	—	—	—	—	—	
15	—	36.1	26.1	0.1	33.1	36	
16	—	.2	.2	.1	—	—	
17	—	.4	.4	.2	.4	32	
18	—	.7	.7	.3	.2	34	
19	—	37.0	27.0	.3	32.0	36	
20	—	.3	.3	.3	—	—	
21	—	.6	.6	.3	31.2	31.5	
22	—	38.0	38.0	.4	31.0	33.5	Lamp in.
23	—	.3	.3	.3	30.8	35	
24	—	.7	.7	.4	.6	36	
25	—	39.0	29.0	.3	.4	36	
26	—	.3	.3	.3	.3	33.5	
27	—	40.0	30.0	.7	.2	30.5	
28	—	.3	.3	.3	.1	28.5	
29	—	.7	.7	.4	30.0	25.5	
30	—	.9	.9	.2	—	—	
31	—	41.2	31.2	.3	31.1	31	
32	—	.6	.6	.4	.0	28.5	
33	—	.9	.9	.3	31.7	30	
34	—	42.2	32.2	.3	.6	27.5	
35	—	.7	.7	.4	—	—	
36	—	43.0	33.0	.3	33.0	34.5	
37	—	.3	.3	.3	32.7	31	
38	—	.8	.8	.5	.4	26	
39	—	44.2	34.2	.4	—	—	
40	—	.8	.8	.6	34.1	32	
41	—	45.2	35.2	.4	33.9	28	
42	—	.8	.8	.6	—	—	
43	—	46.1	36.1	.3	34.8	30	
44	—	.6	.6	.5	.5	26	
45	—	47.0	37.0	.4	—	—	
46	—	.4	.4	.4	36.1	32.5	
47	—	.8	.8	.4	35.9	29	
48	—	48.2	38.2	.4	—	—	
49	—	38.6	.6	.4	36.3	27.5	
50	—	39.0	39.0	.4	—	—	
51	—	.3	.3	.3	37.1	31	
52	—	.6	.6	.3	36.8	28.5	
53	—	.6	.6	—	—	—	
54	—	.7	.7	.1	38.2	35.5	
55	—	.8	.8	.1	37.9	33	
56	—	.8	.8	—	.6	30.5	
57	—	.8	.8	—	.3	28.5	Removed the
7.0	—	—	—	—	36.8	24	[lamp.]
7.14 About 1.5 growth, took fresh control-point on same hypha.							
14	13	22.6	9.6	—	—	—	
15	—	—	—	—	33.2	44	
16	13.0	22.9	9.9	—	.1	44.5	
17	—	23.3	10.3	.4	—	—	
18	—	.6	.6	.3	30.8	27.5	Lamp in.
19	—	.8	.8	.2	.6	28	

1 Time.	2 Control Point.	3 End of Hypha.	4 Length under Observa- tion.	5 Growth per Minute (by difference).	6 Temperature of Bath.	7 Reading of Galvano- meter.	8 Notes.
7.20 p.m.	—	24.0	11.0	.2	.4	29.5	
21	—	.2	.2	.2	.2	30.5	
22	13.0	.3	.3	.1	.1	32	
23	—	.5	.5	.2	.0	32	
24	—	.8	.8	.3	29.9	29.5	
25	—	.9	.9	.1	.7	28	
26	—	25.0	12.0	.1	.6	26.5	
27	—	.1	.1	.1	—	—	
28	—	—	—	—	31.4	37	
29	—	.6	12.5	—	.3	34.5	
30	—	.9	.9	.3	.1	32	
31	14.0	27.0	13.0	.1	30.9	29.5	
32	—	—	—	—	—	—	
33	14.0	27.1	.1	.1	.6	30	
34	—	.2	.2	.1	.4	26	
35	—	.3 ?	.3 ?	.1 ?	—	—	
36	—	.3	.3	.1	32.7	32	
37	—	—	—	—	.4	28.5	
38	—	—	—	—	—	—	
39	—	—	—	—	33.4	30	
40	—	—	—	—	—	—	
41	—	—	—	—	—	—

CURVE 2. Triple standard curve.

The possible Limiting Factors in Standard Culture.

The essential points of the standard culture experiment are as follows:—

Minute fragment of mycelium develops hyphae in a relatively large volume of fresh, rich medium for twenty hours at a temperature which is not higher than 20° C.

A few of these hyphae are then removed, placed in the special damp chamber described above, and provided with a large excess of pure oxygen for their respiration.

The temperature of the chamber is not raised above 20° C. until growth has begun in the hypha under observation.

The temperature is then raised at least 1° C. in every four minutes.

All these precautions have proved to be considerably in excess of what is needed for any one of them.

In such a culture the possible limiting factors are somewhat as follows:—

1. Amount of available oxygen.
2. Rate of diffusion of oxygen through wet semi-permeable membranes.
3. Amount of carbon dioxide present.
4. Rate of diffusion of carbon dioxide.
5. Amount of available food material.
6. Rate of diffusion of food materials.
7. Rate of chemical change in the protoplasm, limited by temperature.
8. Water-supply.
9. Percentage of 'x' in the cell sap.
10. Rate of outward diffusion of 'x'.

This arrangement is not quite logical, because the amounts depend on the rates of diffusion, but it serves as a convenient mode of statement.

1. *The amount of oxygen.* The same results have been obtained with air in the chamber as when oxygen was employed, except in two early experiments when a very small air bubble was used for the respiration of a large mass of mycelium. In order to eliminate any possibility of this factor being limiting I afterwards used pure oxygen in the chamber for all experiments, though I believe it to be quite unnecessary.

2. *Rate of diffusion of oxygen.* This can hardly be a limiting factor at any time, for the use of pure oxygen has no effect on the stopping point, nor (as far as one can judge) on the rate of growth, though the partial pressure under these conditions is five times as great as in ordinary atmospheric air, and the rate of solution in wet membranes is correspondingly increased.

3. *Amount of carbon dioxide.* The culture solution is slightly alkaline, and remains so.

4. *Diffusion of carbon dioxide.* If the diffusion rate of oxygen into the cell is never a limiting factor, it is still less likely that the more soluble carbon dioxide should ever accumulate inside the cell in sufficient amount to influence the protoplasm, seeing that the air of the chamber contains no carbon dioxide whatever.

5. *Available food materials.* The culture solutions employed are fairly concentrated and can be diluted to at least a quarter of this concentration without affecting the stopping point, so that we may assume that there is ample food in the solution, and also (6) that this food passes into the cell by osmosis at a sufficiently rapid rate to satisfy all the cell's demands.

7. *Rate of chemical change.* There is some evidence in the curves obtained that the growth function obeys Van 't Hoff's law, within the limits imposed on it by the rapid staling which takes place at high temperatures. The cessation of growth is obviously not due to any destruction of the protoplasmic machinery, as is the case at the death-point; this is shown by the aberrant result of 40.5°C ., and by the normal behaviour of hyphae which have been heated up as far as 41°C . and then cooled down again. Some effect seems to be produced on such hyphae, however, for the rate of growth is noticeably slower on the second heating, even though the stopping point be practically unaffected¹.

8. *Water supply.* Is never limiting.

8, 9. The hypothesis on which these results find their only present explanation is that some substance (or mixture of substances) is formed by the protoplasm in katabolism, and inhibits growth by its accumulation. Some of the temperature-growth phenomena of the organism depend on the amount of this substance which is present in the cell, which amount is again dependent on its rates of production and of removal.

The formation of this substance takes place at low temperatures as well as at high temperatures, but with far greater rapidity in the latter case.

The hypothesis approximates to a theory in that this katabolite has been isolated from the organism, and some of its properties determined; it has not yet been isolated chemically, nor identified.

Throughout the rest of this discussion this body will be referred to, for convenience, by the designation of '*x*'.

Effects of previous exposure to high temperatures.

Having considered the temperature-growth phenomena under the most favourable conditions obtainable, we will turn to the effect of two modifications of these 'standard conditions' which throw considerable

¹ Curve 2.

light on our hypothesis. The first of these is the effect of prolonged exposure to 'sub-maximal' temperatures.

The effect of such exposure on the morphology of the fungus, and on the time required for the completion of staling, has been described previously. We now proceed to note the effect on the stopping-point.

A difficulty in the experimental method might be noted here in order to excuse the inaccurate temperatures employed; owing to the erratic gas pressure in the laboratory mains, and the absence of a pressure-

CURVE 3. Inoculated tube stored at $34-35\frac{1}{2}^{\circ}\text{C}$. for twenty-four hours. Curve represents the fastest hypha obtainable.

regulator, it is not possible to keep the Hearson incubator at a perfectly constant temperature. A nominal temperature of 33°C . is actually one of $32-34^{\circ}\text{C}$.

The curve plotted from a culture which has been stored at 33°C . before being transferred to the damp chamber for study is similar to that obtained in the standard cultures, except that the rate of growth is slower, and that the stopping-point is lowered.¹ On account of the irregularity

¹ Curve 3.

of the incubator it is not necessary to give the details of each experiment, but in every case some effect was produced, the stopping-point falling to

36.7° C., 34.0° C., 32.5° C., and 29.8° C.

in four experiments.

The offered explanation of this is that the body 'x' has accumulated rapidly in the cell, either in not sufficient amount to check growth, or else checking it completely. The presence of pre-formed 'x' in the cell has the effect of depressing the stopping-point, because it shortens the time required for the accumulation of the 'inhibitory percentage' of 'x'. The corollary of this phenomenon is recorded on p. 559, where the temperature is kept constant and the time prolonged, instead of the temperature being raised and the duration of the experiment reduced to a minimum.

If all that we have assumed about 'x' is true, we should be able to detect it in greater amounts in media which have contained mycelium at sub-maximal temperatures, than in media containing mycelium which had been exposed for an equal time to lower temperatures; in the former case more 'x' should be free to diffuse osmose out into the surrounding medium than in the latter. At present our only test for the presence of 'x' is the biological one.

We have only to remove the mycelium which has formed during the prolonged exposure, reinoculate with a fresh cluster of resting cells, store for a night below 20° C. until the cells of the new mycelium contain the same concentration of 'x' as the medium, and then remove a fragment of mycelium and determine its stopping-point in the usual way. The figures from the most delicate of these experiments are given:—

Tube A. Stored seven days at 20° C.

Volume of medium, 25 cc.

Stopping-point of new inoculation, 37.3° C.

Depression of stopping-point, negligible.

Tube B. Stored four days at 22° C.; one day at 33° C.; and two days at 28° C.

Volume of medium, 25 cc.

Stopping-point of new inoculation, 36.5° C.

Depression of stopping-point, at least 0.5° C.

These results show that 'x' diffuses out into the surrounding medium, and that it is formed more rapidly at sub-maximal than at low temperatures.

The length of time required for the completion of staling is greater at low temperatures than at high ones (p. 559), because less of 'x' is excreted in a unit of time, and it is greater in large amounts of culture medium than in small amounts, because more 'x' has to be formed in order to raise the concentration of 'x' in the medium to the inhibitory percentage. The

extreme case of the latter is seen in the cells of the cotton seedling, where the volume of the culture medium is so small that staling takes place in a few hours at so low a temperature as 30°C .¹

Dilution of stale media.

If the idea of increasing concentration which has been sketched above be true, we ought to be able to simulate increasing staleness by adding the stale media to fresh media.

Before doing so we have to eliminate the possibility that the ratio of 'x' to the available food might have some subsidiary effect. This was done by four parallel experiments, using a solution which was on the verge of complete staleness:—

50 % Stale ;	50 % Fresh ;	stopping-point,	32.1°C .
50 % „	50 % Water ;	„ „	32.5°C .
50 % Fresh ;	50 % „	„ „	37.1°C .
100 % „		„ „	37.5°C .

Hence, if any such effect is exerted it is inconspicuous beside the effects of adding 'x'.

The first experiments gave erratic results, on account of the slow destruction of 'x' by boiling², and owing to the time wasted by this the results of the dilution experiments are not of a quantitative nature at present. The transfer of stale media from one tube to another is now effected through a flame, thus avoiding the necessity for subsequent sterilization.

When the stale medium (or 'solution of x') is added to a fresh medium, we observe a depression of the stopping-point which appears to correspond to the percentage of 'x' which has been added.³ The lowest depression obtained with a 50 % mixture of perfectly stale medium with fresh medium was 8°C ., the stopping-point being 29.5°C .

Parallel with this is the fact that when such graded solutions are inoculated and allowed to stand at a constant temperature, the rate of growth is proportional, as far as macroscopic evidence can be trusted, to the percentage of 'x' which is present,³ provided that the experiment is not carried on so long as to involve secondary staling of the media.

I have not yet been able to show that a proportionate relation exists between the depression of the stopping-point and the amount of 'x' which has been added to the medium. That such a connexion exists is almost beyond doubt, but until its exact nature shall have been determined I consider that the present research is of more interest than value. When we have correlated the influences of the various proportions of 'x' with the

¹ See p. 558.

² Page 581.

³ Note also the three growth-gradients in Curve 2.

depressions of the stopping-point we shall be in a position to put a part of the growth process on a chemical footing.

The causes which have prevented this accurate determination are three-fold. The body 'x' has only been obtained in quantities which are relative to the bulk of the solution, whereas it will have to be isolated and handled in definite amount. The slight instability of 'x' has also to be overcome.

CURVE 4.

Lastly, so much time was spent during the cool months of the Egyptian winter in devising a satisfactory apparatus for the research, that by the time the qualitative experiments had been carried out the summer had arrived.

Decomposition of 'x'.

The first experiments with stale media gave erratic results. The cause of this was traced to the boiling of the media in the steam sterilizer, which partly, or entirely, destroyed the poisonous excreta. This is contrary to the opinion which I formed during the preliminary experiments with mass

cultures, on account of the greater bulk and concentration of the latter, as will be seen below.

The effects of boiling a stale medium are rather erratic. It would seem that the decomposition of 'x' is more rapid in dilute solution, in thin films, and in small bulk of medium. These facts indicate that it is not an enzyme, and that its disappearance is due rather to chemical change than to physical displacement.

In no case was the stopping-point lowered by boiling the stale medium, although some thirty experiments were made, either directly, or in connexion with some other object. The application of heat to the medium either produced no effect, or else caused growth to cease at a higher temperature than had previously been the case.¹

DECOMPOSITION OF 'x'. CURVE 4.

Nearly stale culture medium. Not diluted. Boiled for four hours under reflux condenser, followed next day by two hours' boiling in the steam sterilizer.

Tube inoculated evening of 22/4/07, at 22° C.

Set up 10.15 a.m., 23/4/07. Galvanometer zero, 30 cm.

Readings of micrometer eye-piece scale in length-units of .003 mm. (columns 2, 3, 4, 5).

1 Time.	2 Control Point.	3 End of Hypha.	4 Length under observa- tion.	5 Growth per Minute (by difference).	6 Temperature of Bath.	7 Reading of Galvano- meter.	8 Notes.
10.46 p.m.	1	39.0	38.0	.7			
43	1	.7	.7	.6	26.7	33	
44	1	40.3	39.3	.8	26.7	30.5	
49	0.3	40.4	40.1		26.7	28	
50							
51							
10.52 p.m.	.0	45.5	42.5	—	28.5	27	
53	2.0	45.5	43.5	1.0	—	—	
54	—	—	—	—	30.3	31.5	
55	20.0	47	27	—	—	—	
56	18.0	46	28	1.0	33.6	46.5	
57	—	46.8	28.8	.8	—	41.5	
58	—	48.0	30.0	1.2	33.5	37	
59	19.0	49.0	30.0	0.0	—	33	
11.0	15.0	45.0	30.0	—	—	28.5	
A rapidly growing hypha, branching from the one previously under observation.							
11.1 p.m.	12	36	24	—	—	—	
2	14	38	24	0.0	35.0	31.5	
3	18	42	24	—	34.9	27	
4	19	43	—	—	—	—	

In this connexion it may be pointed out that whereas fresh media either give the same stopping-point in duplex curves (Curve 2), or else a depression due to staling, the artificially staled cultures commonly show an increase in the stopping-point when duplicated; in one case it rose from 31.0°C. to 33.5°C. and in another from 29.5°C. to 37.0°C. This would seem to indicate that in the thin film of solution in the damp chamber the body 'x' is even more readily decomposed than in the large tubes. Further, it is possible that some conditions which protect it from decomposition exist in the cells of the organism.

The series of results given below is typical of the data obtained, and demonstrates all the important points.

Medium from a large flask culture, almost stale; transferred to a series of tubes, and heated at 100°C. in the steam sterilizer for thirty minutes. This solution was then treated in various ways.

(a) Inoculated and tested—growth almost negligible, too slight to enable any determination of the stopping-point to be made. In the tube it grew about five millimetres in ten days.

(b) Boiled for four hours under a reflux condenser, and for another hour in the sterilizer; growth of fungus free, with a stopping-point at 32.5°C. Curve 4.¹

(c) Mixed with own bulk of water, i.e. 50 per cent. solution.

Tested directly	.	.	.	.	.	.	29.5°C.
Boiled in sterilizer for half an hour	.	.	.	.	.	.	35.0°C.
Boiled for another half hour	.	.	.	.	.	.	38.0°C.

(d) Diluted with water to 25 per cent.

Tested directly	.	.	.	.	.	.	31.0°C.
Boiled for half an hour	.	.	.	.	.	.	33.5°C.
Boiled for two hours	.	.	.	.	.	.	37.4°C.

¹ Page 580.

DECOMPOSITION OF 'x'. CURVE 5 a.

Fifty per cent. fresh Salomonson medium, mixed with 50 per cent. stale ditto. Companion experiment to 5 b, but the mixed medium placed in steam sterilizer at 10.30 a.m., boiling by 11.30 a.m., and removed at 1 p.m.

Tube inoculated noon, 11/4/07. Stored at 24° C.

Set up 10 a.m., 12/4/07. Galvanometer zero, 30 cm.

Readings of micrometer eye-piece scale in length-units of .003 mm. (columns 2, 3, 4, 5).

1 Time.	2 Control Point.	3 End of Hypha.	4 Length under Observa- tion.	5 Growth per Minute (by difference).	6 Temperature of Bath.	7 Reading of Galvano- meter.	8 Notes.
10.30 a.m.	7.0	30.5	23.5	—	30.4° C.	33	
31	8.0	32.2	24.2	.7	.4	31	
32	9.0	34.0	25.0	.8	.3	29	
33	—	.8	.8	.8	—	—	
34	—	—	—	—	—	—	
35	6.3	34.2	27.9	—	31.1	31	
36	—	35.3	29.0	1.1	.1	28	
37	—	36.3	30.0	1.0	—	—	
38	—	37.3	30.9	.9	31.9	33.5	
39	6.7	38.3	31.6	.6	—	31.5	
40	—	39.2	32.5	.9	.8	29	
41	—	40.3	33.6	1.1	—	—	
42	—	41.4	34.7	1.1	32.8	34	
43	7.7	43.5	35.8	1.1	—	32.5	
44	7.0	44.0	37.0	1.2	.7	30	
45	31.0	44.5	13.5	—	—	—	New control, old one indefinable.
46	—	45.7	14.7	1.2	33.3	32	
47	—	47.0	16.0	1.3	—	30.5	
48	28.7	48.2	19.5	—	—	—	New control.
49	—	49.2	20.5	1.0	33.5	32.5	
50	—	50.0	21.3	.8	—	30	
51	24.0	34.3	10.3	—	—	—	New control.
52	—	35.5	11.5	1.2	34.3	37	
53	—	36.3	12.3	.8	—	35	
54	—	37.6	13.6	1.3	.2	32	
55	23.7	38.9	15.2	1.6	—	30	Lamp closer.
56	23.5	40.0	16.5	1.3	—	—	
57	23.3	41.2	17.9	1.4	34.9	34	
58	23.1	42.2	19.1	1.2	.9	32.5	
59	23.0	43.2	20.2	1.2	.8	31	
11.0	23.5	44.7	21.2	1.0	.8	28.5	
1	—	45.8	22.3	1.1	—	—	
2	—	46.8	23.3	1.0	35.7	34	
3	24.0	48.0	24.0	.7	.5	32	
4	23.5	48.7	25.2	1.2	.4	29.5	
5	23.2	49.2	26.0	.8	—	—	
6	15.0	42.0	27.0	1.0	36.9	37.5	
7	—	.6	.6	.6	.8	35.5	
8	—	43.0	28.0	.4	.7	32	
9	—	43.7	.7	.7	36.6	29	
10	14.8	44.0	29.2	.5	—	—	
11	.6	.3	.7	.5	37.4	31.5	
12	15.0	45.0	30.0	.3	.3	29	
13	—	—	—	—	—	—	
14	—	—	—	—	38.0	30	
15	—	—	—	—	—	—	
16	—	—	—	—	—	—

CURVES 5a and 5b. Two experiments with same medium.

CURVE 5 b.

Fifty per cent. fresh Salomonson medium, mixed with 50 per cent. stale ditto.
Companion experiment to 5 a, but not boiled.

Tube inoculated, 7/4/07. Stored at 24° C.

Set up, 10 a.m., 8/4/07.

1 <i>Time.</i>	2 <i>Control Point.</i>	3 <i>End of Hypha.</i>	4 <i>Length under Observa- tion.</i>	5 <i>Growth per Minute (by difference).</i>	6 <i>Temperature of Bath.</i>	7 <i>Reading of Galvano- meter.</i>	8 <i>Notes.</i>
11.2 p.m.	6.3	30.3	24.0	—	26.5° C.	32	Lamp in.
3	7.1	32.0	24.9	.9	—	32.5	Apparatus shaken by traffic out- side.
4	—	32.3	25.2	.3	—	30.5	
5	7.0	32.6	25.6	.4	36.4	29	
6	6.8	32.9	26.1	.5	—	—	
7	4.3	31.1	26.8	.7	27.7	37	
8	4.1	31.3	27.2	.4	—	33	
9	—	.8	.7	.5	—	31	
10	4.0	32.2	28.2	.5	—	29.5	
11	3.9	32.5	.6	.4	—	—	
12	—	—	—	—	28.8?	31	
13	3.8	33.2	29.4	—	—	—	
14	4.5	34.3	29.8	.4	29.6	36	
15	.2	.5	30.3	.5	—	33.5	
16	4.0	34.7	30.7	.4	—	30.5	
17	—	.8	.8	.1	—	—	
18	—	.9	.9	.1	30.4	32	
19	3.8	34.8	31.0	.1	—	28.5	
20	4.1	35.2	.1	.1	—	—	
21	4.0	35.2	.2	.1	31.5	34	
22	—	.3?	—	—	—	31.5	
23	—	.3	.3	.05	—	29	
24	—	.4	.4	.1	—	—	
25	—	—	—	—	32.3	31	
26	—	—	—	—	—	28.5	
27	—	—	—	—	—	—	
28	—	—	—	—	—	—	
29	—	—	—	—	—	—	
30	—	—	—	—	—	—	

THEORETICAL ASPECTS AND CONCLUSIONS.

The Components of the Growth-curve.

A growth-curve has been constructed (Curve 6) to simulate the experimental results, and to show, by analysis, the lines along which further work might be attempted.

Two special assumptions will be made of which no accurate proof exists, viz., that—

(a) The depression of the stopping-point is proportional, in some kind of progression, to the amount of 'x' which is present in the cell at the commencement of the experiment.

(b) The decrease in growth-rate at constant temperature is also proportional to the amount of 'x' which is present in the cell.

Until these assumptions have been proved or disproved the following presentment has no direct value.

Let us start by imagining that some substance, or group of substances (probably situate in the nucleus) is, as it were, the governor which controls

CURVE 6. The components of the curve of growth. Varying temperature.

the constructive metabolism of the cell. For convenience in discussion we will again have recourse to a symbol, and denote this governor by G .

We know that the body 'x' is excreted as a result of the growth-process, and that its excretion ceases with the cessation of growth, and that it exerts an injurious effect upon the process—that is to say, upon G . This effect is presumably chemical, and will be affected by rise of temperature.

We also know that ' x ' is excreted with greater rapidity at high temperatures, so we will further assume that both these chemical reactions follow Van 't Hoff's law.

Let us now apply this mass of assumptions to the modification of the theoretical growth-curve which is represented above the base line (upper curve).¹ The practical curve is also shown above the base line (lower curve). The difference between these curves represents the antagonistic force exerted by the action of ' x ' upon G ; this difference is drawn below the base line.

Our object is to find what force, or combination of forces, will simulate this antagonistic curve.

Take first the accumulation of ' x ' with rise of temperature. This will give us a curve below the base line, of less altitude than the theoretical growth-curve, but otherwise similar. Consequently, it can never neutralize the latter, but can only depress it, without even altering its form.

The same applies to a curve which shall represent the action of ' x ' upon G .

As yet we have only taken into account the rise in temperature, and have disregarded the fact that time is also increasing. Now, the production of ' x ' from the protoplasm is obviously a slow reaction; consequently, its rate of accumulation in the cell will be represented by a curve showing an arithmetical progression at constant temperature, and by the combination of this with the previous curve when the temperature is rising.

It is possible that other time-curves may be involved, such as one representing the mass-reaction between ' x ' and G .

The results of three such antagonistic curves acting on the theoretical growth-curve are, however, not unlike the results obtained in practice.

Another curve is appended,² based on the same assumptions, in order to analyse the effects of a constant high temperature. The rates of acceleration have, of course, been deliberately adjusted.

With regard to the manner in which these antagonistic forces might be determined:—

It is clear that the first data will be given by the stopping-point depressions effected by solutions of known concentration of ' x ', and the remainder by comparison of different rates of heating. Lastly, if ' x ' can be isolated (and this should in any case be the next step in the work) we might be able to determine something as to the chemical nature of G .

There are three apparent objections to this scheme of attack:—

It may not be possible to isolate ' x ', which seems to be a somewhat unstable substance; possibly a substitute might be found. The heating apparatus will have to be under much better control than it has been in my experiments. Lastly, we are confronted with a doubt as to whether

¹ Curve 6.

² Curve 7.

CURVE 7. The components of the curve of growth. Constant high temperature, or the Time Factor.

the slight irregularities in the stopping-point, noticed under conditions which are as uniform as I have been able to devise, may not be enough to prevent accurate determinations; some other organism might be employed if this were found to be the case.

The Time Factor in other Life-processes.

This proof of the concrete and chemical nature of the time factor in growth leads one to hope that the same may be found to apply to the time factor in assimilation and respiration. From this point of view a fact recorded by Blackman might be suitable for further investigation; namely, the low assimilatory maximum shown by Cherry Laurel leaves in their second year, as compared with the result which they give in their first year. If this depression of the maximum were due to the accumulation of a specific inhibitory body in the leaf cells, it should be possible to extract this body from two-year-olds, and supply it to the young leaves; when it entered the cells of the latter their assimilatory maximum should at once fall.

If, on the other hand, such treatment produced no effect, there would still be the possibility that this body really existed, but that the young leaves had the power of decomposing it, and that this power was lost in age.

GENERAL CONCLUSIONS.

1. The growth rate at various temperatures accords with the expectations of Van 't Hoff's law.
2. The decrease, and ultimate cessation of growth at high temperatures, is due to the accumulation of katabolic products in the cells.
3. This cessation is distinct from the disorganization of the protoplasm by heat, which results on a further rise of temperature to the death-point.
4. The same products are formed at low, as at high temperatures, but with greater rapidity in the latter case.
5. To the rapid formation of these bodies is due the injurious effect of prolonged exposure to sub-maximal temperatures, commonly known as the time factor. This time factor is identical with the phenomenon of 'staleness' in rich cultures of fungi.
6. In the case of isolated cells these bodies diffuse out into the surrounding liquid. In multicellular organisms they have to be otherwise disposed of, probably by decomposition; since the conditions under which this decomposition takes place must be fairly uniform in the interior of a higher plant, these latter show in consequence a well-marked 'Optimum', which is the expression of the internal struggle between the increasing rapidity of chemical change with rise of temperature, and the inhibiting effects of the accumulating katabolic products.

PRACTICAL CONCLUSIONS.

7. Under the conditions of this research it is unlikely that any other factor should be limiting at the 'stopping-point' than the factor ' x '.

8. The Pfeiffer Hot-Box is quite unreliable for accurate measurements of the varying temperature of objects in the microscope field.

9. The ordinary forms of hanging-drop culture chamber are also unreliable when used with other than constant temperatures, on account of the irregular heating of the various parts, of the temperature-lag induced by the proximity of the objective, and of the changes in drop-concentration which result from this irregular heating.

My sincere thanks are due to Dr. F. F. Blackman, who has given me much assistance in the early stages of the work by advice on apparatus and methods. The present paper is to be regarded as an attempt to extend to growth-phenomena the principles which he has outlined in 'Optima and Limiting Factors'.

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APPENDIX.

Influence of Temperature on Infection.

The artificial infection of a cotton seedling with the Sore-shin fungus can be readily effected by placing the former on several layers of damp blotting-paper in a Petri dish, and then placing a fragment of rapidly growing mycelium from a cool culture in contact with it. The dish is then stored at 20° C., and within twenty-four hours the seedling will be found to have rotted at the point of inoculation. The portion of the seedling stem which is in contact with the damp paper is not usually rotted, because at that point the aeration is usually insufficient.

If the trial is repeated at a temperature of 33° C., a brown superficial scar is formed,¹ but the fungus does not extend its attack into the inner layers of the cortex.

At 38° C. no infection can be found.

On the ' x -hypothesis' it should be possible to bring about rapid infection at the higher temperatures by keeping the infected area in contact

¹ The whole research was founded on this observation.

with water, so as to wash away the body 'x' before it had time to accumulate in such amount as to inhibit the growth of the fungus through the cells of the host. This can actually be done by placing the seedling in the bottom of a Petri dish and adding enough water to submerge all but the upper third of the horizontal stem. If it is then inoculated, and left for a day at as high a temperature as 35° C., the following appearances are observed.

Under water:	No infection, because aeration is insufficient.
In the air:	Slight superficial brown scar.
On the water-level:	Deep brown rotten patch, which follows the line of equilibrium between the two former conditions.

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